IN PERSPECTIVE

Tumor-derived exosomes: Key players in non-small cell lung cancer metastasis and their implication for targeted therapy

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Abstract
Exosomes represent extracellular vesicles of endocytic origin ranging from 30 to 100 nm that are released by most of eukaryotic cells and can be found in body fluids. These vesicles in carrying DNA, RNA, microRNA (miRNA), Long non-coding RNA, proteins, and lipids serve as intercellular communicators. Due to their role in crosstalk between tumor cells and mesenchymal stroma cells, they are vital for tumor growth, progression, and anticancer drug resistance. Lung cancer is a global leading cause of cancer-related deaths with 5-year survival rates of about 7% in patients with distant metastasis. Although the implementation of targeted therapy has improved the clinical outcome of nonsmall cell lung cancer, drug resistance remains a major obstacle. Lung tumor-derived exosomes (TDEs) conveying molecular information from tumor cells to their neighbor cells or cells at distant sites of the body activate the tumor microenvironment (TME) and facilitate tumor metastasis. Exosomal miRNAs are also considered as noninvasive biomarkers for early diagnosis of lung cancer. This review summarizes the influence of lung TDEs on the TME and metastasis. Their involvement in targeted therapy resistance and potential clinical applications are discussed. Additionally, challenges encountered in the development of exosome-based therapeutic strategies are addressed.

KEYWORDS
exosome, lung cancer, metastasis, microRNA, targeted therapy, the tumor microenvironment

Abbreviations: TDEs, tumor-derived exosomes; TME, the tumor microenvironment; NSCLC, non-small cell lung cancer; SCLC, small cell lung carcinoma; EMT, epithelial-to-mesenchymal transition; EVs, extracellular vesicles; MVB, multivesicular body; ILVs, intraluminal vesicles; miRNA, microRNA; ESCRT, Cryo-TEM, cryo-transmission electron microscopy; endosomal sorting complex required for transport; ECM, extracellular matrix; CAFs, cancer-associated fibroblasts; HBECs, human bronchial epithelial cells; MMP, matrix metalloproteinase; EGFR-TKI, epidermal growth factor receptor-tyrosine kinase inhibitor.

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Exosomes are defined as extracellular vesicles (EVs) which play a vital role in cellular communication and disease. The lipid bilayered vesicles are secreted by virtually all types of mammalian cells and carry biomolecules. They were first reported in 1983 by Harding et al. who depicted the recycling of the transferrin receptor in reticulocytes via endocytosis. In the same year, another group delineated the kinetics and internalization of transferrin receptor in a human hematoma cell line. The term ‘exosomes’ was first coined by Johnstone et al. in 1987 when this group also described the isolation of vesicles from sheep reticulocytes by ultracentrifugation.

Studies in the past decade have shown a more multifaceted role of exosomes. The functions of exosome are dependent on the cell types that secrete them, but almost all exosomes have some common physical characteristics. Electron microscopy analysis reveals that they are cup-shaped spheres surrounded by a lipid bilayer and range between 30 and 100 nm in diameter. Exosomes are found in numerous body fluids including plasma, semen, urine, saliva, breast milk, amniotic fluid, ascites fluid, cerebrospinal fluid, and bile. As intercell communicators, exosome vesicles are a source of genetic material along with proteins and lipids. Exosomes carry DNA, messenger RNA (mRNA), microRNA (miRNA), and long noncoding RNAs that can be taken up by recipient cells. The content of exosomes is specific to the cell of origin, which enables signals transmitted from parent cells to the neighboring ones without direct cell–cell contact.

Exosomes are released by a variety of eukaryotic cell types including cancer cells. Tumor-derived exosomes (TDEs) influence tumor invasion and metastasis. Consequently, exosomes have a pivotal role in cancer progression, and they can be used not only as biomarkers for cancer diagnosis and prognosis but also as drug delivery vehicles and reconfigurable therapeutic systems.

Lung cancer is one of the most frequently diagnosed cancers worldwide. Despite progress in our understanding of risk factors, pathogenesis, diagnostic markers, and therapeutic strategies for lung cancer, it remains the leading cause of cancer-related death in both males and females. The 5-year survival of lung cancer is among the worst of all tumor types, varying from 4% to 17%, depending on different tumor stages and regions. At the time of diagnosis, lung carcinomas are most often in an advanced/metastatic stage. A better understanding of the molecular mechanisms for lung cancer progression and identification of potential therapeutic targets for curtailing metastasis will help to achieve a survival benefit for patients with this fatal disease.

Histologically lung cancer is classified into two main groups: small cell lung carcinoma (SCLC), accounting for 15% of all lung cancers, and nonsmall cell lung cancer (NSCLC), containing 85% of all lung cancers. NSCLC are subclassified into adenocarcinoma (ADC), squamous cell carcinoma, and large cell lung carcinoma.

Tumor metastasis is a hallmark of cancer. Metastases are responsible for more than 90% of cancer-related deaths. The most frequently metastatic sites of lung cancer are bone 34.3%, lung 32.1%, brain 28.4%, adrenals 16.7%, and liver 13.4%. The metastatic cascade depicts the process in which malignant tumor cells reach distant organs from their primary site via hematogenous or/and lymphatic circulation. In these complicated processes, migration, invasion, angiogenesis, hypoxia, and epithelial-to-mesenchymal transition (EMT) are involved, which are tightly controlled by gene expression signatures, along with the interaction between tumor cells and the tumor microenvironment (TME). In addition, TDEs and exosomes derived from the components of the TME have been considered as key players in tumor metastasis network recently.

This review briefly summarizes the role of TDEs in lung cancer progression and metastasis, related to the activation of the TME. Also, the involvement of TDEs in NSCLC targeted therapy resistance is addressed, and the potential application of TDEs in lung cancer treatment is discussed.
proteins ALIX, clathrin, and TSG101. In addition, an ESCRT-independent pathway exists which mostly relies on tetraspanins. In this respect, absence of CD9 can cause defective exosome secretion from bone marrow dendritic cells. In another study, CD63 was shown to be directly involved in the formation of lysosome related organelles.

Intracellular vesicle trafficking is primarily regulated by Rab GTPases which are associated with intracellular membranes. Using an RNA interference screen, Ostrowski et al. identified several Rab GTPases which promote exosome secretion including Rab2b, Rab5a, Rab9a, Rab27a, and Rab27b. Most of these Rab proteins have been previously associated with endocytic functions, consistent with the postulated endosomal origin of exosomes.

After release, exosomes then bind to recipient cells and get internalized. In binding to surface receptors, they can trigger intercellular signaling. The internalization of exosomes can be conducted by clathrin-mediated endocytosis and macropinocytosis or clathrin-independent pinocytosis. Alternatively, exosomes can also be taken up by caveolae formation and lipid rafts. Visualizing of the cellular uptake of exosomes by live-cell microscopy revealed that exosome vesicles can be directly endocytosed by cells and their contents internalized.

3.1 Lung TDEs influence the TME

The functional role of exosomes released by most mammalian cells is to allow crosstalk between the cells and their microenvironment. Exosomes carry many biological active molecules and can transfer them from cell to cell to establish intercellular communication. Cancer cells, like other cells, also secrete exosomes, called tumor-derived exosomes (TDEs). TDEs are widely studied to understand the immune activities surrounding a tumor and the interaction between tumor cells and their microenvironment. Recently TDEs have gained much attention. Research on TDEs can help to gain insights into tumor metastasis, progression, and antitumor immune responses. Lung cancer is the most frequently diagnosed form of cancer worldwide causing the greatest number of deaths up to date. It turned out that TDEs are potential diagnostic and prognostic biomarkers of lung cancer, and they can be applied to comprehend the mechanisms responsible for antitumor therapy resistance.

3.2 Tumor-Derived Exosomes

TDEs can induce immune suppression and change the TME to favor tumor progression. Studies have shown that lung TDEs can suppress maturation of immune cells, impair NK cell activation, and induce myeloid-derived suppressor cells. Lung TDEs can be taken up by macrophages and facilitate tumor progression and immune suppression. TDEs can release transforming growth factor beta (TGF-β) to enhance regulatory T cell proliferation and induce effector T cell apoptosis by interacting with Fas/Fasl. Lung TDEs may also regulate tumor cell migration via TGF-β and interleukin (IL)10. Drug-induced COX-2 overexpression could be transferred from lung cancer cells to neighboring cells via exosomes, which resulted in TDE-induced upregulation of PGE2 and VEGF in TDE-binding cells, and induction of inflammatory reactions.

CAFs also secrete exosomes that can alter cellular metabolism. These exosomes can inhibit mitochondrial oxidative phosphorylation and shifting the cancer cell metabolism to glycolysis and glutamine-dependent reductive carboxylation. Moreover, exosomes can carry and supply amino acids, lipids, and tricarboxylic acid cycle intermediates to cancer cells for metabolism.
NSCLC cells inhibit apoptosis and enhance cell proliferation by delivering alpha smooth muscle actin in normal lung fibroblasts and NSCLC cells.61

Activation of the microenvironment is closely associated with EMT. Exosomes derived from highly metastatic lung cancer can induce vimentin expression and EMT in human bronchial epithelial cells (HBECS) and in addition, these TDEs enhance cell migration, proliferation, invasion, and metastasis.62 ZEB1, a master EMT transcription factor, can induce a mesenchymal phenotype in normal cells. It was observed that exosomes derived from transformed mesenchymal HBECS increased ZEB1 expression in parental HBECs, thereby stimulating a mesenchymal phenotype and rendering the HBECs chemoresistant.63 A study by Wu et al.64 showed that TGF-β-mediated exosomal Inc-MMP2-2 promotes lung cancer cell migration and invasion through upregulation of matrix metalloproteinase (MMP2) which is involved in degradation of the extracellular matrix. Exosomes from irradiated lung cancer cells can increase the expression of glycolytic enzymes and glycolytic activity in recipient cells, which in turn controls the motility of these cells via signaling proteins ALDOA and ALDH3A1.65

Accumulating evidence indicates that lung cancers produce more exosomes under hypoxic conditions compared to normoxic ones.66,67 Hypoxic lung cancer-secreted exosomal miRNAs influence the TME and promote tumor metastasis. Hypoxic lung-cancer-derived exosomal miR-103a increases the oncogenic effects of macrophages by enhancing M2 polarization and targeting the tumor suppressor gene PTEN.68 Hypoxic lung cancer-derived exosomal miR-23a inhibits the tight junction protein ZO-1 and induces accumulation of HIF1α in endothelial cells, thus increasing vascular permeability and facilitating tumor cell spread.69 Hypoxic lung tumor-derived exosomal miR-150 decreases anticancer activity of NK cell by targeting CD226,70 a member of the immunoglobulin superfamily. Collectively, these data suggest that lung TDEs, interacting with the TME, have a tremendous impact on tumor progression and development (Figure 2).

3.2 | Tumor-derived exosomal miRNAs in lung cancer metastasis

miRNAs are small noncoding RNAs with length between 21 and 24 nucleotides that have a key role as regulators of gene expression at posttranscriptional level.71 Exosome-derived miRNAs (exosomal miRNAs) are associated with many pathophysiological processes in lung cancer like EMT, proliferation, migration, invasion, and angiogenesis, which ultimately lead to tumor progression and metastasis.72

As already discussed, crosstalk between tumor cells and the TME plays a critical role in tumor progression. A study by Fang et al.73 showed that liver cancer derived exosomal miR-1247-3p activated CAFs to facilitate formation of a premetastatic niche in the lung.72 It was found that exosomal miR-1247-3p directly targeted B4GALT3, resulting in activation of the β1-integrin-nuclear factor kappa B pathway in CAFs.73 Lung ADC-derived exosomal miR-21 was involved in bone metastasis through facilitating osteoclastogenesis via targeting Pdcd4, a known regulator of osteoclastogenesis.74 miR-192 was identified as a repressor of tumor metastasis by comparative transcriptomic profiling using an in vivo murine model of bone metastasis.75 Treatment of NSCLC cell line A549 with miR-192-enriched exosome-like vesicles abrogates angiogenesis by inhibition of IL-8, ICAM, and CXCL1 in vitro and reduces the metastatic burden and tumor colonization in the bone in vivo.75 NSCLC-derived exosomal miR-619-5p promotes metastasis through modulation of angiogenesis and inhibition of the regulator of calcineurin 1 gene

![Figure 2](wileyonlinelibrary.com)
a tumor suppressor in various cancer cells. Exosomal miR-494 and miR-542-3p derived from metastatic rat ADC BSp73ASML modulate draining lymph nodes and lung tissue to support tumor spread via targeting cadherin-17 and upregulation of MMP2, MMP3, and MMP14. Recently accumulating research data have implied that exosomal miRNAs influence NSCLC progression and metastasis through interacting with the Wnt/β-catenin signaling pathway. Liu et al. observed that the plasma exosomal miR-433 level was lower in NSCLC patients with chemoresistance compared to patients with chemosensitive NSCLC, which was negatively associated with distant metastasis. Furthermore, it shown that miR-433 inhibited NSCLC progression via incremental infiltration of CD4 and CD8 cells and inactivation of the Wnt/β-catenin signaling pathway. Exosomal miR-1260b derived from NSCLC promotes tumor metastasis via targeting homeodomain-interacting protein kinase-2, and previously, this miR-1260b was found to be able to promote tumor cell invasion in lung ADC through activation of Wnt/β-catenin signaling pathway. Analysis of the association between exosomal microRNA clusters and bone metastasis from NSCLC revealed that miR-574-5p, a suppressor of Wnt/β-catenin pathway, was downregulated in NSCLC patients with bone metastasis. Evidence supporting the notion that TDEs facilitate metastasis in the context of lung cancer is still coming. For example, miR-499a-5p promotes lung ADC cell proliferation and EMT and, therefore, facilitates tumor cell metastasis via mTOR signaling. Exosomal miR-106b acts as a novel biomarker for lung cancer and promotes cancer metastasis through inhibition of the tumor suppressor gene PTEN. Chen et al. found that exosomal mir-3180-3p inhibits proliferation and metastasis of non-small cell lung cancer by downregulating FOXP4. Breast cancer-derived exosome transfected with miR-126.
migration through interrupting the PTEN/PI3K/AKT pathway and suppress lung tumour metastasis in vivo. Exosomal miRNAs from hypoxic bone marrow-derived mesenchymal stem/stromal cells enhance lung cancer metastasis via STAT3-induced EMT.86

3.3 The role of tumor-derived exosomal proteins in lung cancer metastasis

It is widely believed that TDE proteins carrying oncogenic proteins are involved in lung cancer progression and metastasis. Taverna et al.87 observed that NSCLC-derived exosomes containing amphiregulin activated the EGFR pathway in preosteoclasts that in turn fostered bone metastasis by upregulation of RANKL. Another study showed that exosomes derived from the highly metastatic lung cancer cell line 95D promoted metastasis in the lung cancer cell line A549, the lung fibroblast cell line MRC-5, and the poorly metastatic cell line 95C through activation of the HGF/c-Met pathway. Additionally, quantitative proteomics analysis revealed that 268 exosomal proteins differentially expressed in 95D cells might contribute to the enhanced metastatic behavior.88 Wnt proteins have been identified as exosomal cargoes, contributing to tumorigenesis and metastasis. Golgi phosphoprotein 3 was found to be interacted with cytoskeleton-associated protein 4, which enhanced the secretion of exosomal WNT3A and promoted NSCLC cell metastasis.89 Tumor metastasis is closely associated with EMT. The main features of EMT related to tumor invasiveness and metastasis are the loss of epithelial cell properties and gain of mesenchymal phenotype.90 A study by Kim et al.91 reported that the exosomal β-catenin protein was upregulated in A549, stimulated by the EMT inducer TGF-β, and autologous treatment of exosomes led to a significantly increased TCF/LETS transcriptional activity in A549 cells, indicating that exosomes might induce phenotypic switches via autocrine signaling. In addition, exosomes derived from highly metastatic lung cancer cells and advanced stage patient serum induced a mesenchymal phenotype alteration and increased expression of the mesenchymal marker protein vimentin in normal HBECs.62

Exosomes from nonmalignant cells also affect lung metastasis. For example, exosomes derived from adipocytes increase MMP activity by transferring MMP3 to lung cancer cells, thereby promoting lung cancer metastasis.92 Recently, it was found that exosomal PD-L1 could promote tumor growth through immune escape in NSCLC.93 The main findings of tumor-derived exosomal proteins in lung cancer metastasis are summarized in Table 2.

The potential role of tumor-derived exosomal proteins in diagnosis of metastatic lung cancer was revealed by several studies.94,95 The concentration of exosomes isolated from metastatic NSCLC was found to be significantly higher in comparison with those from healthy individuals, and additionally, the exosomal levels of alpha-2-HS-glycoprotein and ECM1 increased significantly in the metastatic NSCLC patients.95 In a study by Wang et al.,96 tandem mass tags combined with multidimensional liquid chromatography and mass spectrometry analysis were applied for screening the proteomic profiles of serum samples from metastatic, non-metastatic NSCLC patients, and healthy individuals. It turned out that the lipopolysaccharide-binding proteins were highly expressed in serum exosome from metastatic patients. These data suggest that tumor-derived exosomal proteins might be predictive biomarker for NSCLC metastasis.

### 3.4 Exosomes in lung cancer molecular diagnosis: current developments

Exosomes are found stable in most body fluids and their contents share common features to the parental cells. These features make them a great tool for liquid biopsy to detect various diseases including cancer.97 In contrast to tissue biopsy requiring surgery, liquid biopsy provides a noninvasive approach.98 Decades of scientific research have led to the identification of predictive

| Exo-protein                  | Origin of exosomes | Receptant cell | Target/mechanism | Function                              | Ref |
|-----------------------------|--------------------|----------------|------------------|---------------------------------------|-----|
| amphiregulin                | plasma of NSCLC patients | primary osteoclasts | EGFR             | triggering osteolytic bone metastasis | 87  |
| 268 differentially expressed| NSCLC cells 95D    | A549, MRC-5     | HGF/c-Met        | promoting metastasis                  | 88  |
| exosomal proteins           |                    |                |                  |                                       |     |
| exosomal-WNT3A              | GOLPH3 overexpressing | A549 and H460  | Wnt/β-catenin    | promoting metastasis                  | 89  |
| proteins involved in EMT    | TGF-β1 treated A549 cells | A549, MRC-5   | β-catenin        | promoting metastasis via EMT          | 91  |
| proteins involved in EMT    | serum from NSCLC patients | HBEC          | EMT              | enhancing migration and invasion      | 61  |
| MMP3                        | 3T3-L1 adipocyte   | 3LL NSCLC cells | MMP9             | promoting lung cancer metastasis      | 92  |
| PD-L1                       | NSCLC cells H460, H1975 | T cell         | immune escape    | promoting tumor growth                | 93  |

Abbreviations: EMT, epithelial-to-mesenchymal transition; HBEC, human bronchial epithelial cells; MRC-5, lung fibroblast cells; NSCLC, nonsmall cell lung cancer; TGF, transforming growth factor.
molecular markers including EGFR-activating mutations, EML4-ALK rearrangements, and PD-L1 expression for targeted therapy with EGFR-tyrosine kinase inhibitors (EGFR-TKIs), ALK inhibitors, and immune therapy with PD-L1 inhibitors, respectively, in patients with NSCLC. However, in some cases, no adequate tumor tissues are available for molecular analysis. Since TDEs containing biological information from the parental cells are found in almost all body fluids and are more representative than cell-free DNA, they can be applied as liquid biopsy in clinical settings, and they may also contribute to novel biomarker discovery in drug resistance. Indeed, exosome-based detection of EGFR-activating and resistance mutations from plasma of NSCLC patients has been successfully performed.97,100

### 3.5 TDEs in NSCLC-targeted therapy resistance

Molecular mechanism analyses revealed targetable driver mutations including EGFR, ALK, c-met, BRAF, and reactive oxygen species in metastatic NSCLC.101 These molecular features provide the basis for personalized targeted therapy and lead to development of FDA-approved EGFR-TKIs, c-met-TKIs, BRAF-TKIs, and TRK-TKIs for treatment of patients with metastatic NSCLC. However, drug resistance remains an obstacle and is a main limitation for targeted therapy. Despite the initially great response to therapy, most of the NSCLC patients ultimately develop drug resistance within 9–12 months.102 Therefore, better understanding of the molecular mechanisms for drug resistance and identification of predictive biomarkers for targeted therapy are essential to improve the clinical outcome of NSCLC.

A growing body of evidence demonstrates that TDEs are involved in drug resistance to EGFR-TKIs via transfer of active cargoes, particularly exosomal miRNAs. Jing et al.103 reported that exosomes released by EGFR-TKI-resistant H827R cells decreased the sensitivity of the NSCLC HCC827 cells to gefitinib. Moreover, miR-21 inhibition abrogated exosome-mediated drug resistance in HCC827 cells,104 consistent with the previously reported role of miR-21 in EGFR-TKI-resistant NSCLC cells.103 Treatment of the EGFR-TKI gefitinib-sensitive cell line PC-9 with exosomes from a gefitinib-resistant cell line PC-9/ZD led to an increased proliferation of the PC-9 cells, and a microRNA array analysis showed that exosomal miRNAs including miR-564, miR-658, miR-3652, miR-3126-5p, and miR-6810-5p were significantly upregulated in PC-9/ZD compared to PC-9.105 Liu et al.106 observed that exosomes derived from the NSCLC cell line H1975 containing a secondary T790M mutation of EGFR could induce drug resistance in the EGFR-TKI-sensitive PC-9 cells in vitro and in vivo through activating the PI3K/AKT signaling pathway, and this process was accompanied by an enhanced expression of exosomal miR-3648 and miR-522-3p. Similarly, miR-214 was found to be upregulated in gefitinib-resistant PC-9GR cells compared to gefitinib-sensitive PC-9 cells, and inhibition of exosomal miR-214 with antagonim was able to reverse gefitinib resistance conferred by PC-9GR-derived exosomes.107 Exosomes derived from gefitinib-treated PC-9 cells decreased the antitumor effects of cisplatin by induction of autophagy and reduction of apoptosis. This observation might partially explain the reason why combination of EGFR-TKIs with chemotherapy agents failed to improve clinical outcome in metastatic NSCLC patients, as revealed by several clinical trials.108 Besides exosomal miRNAs, exosomal...

### Abbreviations
- miR, microRNA
- NSCLC, nonsmall cell lung cancer
- Ref, reference
- TKI, tyrosine kinase inhibitor

### Table 3: Tumor-derived exosomal miRNAs (Exo-miRNAs) in NSCLC TKI targeted therapy resistance

| Exo-miRNA | NSCLC cells | Therapy | Role of Exo-miRNAs in therapy resistance | Ref |
|-----------|-------------|---------|-----------------------------------------|-----|
| miR-21    | HCC827, HCC827R | EGFR-TKI | upregulation of exo-miR-21 is related to gefitinib resistance | 103 |
| miR-564   | PC-9 and PC-9/ZD | EGFR-TKI | PC-9 cells transfected with miR-564 or miR-658 | 105 |
| miR-658   | showed gefitinib resistant phenotypes | | |
| miR-3652  | PC-9 and PC-9/ZD | EGFR-TKI | these miRNAs were upregulated | 105 |
| miR-3126-5p | PC-9 and PC-9/ZD | | in gefitinib resistant PC-9/ZD cells |
| miR-3682-3p | PC-9 and PC-9/ZD | | |
| miR-6810-5p | PC-9 and PC-9/ZD | | |
| miR-3648  | PC cells treated with | EGFR-TKI | upregulation of Exo-miR-3648 or Exo-miR-522-3p | 106 |
| miR-522-3p | H1975-derived exosomes | | is linked to gefitinib resistance |
| miR-214   | Exosomal transfer of miR-214 | | mediates gefitinib resistance | 107 |
| miR-21-5p | ALK-Translocated cells FA34 | ALK-TKI | miR-21-5p and miR-486-3p levels were | 110 |
| miR-486-3p | and FA121 | | significantly increased in crizotinib resistant subclones | |
mRNAs also contribute to drug resistance. As exemplified by the study of Yu et al.,\textsuperscript{109} upregulation of the oncogene MET was found in exosomes released by EGFR-TKI ictitinib-resistant NSCLC cells and exosomes isolated from metastatic NSCLC patients.

TDEs also participate in ALK-TKI resistance. A study by Kwok et al.\textsuperscript{110} showed that exosomes from an ALK-TKI-resistant NSCLC subclone could induce drug resistance in the originally sensitive subclone, and miRNAs including miR-21-5p and miR-486-3p, as well as lncRNAs such as MEG3 and XIST were found to be differentially expressed in the exosomes secreted by the resistant subclones. The role of TDEs in NSCLC targeted therapy resistance is summarized in Table 3.

4 | THE POTENTIAL APPLICATION OF TDES IN THE PERSONALIZED THERAPY OF NSCLC

As mentioned above, an increasing number of studies revealed that TDEs participate in progression, metastasis, and drug resistance of NSCLC, which implies potential application in targeted therapy of NSCLC using exosomes.

Exosomes have characteristics that make them suitable for drug delivery. Recombinant proteins and siRNA can degrade before reaching the target cells and can also elicit immune responses, while EVs can overcome this. Drugs delivered by exosomes can be protected from biodegradation, since exosomes at the nanoscale contain lipid bilayer membranes.\textsuperscript{111} Additionally, exosomes carrying cargoes like RNA, DNA, and miRNA are well-tolerated, have low immunogenicity and a longer circulating half-life in human body, and can cross the biological barrier, for example, the blood-brain barrier.\textsuperscript{72,112,113} Exosomes can be engineered to deliver anticancer drugs by different approaches. Electroporation/lipofection can be applied to transfer molecules and proteins of interest into cells secreting exosomes.\textsuperscript{114} Loading of anticancer drugs can also be achieved by simple incubation with exosomes. Aqil et al. observed that celastrol, a plant-derived triterpenoid, loaded into exosomes enhanced its anticancer effects with reduced dose-related toxicity.\textsuperscript{115} It was found that exosomes carrying doxorubicin exhibited potent anticancer activity in the NSCLC cell lines H1299 and A549.\textsuperscript{116} Treatment of the lung cancer cell line A549 with the anticancer drug taxol delivered by mesenchymal stem cell-derived exosomes resulted in a significantly reduced proliferation in vitro and organ metastasis in vivo.\textsuperscript{117} Besides, studies demonstrated that chemotherapeutics including anthocyanidins and paclitaxel encapsulated in exosomes were able to inhibit lung cancer metastasis in nude mice.\textsuperscript{118,119}

Since TDEs are closely associated with tumor progression and metastasis, they may be potential targets for anticancer therapy. Suppression of exosome release can be achieved by blood purification, changing the pH values of the tumor environment, and application of drugs.\textsuperscript{120} Compounds targeting exosomal proteins and different stages of the exosome biogenesis process can serve as potential exosome inhibitors.\textsuperscript{121} For example, GW4869, the first sphingomyelinase inhibitor, has been used to inhibit the production of exosomes. Inhibition of exosome secretion by GW4869 reversed the antagonistic effects of gefitinib and cisplatin in NSCLC cells when TKIs and chemotherapeutic agents are co-administered, implying a feasible and promising strategy for NSCLC treatment.\textsuperscript{122} Additionally, recent studies showed the therapeutic efficiency of anti-CD9 and anti-CD63 monoclonal antibodies (mAbs), in gastric cancer and breast cancer.\textsuperscript{122,123} Treatment with these two antibodies significantly decreased metastasis to the lungs in mice.\textsuperscript{123}

The significant role of exosomal cargo including miRNA and proteins in regulation of NSCLC metastasis and induction of drug resistance also provides targets for potential therapeutic intervention. A study by Li et al.\textsuperscript{103} depicted that miR-21 was overexpressed in the EGFR-TKI-resistant cell line PC9R, and inhibition of miR-21 induced tumor cell apoptosis in vitro and suppressed tumor growth in vivo. Downregulation of miR-let-7e was found in serum-derived exosomes from NSCLC patients, and miR-let-7e overexpression in serum-derived exosomes inhibited metastasis of NSCLC nude mice.\textsuperscript{124} These findings imply a potential application of the exosomal miRNAs. Collectively, the potential application of TDEs in treatment of NSCLC can be considered in three aspects: (1) loading of anticancer drugs, (2) suppressing TDEs release, (3) targeting exosomal miRNA and proteins.

5 | MAJOR CHALLENGES AND ASPECTS

Exosomes are the natural carriers of biomolecules which make them ideal for therapy of complex diseases including lung cancer. Exosomes have emerged as key players in nanomedicine, but certain challenges still remain.

The first step in any study regarding exosomes is the isolation procedure which is challenging. Exosomes can be isolated from body fluids or in vitro cell cultures by different techniques, but a standardized protocol is still missing. The amount of exosomes obtained is also variable and differs in every experiment.\textsuperscript{125} The primarily used isolation technique is ultracentrifugation as it is cost-effective, but it has many drawbacks as well. It is time-consuming, bears low yield of exosomes, and therefore is not suitable for clinical use.\textsuperscript{126} Other methods including differential centrifugation, size exclusion chromatography, immunoaffinity capture, precipitation, and microfluidics techniques also have their own disadvantages.\textsuperscript{127} Additionally, absence of suitable exosomal markers together with technical challenges makes it difficult to purify specific exosomes from a mixture of different cells and vesicle types.\textsuperscript{127}

Second, the storage conditions, which may affect the stability of exosomes, are not yet fully understood. It is reported that storage of exosomes at 4°C or −80°C may destabilize the surface characteristics, morphological features, and protein content of
Exosome-based therapeutics can only be realized in a clinical setting if the barriers of improper isolation and storage approaches have been overcome. Thirdly, challenges remain regarding application of functional exosomes as therapeutic cargo. So far, there is no sufficient data about the efficiency and safety of the exosome miRNA/protein delivery system in cancer. Cellular toxicity of imported exosomal miRNAs could be a major problem for clinical application. Moreover, exosomes contain heterogeneous components and may exhibit immunogenicity effects based on the nature of parent donor cells. Therefore, improving delivery efficiency and therapeutic potential is required for the development of exosomes for clinical applications.

6 | CONCLUSION

In this review, recent developments of TDE in lung cancer progression, metastasis, their interactions with the TME, and their potential therapeutic applications have been discussed. Currently, the application of exosome in diagnosis and cancer therapy is still at the early stage. In the near future, intensive research, particularly in vivo studies on exosomes, will improve our knowledge on exosome biogenesis, sorting mechanisms of miRNAs and proteins into exosomes, and precise procedure of exosomal cargo delivery. Additionally, developments of standardized exosome isolation technique, storage conditions, and delivery system are largely required for a successful application of exosomes in clinical settings.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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