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REVIEW

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Formation, contents, functions of exosomes and their potential in lung cancer diagnostics and therapeutics

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Abstract

Lung cancer is the leading cause of cancer-related death worldwide due to diagnosis in the advanced stage and drug resistance in the subsequent treatments. Development of novel diagnostic and therapeutic methods is urged to improve the disease outcome. Exosomes are nano-sized vehicles which transport different types of biomolecules intercellularly, including DNA, RNA and proteins, and are implicated in cross-talk between cells and their surrounding microenvironment. Tumor-derived exosomes (TEXs) have been revealed to strongly influence the tumor microenvironment, anti-tumor immunoregulatory activities, tumor progression and metastasis. Potential of TEXs as biomarkers for lung cancer diagnosis, prognosis and treatment prediction is supported by numerous studies. Moreover, exosomes have been proposed to be promising drug carriers. Here, we review the mechanisms of exosomal formation and uptake, the functions of exosomes in carcinogenesis, and potential clinical utility of exosomes as biomarkers, tumor vaccine and drug delivery vehicles in the diagnosis and therapeutics of lung cancer.

KEYWORDS
biomarker, diagnosis, exosome, lung cancer, tumor-derived exosome

INTRODUCTION

Lung cancer, which accounts for 11.6% of all cancer cases and 18.4% of cancer related mortalities worldwide, represents a serious public health problem.[1] Non-small cell lung cancer (NSCLC), consisting of adenocarcinoma, squamous cell carcinoma and large cell carcinoma, is the predominant histological subtype of lung cancer encompassing more than 80% of total cases.[2] Chemotherapy is currently the primary therapy for advanced lung cancer. Targeted therapies with EGFR-, ALK-, BRAF- or MET-inhibitors and immunotherapies with PD-L1, PD1 or CTLA4 antibody have also been developed in NSCLC treatment.[3] While a number of potential biomarkers have been explored, none are recommended for lung cancer screening, which accounts for the current situation whereby only 15% of lung cancer patients are diagnosed at an early stage.[4] Improvement of lung cancer outcome calls for the development of biomarkers for lung cancer management.

Exosomes are a type of extracellular vehicle (EV) of endosomal origin ranging from 30 to 150 nm in diameter which are secreted by most cells and found in various body fluids, such as plasma, saliva, urine, and ascites.[5] Despite the diversity in size and type of body fluid, all exosomes contain a subgroup of membrane proteins, including TSG101, ALIX and CD63, owing to their common endosomal origin.

When first discovered in 1983,[6,7] exosomes were regarded as garbage cans for unwanted materials from the cell of origin. Accumulative studies have subsequently illustrated that exosomes are capable of conducting intracellular communication by transporting DNA, RNA, and proteins, which in turn affects the physiological condition of the recipient cell. Tumor-derived exosomes (TEX) can remold the tumor microenvironment to favor tumor progression and metastasis, for example, by transporting oncoproteins K-RAS and MET or oncogenic miRNAs to surrounding healthy cells,[8] or by initiating a premetastatic niche and guiding tumor cells to

prospective metastatic spots mediated by exosomal integrins.[9–11] TEXs can also serve as tumor vaccine to induce immune response against tumor by affecting activities of natural killer (NK) cells.[12] Moreover, the ability of exosomes to transfer biomolecules and drugs to recipient cells makes them promising drug delivery vehicles,[13] and exosomal nucleic acids (miRNA, mRNA, DNA) and proteins have shown potential in serving as diagnostic, prognostic, and predictive biomarkers for various cancers.

In the current review, we first portray recent studies on the mechanisms of formation and uptake of exosomes. We summarize tumor-promoting functions of exosomes in correlation with crosstalk between cancer cells and tumor microenvironment. Finally, we discuss the clinical potential of exosomes as biomarkers, tumor vaccine and drug carrier in lung cancer diagnosis and therapeutics.

FORMATION OF EXOSOMES

Exosomes are generated by inward budding of the membrane of a type of endosomes called multivesicular bodies (MVBs) to form intraluminal vesicles (ILVs)[14,15]followed by release of the ILVs into the extracellular space upon fusion of the MVBs with the cellular membrane[16] (Figure 1). Alternatively, MVBs can fuse with the lysosome, which results in the degradation of ILVs-containing MVBs and their contents, and may therefore modulate the secretion of exosomes.[17]Biogenesis of exosomes starts with the formation of ILVs within MVBs. The endosomal membrane is first restructured and gets enriched with tetraspanins (such as CD9 and CD63) which play a crucial role in exosomal formation.[18] The endosomal sorting complexes required for transport (ESCRTs) are then anchored to the site of ILV formation.[15,19] ESCRT I/II initiate and drive the inward budding of endosomal membrane and ESCRT III terminates this course, respectively.[20,21] Recruitment of ESCRT I/II to the cytoplasmic side of the early endosomal membrane is stimulated by a variety of factors, such as hepatocyte growth factor-regulated tyrosine kinase substrate (HRS), the ubiquitination of the cytosolic tail of endocytosed proteins, phosphatidylinositol 3-phosphate (PIP3), and curved membrane topology.[22–27]Despite the predominant role of the ESCRT pathway in exosomal biogenesis, Stuffers and colleagues revealed that, depending on the cell type, depletion of the ESCRTs did not block the formation of MVBs, indicative of the existence of ESCRT-independent mechanisms of exosomal biogenesis in parallel to the ESCRT pathway.[28] In a recent study, Baietti et al. have shown that an alternative pathway, the syndecan-syntenin-ALIX pathway which includes heparanase, syndecan heparan sulfate proteoglycans, ADP ribosylation factor 6 (ARF6), phospholipase D2 (PLD2) and syntenin, is involved in mediating exosomal biogenesis.[14]Release of exosomes into the extracellular milieu is initiated by fusion of the MVB membrane with the plasma membrane, a process revealed to involve a variety of mechanisms and facilitated by a couple of Rab GTPases, including RAB11 and RAB35, or RAB27A/B.[29–31]

EXOSOMAL CONTENTS AND THEIR SELECTIVE LOADING INTO EXOSOMES

Exosomes contain various cytoplasmic proteins, lipids, and genetic materials including DNA, mRNA, and non-coding RNAs from the cell of origin (Figure 1). These data along with the purification procedures used to isolate the exosomes have been assembled into four publicly accessible databases: Exocarta,[32] EVpedia,[33] Vesiclepedia[34] and exoRBase.[35] Extensive research has revealed that exosomal contents change with different cell types and physiological conditions.Proteins involved in exosomal biogenesis, including different types of tetraspanins (for example, CD9, CD63, and CD81), in exosomal release (such as RAB27A and RAB11) and in the endosome-related pathways (for example, ESCRT components, ALIX, ARF6, and TSG101, etc.), are universally present in exosomes. In addition, endosomal trans-membrane proteins and proteins involved in signal transduction and antigen presentation (such as TfR, LAMP1, EGFR, MHC I/II), are also commonly found in exosomes. In contrast, resident proteins of the endoplasmic reticulum, Golgi, and nucleus are barely present in exosomes.[36] A large amount of RNAs, including mRNAs, rRNAs, tRNA, miRNAs, long non-coding RNAs (lncRNAs) and circular RNAs, have been characterized in exosomes by next-generation sequencing (NGS) and other techniques.[37–40] The majority of exosomal RNAs are no longer than 200 nucleotides and only a minority can reach up to 4 kb.[41] After exosomes are released from the donor cell, the exosomal membrane is believed to protect the RNAs in the exosomes from RNase digestion in the extracellular environment.[42]Despite that, exosomes share similar lipid composition with the donor cells, sphingomyelin, cholesterol, ganglioside GM3, phosphatidylserine, and ceramide were revealed to be enriched in exosomes, whereas phosphatidylcholine and diacyl-glycerol are reduced in exosomes comparing to the cell of origin.[43,44] Studies have indicated that phosphatidylserine enrichment in exosomal membrane may promote their uptake into recipient cells via the internalization pathway.[45,46] Sphingolipid ceramide has been found to be required for the formation of ILVs by facilitating the inward budding of MVB membrane through its cone-shaped structure.[46] In accordance with this observation, ceramide and its derivatives were detected to be abundant in exosomes.[47,48] However, inactivation of neutral sphingomyelinase (nSMase), a protein which produces ceramide, does not inhibit MVB formation or exosomal release in some cell types. Different molecular machineries, therefore, may be involved in exosomal formation in different cell types and related to the difference in the exosomal contents.Indeed, exosomes released via RAB11 and RAB35 were enriched with flotillin and cell-specific proteins including Wnt, PLP and the transferrin receptor (TfR),[44] while exosomes released via RAB27A/B were enriched with late endosomal resident proteins, such as CD63, ALIX, and TSG101.[30,49] Yang and colleagues indicated proteins can be loaded into vesicles by associating with the plasma membrane as an oligomeric complex.[50] More

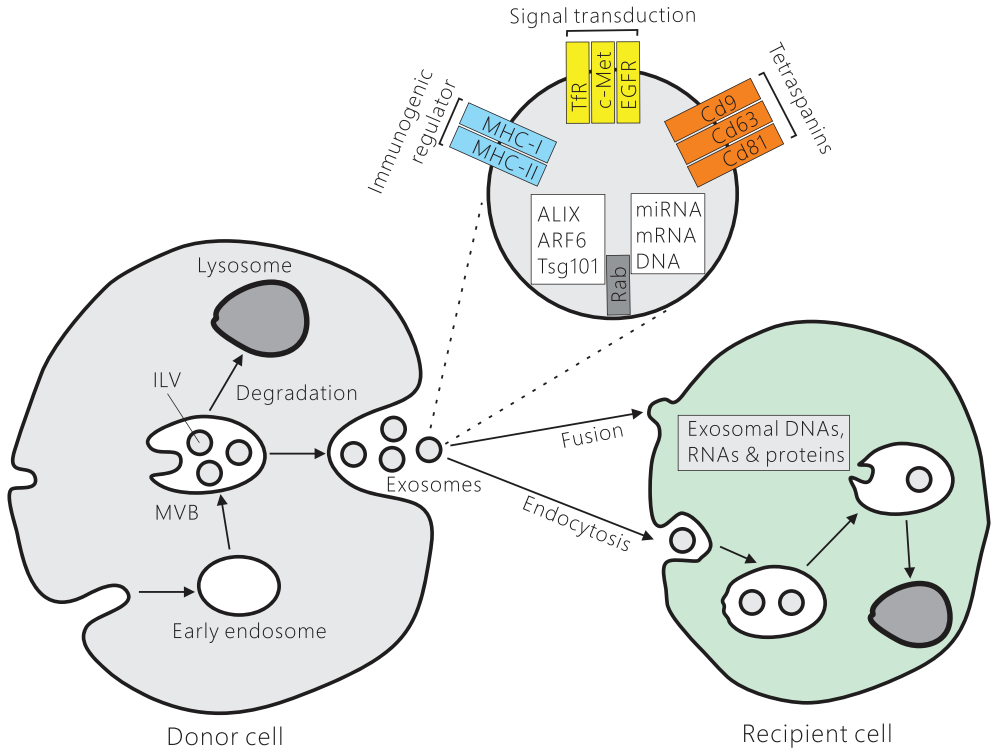

FIGURE 1 Biogenesis and secretion of exosomes by donor cells and uptake of exosomal contents by recipient cells. Exosomes originate by membrane invagination of the multivesicular bodies (MVBs) to form intraluminal vesicles (ILVs). MVBs fuse either with lysosomes or with the cellular membrane, leading to degradation of the MVBs or secretion of the exosomes into the extracellular space. Exosomes merge with the recipient cell either by fusion of the exosomal membrane with the cellular membrane or via the endocytosis pathway, leading to the discharge of exosomal contents into the cytosol of the recipient cell. Components generally found in the exosome include miRNA, mRNA, DNA and proteins such as tetraspanins (CD9, CD63, and CD81) involved in exosomal biogenesis, RAB GTPases (RAB11, RAB27A/B and RAB35) involved in exosomal secretion, ALIX, ARF6 and TSG101 in the endosome-related pathways, and proteins involved in the signal transduction and antigen presentation (such as TfR, c-Met, EGFR, MHC I/II). Abbreviations: miRNA, microRNA; ARF, ADP-ribosylation factor; TSG101, tumor susceptibility gene 101; MHC, major histocompatibility complex; EGFR, epidermal growth factor receptor; TfR, transferrin receptor

efforts are required to unveil molecular mechanisms involved in the selective loading of exosomal proteins.Studies have shown that exosomal RNA profiles, to some extent, were different from the cytosolic counterparts of the cells of origin, indicating selective recruitment of RNA cargos into the exosmes.[51–53] Various molecular mechanisms have been unveiled in selective loading of miRNAs into the exosome. An interaction between a four nucleotide (GGAG) motif enriched in exsomal miRNAs and the ribonucleoprotein hnRNPA2B1 has been proposed to sort these miRNAs into MVBs.[54] In addition, 3′-uridylation of miRNAs may contribute to recruitment of the miRNAs into exosomes while 3′-adenylation can block exosomal loading of the miRNAs.[55]

UPTAKE OF EXOSOMES

Apart from components on the exosomal membrane, such as EGFRvIII, Notch1 and Rheb, which can trigger signaling in the recipient cell by ligand-receptor interactions without exosomal merge, functionality of exosomal contents necessitate their discharge into the recipient cell to work.[56,57] Indeed, a number of studies have observed the transfer of exosomal RNAs into the recipient cell, both in vitro and in vivo.[58–60]Exosomes released from donor cells are either taken up by neighboring cells or travel through the circulation system followed by merging into cells at distance. Merging of exosomes into the recipient cell can be accomplished either by fusion of the exosomal membrane with the plasma membrane, resulting in release of exosomal contents into the cytosol, or via the cellular endocytosis pathway[61,62] (Figure 1). By the endocytosis pathway, exosomes are first encompassed in the endosomal compartments followed by fusion of the exosomal membrane with the endosomal membrane to escape their destination for lysosomal degradation, leading to discharge of exosomal cargos into the cytosol of the recipient cell.[63] Spatiotemporal tracking by fluorescence microscopy has shown that exosomes in the medium were first docked onto plasma membrane and diffused slowly in the cytoplasm followed by switching to a rapid and directed movement mode, indicative of active trafficking along actin filaments or microtubules.[64] The mechanisms of exosomal cargo discharge from the endosome require further investigation.

FUNCTIONALITY OF EXOSOMES

Abundant research has illustrated that tumor-derived exosomes (TEXs) play important roles in regulation of cell proliferation, migration and invasion. TEXs regulate tumor progression and metastasis by modulating local immune response,[65,66] epithelial-mesenchymal transition (EMT),[67] angiogenesis[68] and drug resistence.[69] Various molecular mechanisms have been unveiled to be involved in the modulation of tumor progression and metastasis conducted by TEX contents, such as proteins and microRNAs.The immune response significantly affects cancer outcomes.[70] As important roles of EVs being revealed in regulation of inflammatory reactions in different inflammatory diseases including lung inflammation and injury,[71,72] TEXs can trigger the release of cytokines/chemokines from immune cells which result in the stimulation of anti-tumor immune reactions or in a systemic immunosuppression.[73] TEXs have been reported to suppress CD8+ T cells-induced anti-tumor immune activities stimulated by tumor-specific antigens, resulting in promotion of tumor growth.[74] Exosomes derived from exhausted CD8+ T cells could be uptake by non-exhausted CD8+ T cells and subsequently impaired the anticancer function of normal CD8+ T cells.[75] Rather than suppress tumor growth by undermining cancer cells, the affected immune system promotes tumor progression by supporting the chronic inflammation and suppressing anti-tumor immunity.[76] Epithelial–mesenchymal transition (EMT) is a process by which epithelial cells acquire mesenchymal cell properties, which enables the cells to be invasive and migrate to distant sites leading to metastasis and tumor progression.[77,78] The importance of EMT in lung cancer has also been illuminated in various studies.[79,80] Serum TEXs isolated from patients with late stage lung cancer can induce EMT in recipient human bronchial epithelial cells.[81] These TEXs contain high levels of vimentin, which is a member of the type III intermediate filament protein family and a marker for EMT. The correlation between vimentin expression level and metastasis and invasion ability has been observed in lung cancer[81–83] and many other cancers, including prostate, colorectal and gastric cancers. Angiogenesis, a process regulated by various mechanisms and angiogenic factors, is crucial for tumor progression and metastasis. Hypoxia, a hallmark of the tumor microenvironment, has been reported to cause enhanced TEX production and a change in their content which enables TEXs to induce angiogenesis.[84–86]Among the exosomal contents, miRNAs are most studied. MiRNAs are a type of short noncoding RNAs which can mediate paracrine and endocrine effects by post-transcriptionally modulating gene expression and cellular function in the recipient cells.[87] Specifically, TEX production and the level of exosomal miR-23a were detected to be increased during hypoxia-induced angiogenesis in CL1-5 lung adenocarcinoma cells. Uptake of TEX-associated miR-23a, in turn, leads to targeting of prolyl hydroxylase 1 and 2 (PHD1 and 2), the accumulation of hypoxia-inducible factor (HIF)-1α, and the boost of angiogenesis.[85] Studies have demonstrated that hepatocellular carcinoma (HCC) cell-derived exosomal miRNA-21 could convert hepatocyte stellate cells to cancer-associated fibroblasts and thus promote tumor progression by secreting angiogenic cytokines selected,[88] while metastatic breast cancer cells secrete miR-105 to boost cell migration by down-regulating expression of the tight junction protein ZO-1.[89] Additionally, studies have identified exosomal proteins that may play crucial roles in the recipient cells.[90] EVs of tumor origin induced tumor angiogenesis by transporting proangiogenic peptides (for example, EGFRvIII) to the surrounding endothelial cells of microvessels, leading to activation of transforming signal pathways and regulation of the expression levels of vascular endothelial growth factor (VEGF).[91] In recent years, functional research on exosomal dsDNAs,[92] lncRNA[93] and circRNA[94] has also greatlyincreased.Formation of a premetastatic niche is the primary step required for metastasis, and is initiated through various mechanisms that promote a series of events beginning with vascular leakage which facilitates colonization of CTCs to the premetastatic site.[95,96] Exosomes released by breast cancer play an important role in promoting breast cancer bone metastasis, which is associated with the formation of a premetastatic niche via transferring miR-21 to osteoclasts.[97] Hypoxia-induced exosomal miR-135a-5p could initiate LATS2-YAP-MMP7 axis to form a premetastatic niche, and eventually promote the occurrence of CRC liver metastasis.[98]MiR-25-3p, a metastasis-promoting miRNA of colorectal cancer (CRC), can be transferred from CRC to endothelial cells via exosomes, and promotes premetastatic niche formation by inducing vascular permeability and angiogenesis.[99] TLR3 in lung epithelial cells can be activated by the small RNA content of TEXs, and subsequently stimulate chemokine secretion and neutrophil recruitment to the lung, which together promote the niche formation and tumor lung metastasis.[100] Tumor exosome integrins also play important roles in preparing the premetastatic niche. A different tumor exosomal integrin subtype has been linked to specific organ metastasis and exosomal integrins have been suggested to be used for predicting organ-specific metastasis.[10]TEXs are also involved in drug resistance in cancer. Recent studies indicated that exosomal delivery of functional P-glycoprotein and multidrug resistance associated protein-1 (MRP-1) from drug resistant cancer cells led to acquired multidrug resistance by drug sensitive cancer cells.[101,102] Tumor-associated macrophage-derived mir21 can be transferred to the gastric cancer cells, where it suppresses cell apoptosis and enhances activation of PI3K/AKT signaling pathway by down-regulation of PTEN, thus confer cisplatin resistance in gastric cancer.[103] In lung cancer, hypoxia-induced exosomal PKM2 reprogrammed CAFs to create an acidic microenvironment promoting NSCLC cells proliferation and transmitted cisplatin-resistance to sensitive NSCLC cells, led to cisplatin resistance in vitro and in vivo.[104] Therefore, exosomes play an important role in drug resistance by transfer of biomolecules to affect the characteristics of receptor cells or microenvironment, and exosomes may be used as

drug delivery vehicles to tumor drugs or gene therapy.[105] Accordingly, exosomal miRNAs and mRNAs may predict drug resistance and help improve treatment options.Epigenetic modification plays an important role in tumor occurrence and development. TEX signaling participates in the adjustment of epigenetic modification. Exosome-derived ncRNAs may serve as potential drivers of epigenetic reprogramming of cancer stem cells.[106] In NSCLC cell lines (A549 and H1299), exosome-transmitted UFC1 promote progression by inhibiting PTEN expression via EZH2-mediated epigenetic silencing.[107]Normal human gastric epithelial GES-1 Cells absorbed gastric cancer cells released exosomal lncHEIH can upregulate EZH2 expression, which inhibited the expression of the tumor suppressor GSDME by methylation of the GSDME promoter, thus promoting the malignant transformation of normal gastric cells.[108] SNHG9, a papillary thyroid cancer cell exosome-enriched lncRNA, inhibits cell autophagy and promotes cell apoptosis of normal thyroid epithelial cell.[109] LINC00470 in exosomes from glioma patients inhibiting autophagy and enhancing the proliferation of glioma cells by regulating WEE1 expression and activation of the PI3K/AKT/mTOR pathway.

The tumor microenvironment (TME) is a highly heterogeneous system incorporating cancer cells, endothelial cells, fibroblasts, adipocytes, mesenchymal stem cells, immunocyte and extracellular matrix. Tumor cells are closely connected with immune and stromal cells in TME and interact to form an environment of chronic inflammation and immunosuppression. Tumor-associated macrophages (TAMs) are macrophages derived from peripheral blood monocytes recruited into solid tumor tissue microenvironment. Increased infiltration of tumor-associated macrophages (TAMs) is observed in most cancer tissues compared with paracancer or normal tissues.[110,111] TAMs lose their killing ability and acquire an inhibitory phenotype, which promotes tumor development. Generally, macrophages differentiate into two main phenotypes: classically activated (M1) and alternatively activated (M2)[112] .TEXs can polarize M1[113] or M2 macrophage[114] and consequently inhibit or promote tumor metastasis. Exosomes from M2 macrophage promote the development of cancer.[115] Cancer-associated fibroblasts (CAFs) are activated fibroblasts in tumor tissues. Extensive evidence suggests that CAFs are involved in stimulating cancer cell proliferation and progression.[116] TEXs can activate fibroblasts and promote CAF conversion. In cervical cancer, tumor-secreted exosomal Wnt2B activates fibroblasts and promotes CAF conversion to promote cervical cancer progression.[117] Tumor-secreted exosomal lncRNA POU3F3 promotes cisplatin resistance in ESCC by inducing fibroblast differentiation into CAFs.[118]

EXOSOMES AS DISEASE BIOMARKERS

Abundant evidence has shown that cells from individuals with diseases and healthy subjects secreted exosomes containing different proteins and RNAs into the circulation and body fluid, which makes exosomes applicable for liquid biopsy as potential diagnostic biomarkers.[119,120] Melo and coworkers found that exosomal proteoglycan glypican-1 (GP1) was specifically present in the serum of patients with pancreatic cancer with high sensitivity and exosomal GP1 level is highly positively correlated with the tumor burden. Correspondingly, exosomal GP1 level is also correlated with survival of pre- and post-surgical patients, indicating exosomal GP1 ideal to serve as diagnostic and prognostic biomarkers for pancreatic cancer.[121] Exosomal cytoskeleton-associated protein 4 (CKAP4) was secreted by pancreatic ductal adenocarcinoma (PDAC) cells and was highly detected in pancreatic tumor-bearing xenografted mice and patients with PDAC, whereas CKAP4 was barely detectable in normal mice and postoperative patients, suggests that CKAP4 secreted in exosomes may represent a biomarker for PDAC.[122] In addition, exosomes released by tumor tissues are enriched with miRNAs and exosomal miRNAs may be explored as potential biomarkers for early diagnosis of cancers.[123,124] A number of clinical studies on exosomes as diagnostic biomarkers of cancer are ongoing.Potential of exosomes as biomarkers for noncancer diseases have been investigated as well. The expression of exosomal miR-331-5p and miR-505 were significantly higher in patients with Parkinson's disease (PD) compared with healthy controls with the ROC curve 0.849 and 0.898, respectively, which suggests that exosomal miRNAs could potentially act as biomarkers for PD.[125] Exosomes collected from bronchoalveolar lavage fluid from patients with asthma and healthy subjects contain different miRNA contents, exhibiting potential to serve as diagnostic biomarker of asthma.[126]

EXOSOMAL miRNAs AS DIAGNOSTIC, PREDICTIVE AND PROGNOSTIC BIOMARKERS FOR LUNG CANCER

Studies have illustrated that the composition of exosomal miRNAs differs among NSCLC subtypes and may serve as biomarkers for diagnosis, therapeutics and prognosis of NSCLC[127,128](Figure 2).As early as in 2009, Rabinowits et al.[129] described a set of 12 miRNAs (including miR-17-3p, miR-21, miR-106a, miR-146, miR-155, miR-199, miR-192, miR-203, miR-205, miR-210, miR-212 and miR-214) isolated from serum exosomes of NSCLC patients while not from those of healthy subjects, suggesting that exosomal miRNAs could be used for NSCLC diagnosis. In another study, 746 exosome-derived miRNAs were globally screened in lung adenocarcinoma (LAC) patients, lung granuloma patients and healthy controls. 2 miRNA panels consisting of 4 miRNAs (miR-378a, miR-379, miR-139-5p and miR-200-5p) and 6 miRNAs (miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100 and miR-154-3p), respectively, were explored for screening of LAC against healthy subjects and patients with benign lung nodules with high sensitivity

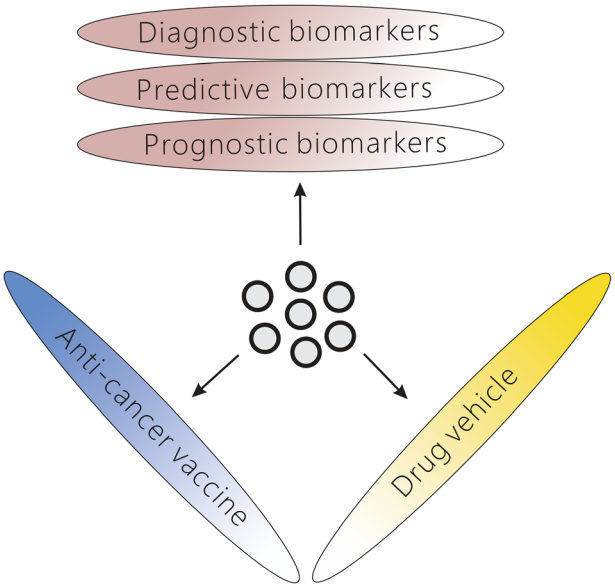

FIGURE 2 Potential applications of exosomes in lung cancer management. Tumor-derived exosomes can serve as promising biomarkers for diagnosis, prognosis and treatment efficacy of lung cancer. Exosomes could also act as drug delivery vehicles and anticancer vaccine in lung cancer therapy

(96% and 97.5%) but probably insufficient specificity (60% and 72%).[130] More recently, an miRNA panel containing let-7b-5p, let-7e-5p, miR-23a-3p, and miR-486-5p were developed for NSCLC diagnosis with sensitivity of 80.25%, specificity of 92.31% and AUC value of 0.899, respectively. In the meanwhile, levels of miR-181b-5p and miR-361b-5p, and of miR-10b-5p and miR-320b were indicated to identify lung adenocarcinoma (LAC) and lung squamous cell carcinoma (LSCC), respectively, with sensitivity of 80.65% and 83.33%, specificity of 91.67% and 90.32%, and AUC value of 0.936 and 0.911, respectively.[131]Exosomal miRNAs have also been illustrated in prediction of treatment response of lung cancer patients. Two studies showed that upregulation of miR-96, and miR-208-a in NSCLC promoted tumor growth and resistance to radiotherapy, indicative of potential novel therapeutic targets.[132,133] Level of exosomal miR-146a-5p was demonstrated to predict sensitivity of NSCLC to the cisplatin therapy through regulating autophagy pathway.[134] Similarly, exosomal miR-100-5p was shown to be downregulated in cisplatin-resistant A549 (A549/DDP) cells in comparison with that in wild-type A549, and A549/DDP-derived exosomes were capable of endowing cisplatin resistance to wild-type A549 cells mediated by mTOR pathway.[135] In a recent study, Poroyko et al.[136] analyzed exosomal miRNAs from the serum of NSCLC, SCLC and healthy controls by shotgun sequencing and identified 17 differentially displayed miRNAs between lung cancer patients and healthy subjects. A set of exosomal miRNAs were differentially displayed in the patient cohort before and after chemotherapy, indicative of the potential of exosomal miRNA profiling in disease subtyping and treatment efficacy evaluation of lung cancer. exosomal could be a noninvasive diagnostic and prognostic marker of radioresistant NSCLC For patients who received tyrosine kinase receptor inhibitors, exosomal miRNAs has also shown the predictive effect in drug response. MiR-184 and miR-3913-5p derived from exosomes in the peripheral blood of NSCLC patients could be used as biomarkers to indicate osimertinib resistanceLevels of miR-21 and miR-4257 were found to be significantly increased in relapsing NSCLC patients after surgery and predict low disease free survival.[137] Low level of miR-146a-5p in serum exosomes indicated poor progression free survival of NSCLC patients.[134] Higher level of plasma exosomal miR-451a from NSCLC patients of stages I, II or III before surgery were detected in those with recurrence and poor disease-free and overall survival, indicative of the potential of exosomal miR-451a serving as the prognostic biomarker for NSCLC patients.[138] In another study, 83 tumor-related miRNAs in serum exosomes were screened and nine miRNAs were detected to be differentially present in exosomes of NSCLC patients. Among these nine miRNAs, miR-23b-3p, miR-10b-5p and miR-21-5p were upregulated in NSCLC compared to healthy subjects, and higher levels of the three miRNAs predicted low overall survival of the patients.[139]

EXOSOMAL PROTEINS AS DIAGNOSTIC AND PROGNOSTIC BIOMARKERS FOR LUNG CANCER

Recent studies by proteomic analysis have detected a number of lung cancer exosome-enriched proteins that are involved either in biogenesis, transport and fusion of exosomes or play important roles in tumor metastasis, angiogenesis and immunoregulation.[140] These exosomal proteins can reflect the donor cells and pathological state of disease, which make them potential biomarkers for diagnosis and prognosis of lung cancer (Figure 2).Quantitative proteomic analyses of exosomal proteins in NSCLC cells and normal bronchial epithelial cells have identified NSCLC exosome-enriched proteins involved in cell signaling, cell adhesion and extracellular matrix remodeling. Levels of EGFR and SRC as well as their downstream effectors GRB2 and RALA, and MET, RAC1 and KRAS proteins were detected to be upregulated in NSCLC exosomes.[141] In contrast to similar plasma EGFR levels between lung cancer patients and normal subjects, remarkable higher level of exosomal EGFR was observed in lung cancer patients in comparison to normal subjects[142] with 80% of serum exosomes from NSCLC being identified to be EGFR positive.[143] These studies indicated exosomal EGFR as potential diagnostic biomarker for lung cancer. Jakobsen and coworkers used an extracellular vesicle array containing 37 antibodies against lung cancer-related proteins to capture and phenotype serum exosomes of NSCLC patients. A combined 30-marker model was explored to distinguish NSCLC with sensitivity of 75%, specificity of 76% and diagnostic accuracy of 75.3%.[144] Another study of 581 patients by the same team indicated three markers CD151, CD171, and tetraspanin 8 as the strongest separators

of patients with cancer of all histological subtypes versus patients without cancer.[145] Recently, Wang et al.[146] detected the differential expression protein in exosomes of distant metastatic and nonmetastatic NSCLC patients identified by multidimensional liquid chromatography and mass spectrometry analysis and found lipopolysaccharide-binding proteins (LBP) in the exosomes to be well distinguished between patients with metastatic and patients with nonmetastatic NSCLC. The area under the curve (AUC) was 0.803 with a sensitivity of 83.1% and a specificity of 67%, suggesting exosome LBP might be promising and effective candidates of metastatic NSCLC. Further research by the same team found that a combination of AHSG, ECM1, and carcinoembryonic antigen improved the diagnostic potential of NSCLC with the diagnostic values AUC of 0.938 for NSCLC and 0.911 for early stage NSCLC versus healthy individuals, further suggesting the potential diagnostic value of serum exosome proteins.[147]Sandfeld-Paulsen et al. performed proteomic analyses of 49 exosomal membrane bound proteins from a cohort of 276 NSCLC patients and identified nine exosomal membrane bound proteins to be potential prognostic biomarker of NSCLC. Specifically, high levels of EGFR, NYESO-1 and PLAP are indicative of poor prognosis of NSCLC patients.[148]Recently, saliva has emerged as a novel medium for cancer detection as its collection is simple and noninvasive.[149] Exosomes released by cells or organs could also be detected in saliva.[150] In a xenografted mouse model of human lung cancer, salivary exosome-like microvesicles were found to carry tumor cell-specific mRNA and protein from blood to saliva.[151] Salivary exosomal proteins were systematically quantitatively compared by LC-MS/MS between lung cancer patients and normal subjects, and 150 proteins in salivary exosomes were identified to be dysregulated in lung cancer, among which 25 proteins were from remote organs and five were lung-associated proteins. These studies indicated that salivary exosomal proteins could also be explored as a diagnostic biomarker of lung cancer.[152]Exosome-based detection of *EGFR* mutation in plasma from NSCLC patients has also achieved favorable diagnostic results.[153] Detection of the T790M mutation on exosomal cfDNA achieved 92% sensitivity and 89% specificity using tumor biopsy results as gold standard.[154] In another study, the sensitivity was 98% for detection of activating *EGFR* mutations and 90% for EGFR T790M based on exosomal cfDNA.[155] Recently, an exosome-focused translational research for afatinib (EXTRA) study has been carried out to identify a novel predictive biomarker and a resistance marker for patients who received afatinib treantment.[156] All these studies demonstrate that exosomal EGFR mutation detection might be used as diagnostic and predictive biomarker for EGFR-TKI treatment and help to avoid unnecessary tumor biopsies.

EXOSOMES IN LUNG CANCER THERAPY

The overall patient outcomes of lung cancer therapy remain unsatisfactory. Improvement of efficacy necessitates the exploration of novel therapeutic approaches for lung cancer. Immunotherapy by targeting PD-1 and PD-L1, which negatively regulate T cell activation, represents a novel treatment approach for broad range of cancers, including lung cancer. Exosomes are involved in the regulation of inflammatory signals in the tumor microenvironment and therefore affect the immunotherapeutic efficacy in lung cancer.[70] As a result, inhibition of TEX release or of integrin-mediated TEX uptake by blocking integrins may restrain the development of an amicable tumor microenvironment, which leads to the repression of tumor progression.[157,158]In addition, the exosome represents a potential delivery tool of biological molecules and drugs (Figure 2). In comparison to other drug delivery tools developed, including nanoparticles and liposomes,[159] exosomes display numerous advantages, such as less toxicity, low immunogenicity, targeting specific recipient cells mediated by ligands and peptides on the exosomal membrane, and the ability to transport across the blood brain barrier.[160] Natural compounds can be loaded into exosomes during exosomal biogenesis process or through in vitro incubation, transfection or electroporation of purified exosomes.[161] Docetaxel (DTX), the first-line of the antitumor agent used to treat NSCLC, was selected payload into exosome by electroporation, compared to the free DTX, exosomes significantly increased the cellular uptake in vitro evaluation and showed better targeting to tumor tissue in the mice.[162] In another research, engineered targeting tLyp-1 exosomes had high transfection efficiency into lung cancer and cancer stem cells and were able to knockdown the target gene of cancer cells and to reduce the stemness of cancer stem cells.[163] All these researches suggest that exosomes might offer a promising gene delivery platform for future cancer therapy. Exosomes have been explored as anticancer vaccine based on the consideration that they contain tumor-specific antigens[164] (Figure 2). Yaddanapudi et al.[165] recently reported that vaccination with GM-CSF positive but not GM-CSF negative exosomes from murine embryonic stem cells (ESCs) delayed or prevented tumorigenesis in mice by boosting tumor-specific responses and Th1 cytokine reactions of the tumor-infiltrating lymphocytes, suggesting potential roles of GM-CSF positive human ESCs in cancer-preventing vaccination of susceptible individuals. In a clinical study, MAGE peptides loaded exosomes derived from autologous dendritic cells (DEXs) of NSCLC patients were evaluated for their safety and efficacy as tumor vaccine.[166] Enhanced immune responses with NK activities and T cell response against MAGE peptides were observed and long term disease stability were achieved in some patients. In another clinical study, clinical benefits of cancer antigens-loaded IFN-g-DEXs were evaluated in NSCLC patients without disease progression after chemotherapy.[167] Augmented antitumor function of NK cells induced by DEXs has been established in advanced NSCLC patients with defective NKp30 expression. These studies reveal potential applications of dendritic cell-derived exosomes as anticancer vaccine.

DISCUSSION

Crosstalk between cancer cells and the tumor microenvironment mediated by tumor derived exosomes has been established to play an important role in tumor progression and metastasis. However, little is known about how TEXs interact with tissues in distance. Further investigation is necessary to understand the function of TEXs in tumor progression and metastasis, which will facilitate the development of novel cancer therapeutic approaches.Exosomes appear to be desirable biomarkers for prognosis, diagnosis and treatment prediction of lung cancer on account of exosomal cargos mirroring tissue expression patterns, the noninvasiveness of liquid biopsy which makes consecutive surveillance possible, and the stability of genetic materials safeguarded by the exosomal membrane. There has been extensive research on exosomal miRNAs and proteins as potential diagnostic biomarker for lung cancer. These studies suggest that miRNA and protein panels, instead of single miRNAs and proteins, present more clinical values. The outcomes from different studies, despite being inconsistent, do partially overlap. In consideration of the variations in study subjects, exosome origins and exosome isolation approaches, production of comparable outcomes necessitates larger-scale investigation with unified patient classification and exosome isolation methods. Moreover, possible therapeutic applications of exosomes could open up new avenues in lung cancer treatment. The potential of exosomes to serve as drug delivery vehicles have been proposed. Exosomes derived from dendritic cells serving as cancer vaccine have been proven to be promising in lung cancer therapy. While vaccination of DEXs has been illustrated to enhance NK cell activity in some lung cancer patients, little is known about the application of exosomes in adjuvant therapy of lung cancer or as drug delivery tool. Further clinical studies are warranted to confirm the capacity of exosomes in lung cancer management.In conclusion, exosomes are a new exciting field of research which have opened a new window to the diagnosis, prognosis and treatment of lung cancer. Although a great number of inspiring findings and potential applications for exosomes have been published, methods for exosome isolation remain to be standardized. Future investigations should be conducted on approaches to manage the biogenesis, cargo loading, release, and interaction of lung cancer exosomes so as to better understand their molecular mechanisms and develop novel therapeutics with maximal on-target efficiency.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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