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REVIEW

EV-Mediated Oncogenic Regulation in Lung Cancer and Clinical Translation: From Liquid Biopsy to Targeted Delivery Systems

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Abstract: Lung cancer (LC) remains the leading cause of cancer-associated mortality globally. Delayed diagnosis, therapeutic resistance and high postoperative recurrence are major hurdles that compromise its clinical outcomes. Extracellular vesicles (EVs) are cell-secreted nanoscale lipid vesicles that mediate intercellular crosstalk via the transfer of bioactive cargo, such as nucleic acids and proteins. In the lung cancer microenvironment, EVs remodel core signaling axes including PI3K/AKT and NF-κB, consequently driving hallmark malignant phenotypes: angiogenesis, distant metastasis, immune evasion and multidrug resistance. Boasting robust physicochemical stability, complete molecular cargo signatures and minimally invasive sampling feasibility, EVs represent ideal tumor biomarkers for liquid biopsy. Additionally, their intrinsic low immunogenicity renders EVs versatile nanovehicles capable of amplifying anti-tumor effects elicited by conventional radiotherapy, chemotherapy and immunotherapy. However, substantial inherent heterogeneity, a lack of unified standards for EVs isolation and characterization, obstacles to scalable production and inadequate tumor-targeting efficiency collectively impede their clinical translation. This review systematically dissects the molecular mechanisms through which EVs orchestrate lung cancer malignant progression, comprehensively outlines state-of-the-art progress of EVs applications in liquid biopsy-based early screening, prognostic stratification, targeted drug delivery and cellular immunotherapy, thoroughly discusses prevailing translational bottlenecks, and envisions future directions of EVs in precision oncology for lung cancer. The work offers theoretical guidance for developing next-generation EV-centric diagnostic and therapeutic platforms.

Keywords: extracellular vesicles, lung cancer, cancer biomarkers, targeted drug delivery, liquid biopsy

Introduction

Despite advances in oncology, LC remains a dominant contributor to global cancer mortality and continues to impose a substantial healthcare burden worldwide.[1] Recent epidemiological estimates from the International Agency for Research on Cancer (IARC) indicate that LC accounts for approximately 12.4% of all newly diagnosed malignancies, representing a substantial and persistent global health burden.[2,3] From a histopathological perspective, LC is broadly categorized into small cell LC (SCLC) and non-small cell LC (NSCLC), with NSCLC comprising nearly 85%–90% of cases. The major NSCLC subtypes include lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC).[4] At the molecular level, LC development is driven by a heterogeneous landscape of genetic and signaling alterations. Clinically actionable driver events, including *EGFR* mutations, *ROS1* rearrangements, and *KRAS* mutations, play central roles in NSCLC pathogenesis. Dysregulation of key oncogenic pathways such as PI3K/AKT, together with loss of tumor suppressor function in genes including *TP53* and *RB1*, contributes to tumor initiation and progression.[5] Environmental and lifestyle-related exposures further modulate disease risk, with tobacco smoking, air pollution, and occupational exposure to asbestos and particulate matter representing well-established risk factors.[6] Despite advances in molecular

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diagnostics and therapeutic strategies, the clinical management of LC continues to face substantial limitations. Early-stage disease is often clinically silent, and current screening modalities, including imaging and serum-based biomarkers, lack sufficient sensitivity for reliable early detection. As a result, a significant proportion of patients are diagnosed at advanced stages. Therapeutic resistance to radiotherapy, chemotherapy, and targeted agents remains a major challenge, frequently accompanied by disease recurrence, metastatic progression, and immune evasion, collectively contributing to poor long-term survival outcomes.[7] These limitations underscore the urgent need for more effective, minimally invasive diagnostic strategies and improved therapeutic approaches with higher precision.

In this context, EVs are nanoscale, lipid-bilayer-enclosed vesicles actively secreted by virtually all cell types and are widely detectable in diverse biological fluids. They carry a complex cargo of bioactive molecules, including nucleic acids, proteins, and lipids, and participate in the dynamic regulation of the tumor microenvironment. In LC, EVs have attracted increasing attention due to their inherent stability, molecular specificity, and accessibility through minimally invasive sampling, making them promising candidates for applications in early detection, disease monitoring, and therapeutic response assessment.[8,9] Furthermore, EVs are being actively investigated as natural nanocarriers for drug delivery and as engineered platforms for targeted therapeutic intervention in LC.[10] This review provides a comprehensive overview of EV-mediated mechanisms involved in LC progression, evaluates current liquid biopsy approaches, and summarizes recent advances in EV-based diagnostic and therapeutic strategies. Finally, it discusses key challenges limiting clinical translation and highlights future perspectives for the development of EV-based precision oncology approaches.

Overview of EVs

In accordance with the MISEV 2023 guidelines, EVs are defined and characterized based on their biogenesis, size distribution, and molecular composition, although no single definitive marker uniquely distinguishes EVs subtypes.[11] EVs are heterogeneous, lipid bilayer-enclosed vesicles that are conventionally classified into three major categories: exosomes, microvesicles (MVs), and apoptotic bodies, reflecting distinct biogenetic pathways and biophysical properties (Figure 1).[12]

Exosomes

Exosomes are nanoscale EVs actively released by virtually all cell types into the extracellular microenvironment, with a typical diameter of approximately 30–150 nm.[13] Their biogenesis originates from the endosomal system, beginning with plasma membrane invagination and the formation of early endosomes that internalize extracellular and membrane-associated cargo. During endosomal maturation, selected cargo is either directed toward recycling pathways or retained within intraluminal vesicles (ILVs) formed by inward budding of the endosomal membrane, leading to the generation of multivesicular bodies (MVBs).[14] ILV formation is predominantly regulated by the endosomal sorting complex required for transport (ESCRT), comprising ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III complexes. ESCRT-0 is responsible for cargo recognition and clustering; ESCRT-I and ESCRT-II facilitate membrane deformation and budding, while ESCRT-III mediates membrane scission and vesicle release.[15,16] In addition to ESCRT-dependent mechanisms, ESCRT-independent pathways involving lipids, tetraspanins, and heat shock proteins (HSPs) also contribute to ILV biogenesis.[17] MVBs follow two main fates: fusion with lysosomes for degradation or fusion with the plasma membrane, resulting in the extracellular release of ILVs as exosomes. Exosomes are enriched in proteins such as CD63, LAMP1, and LAMP2, and commonly express conserved markers including Alix, TSG101, HSC70, and HSP90β, although no single universal marker definitively defines their origin.[18,19] They appear as round to oval vesicles and often exhibit a cup-shaped structure under an electron microscope.[20]

MVs

MVs are a heterogeneous population of EVs typically ranging from 150 to 1000 nm in diameter.[16] Unlike exosomes, MVs are formed through direct outward budding of the plasma membrane, a process driven by alterations in membrane lipid composition, intracellular calcium (Ca^{2+}) flux, and cytoskeletal reorganization. A key early event in MV biogenesis is the translocation of phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane, which

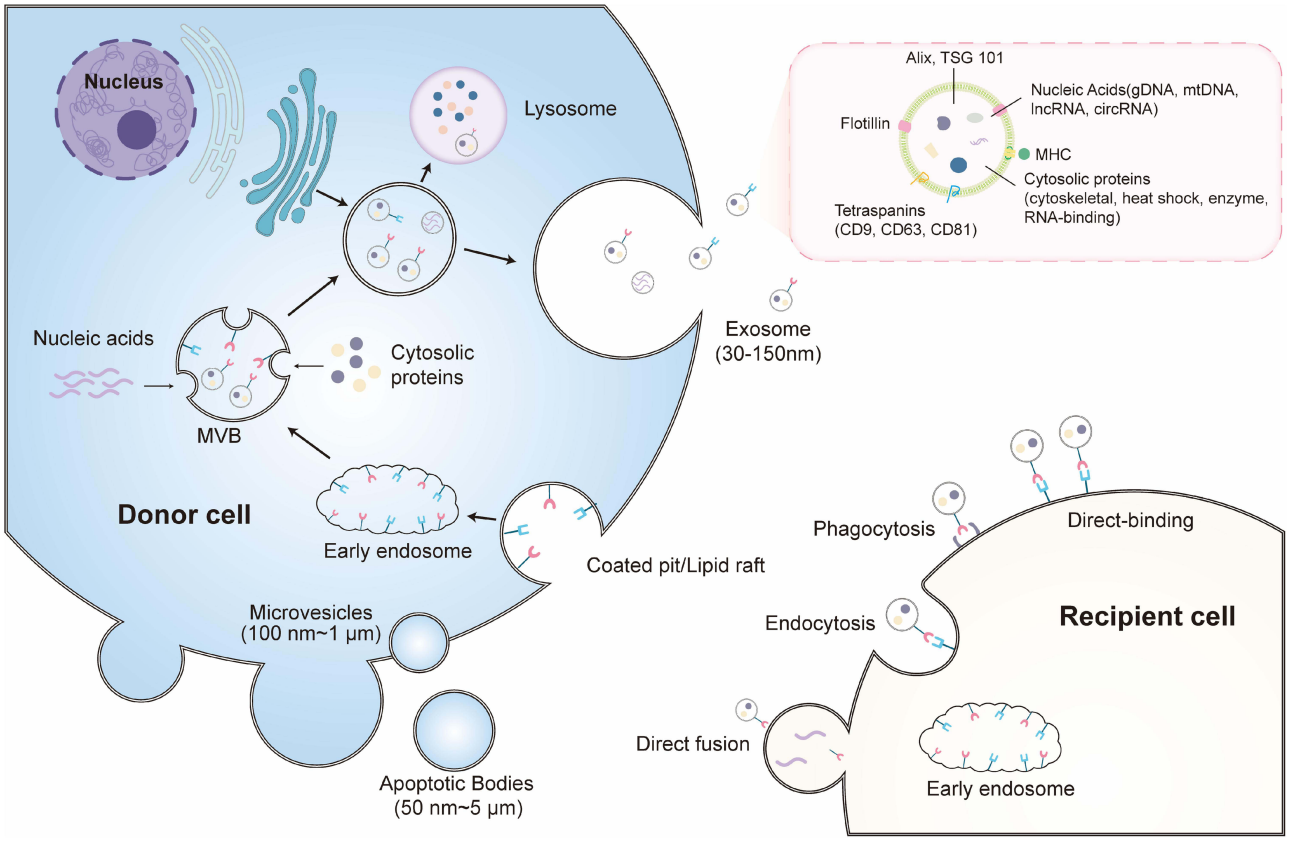

Figure 1 Biogenesis, secretion, and cellular uptake pathways of EVs: EVs are broadly classified into exosomes, microvesicles (MVs), and apoptotic bodies based on their biogenesis and size distribution. Exosomes originate from the endosomal system, where early endosomes undergo inward budding to generate intraluminal vesicles (ILVs), forming multivesicular bodies (MVBs). MVBs subsequently follow two distinct fates: fusion with lysosomes leading to cargo degradation, or fusion with the plasma membrane, resulting in the release of mature exosomes into the extracellular space. Microvesicles are generated through direct outward budding and fission of the plasma membrane, whereas apoptotic bodies are formed as membrane-enclosed vesicles released during programmed cell death. Following their release, EVs are internalized by recipient cells via multiple mechanisms, including endocytosis, phagocytosis, and direct membrane fusion, enabling the transfer of bioactive cargo that modulates recipient cell function.

promotes membrane curvature and budding.[14] This process is tightly regulated by cytoskeletal remodeling and signaling pathways involving Rho family GTPases and Rho-associated protein kinase (ROCK) activity.[21] Cargo sorting into MVs occurs through multiple mechanisms, including lipid raft-associated localization, glycosylphosphatidylinositol anchoring, and sequence-dependent RNA and protein recruitment.[22,23] Subsequent actin– myosin contraction and ATP-dependent cytoskeletal forces facilitate membrane scission and release of MVs into the extracellular space.[24] Intracellular Ca^{2+} signaling, CDC42 activation, and actin dynamics further regulate this shedding process.[25]

Apoptotic Bodies

Apoptotic bodies are large EVs generated during programmed cell death, with a broad size range of approximately 50–5000 nm.[26] Their formation is closely linked to apoptotic membrane remodeling and reflects the structural and molecular composition of the parent dying cell. During apoptosis, increased intracellular pressure and cytoskeletal breakdown lead to membrane blebbing, resulting in the formation of dynamic membrane protrusions surrounding nuclear and cytoplasmic material.[27] Progressive nuclear condensation and fragmentation further contribute to the encapsulation of cellular components into discrete vesicular structures.[28] These fragmented cellular components are ultimately packaged into apoptotic bodies, which contain proteins, nucleic acids, and organelle remnants derived from the parent cell.[29] Apoptotic bodies may express markers such as caspase-3-associated components and histones, reflecting their apoptotic origin.[30] Under physiological conditions, they are efficiently cleared by phagocytic cells, particularly macrophages, through phosphatidylserine-dependent recognition pathways involving Annexin V binding.[31]

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Exosomes, microvesicles, and apoptotic bodies represent distinct yet partially overlapping populations of EVs that differ in their biogenetic origin, size distribution, and molecular composition. In LC, these EV populations participate in multiple aspects of tumor progression by shaping the tumor microenvironment and influencing cellular behavior. Their biological diversity also has important implications for the development of EV-based diagnostic and therapeutic approaches, while simultaneously creating challenges for standardization and clinical translation.

The Role of EVs in LC Progression

Tumor cells exhibit high proliferative activity and release abundant EVs, which serve as key mediators of intercellular communication within the tumor microenvironment. In LC, EVs are implicated in multiple hallmarks of disease progression, including tumor proliferation, immune modulation, and the development of therapeutic resistance.[32] Through both paracrine and systemic signaling, EVs facilitate bidirectional communication between malignant and stromal cells, contributing to tumor microenvironment remodeling and establishing a permissive niche for tumor growth and metastatic dissemination.[33] Building on these concepts, the following sections explore the key mechanistic pathways through which EVs regulate LC progression.

Angiogenesis

Tumor angiogenesis is a critical pathological process that supports cancer cell survival and promotes tumor growth, invasion, and metastatic dissemination.[34] In the early stages of tumor development, hypoxic stress and microenvironmental alterations stimulate tumor cells to release abundant EVs.[35] These EVs circulate through biological fluids and are subsequently taken up by vascular endothelial cells within the tumor microenvironment, where they modulate key cellular functions including proliferation, migration, and vascular remodeling, facilitating angiogenic activation.[36] At the mechanistic level, EV-derived bioactive cargo such as non-coding RNAs and functional proteins disrupt endothelial junction integrity by downregulating cadherin and β-catenin expression, resulting in reduced intercellular adhesion and enhanced endothelial motility.[37] EVs signaling activates transcriptional regulation associated with angiogenesis, leading to upregulation of pro-angiogenic factors and receptors including VEGFA, FGF2, PDGFB, and ANGPT2.[38] This molecular reprogramming promotes endothelial cell proliferation and contributes to neovessel formation. Subsequently, activated endothelial cells secrete cytokines and growth factors that further amplify angiogenic signaling. This process is reinforced through the PI3K/Akt signaling axis, which facilitates pericyte activation and stabilization of newly formed vascular structures, sustaining progressive vascular expansion.[39] In LC, multiple EV-associated molecular regulators have been implicated in angiogenic modulation. Tumor-derived EVs enriched with miR-671-3p suppress KLF2 expression in endothelial cells, leading to increased vascular endothelial growth factor receptor 2 (VEGFR2) expression and enhanced angiogenic signaling; inhibition of miR-671-3p effectively disrupts this regulatory axis.[40] Similarly, EVs carrying miR-197-3p, miR-23a, and miR-141 promote angiogenesis through distinct mechanisms: miR-197-3p downregulates TIMP2 and TIMP3 expression to activate the ERK pathway, miR-23a suppresses PTEN signaling, and miR-141 targets KLF12 to induce abnormal endothelial proliferation and vascular remodeling.[41,42] EVs containing YAP protein enhance angiogenic potential in a concentration-dependent manner and significantly increase endothelial tube formation when co-cultured with HUVECs.[43] EVs orchestrate angiogenic processes in LC through coordinated effects on endothelial signaling, gene regulation, and microenvironmental remodeling, contributing to sustained tumor vascularization and disease progression.

Metastasis

The invasion and metastasis of LC represent a highly complex, multi-step cascade process that includes local invasion of the primary tumor, intravasation into the vasculature, survival in circulation, extravasation into distant tissues, and eventual colonization within the microenvironment of secondary organs to establish metastatic lesions.[44] Accumulating evidence indicates that EVs, derived from both tumor and stromal cells within the tumor microenvironment, act as critical mediators of intercellular communication and play a central role in regulating both local invasion and distant metastatic colonization in LC.[45] During the early phases of metastasis, tumor-derived EVs (TDEs) promote metastatic dissemination through complementary mechanisms.[46] On one hand, they enhance tumor angiogenesis, while on the other

hand, they compromise vascular integrity by disrupting tight junctions and adherens junctions in vascular endothelial cells, degrading the vascular basement membrane, and weakening extracellular matrix barriers. These alterations increase vascular permeability, facilitating tumor cell intravasation and entry into the systemic circulation, establishing the structural basis for distant metastasis.[47] Beyond vascular remodeling, TDEs actively contribute to the formation of a pre-metastatic niche in distant organs. This is achieved through the activation of key signaling pathways, including NLRP6/NF-κB, OSMR/JAK/STAT3, and PTPRD/STAT3, which collectively promote immunosuppressive and tumor-supportive microenvironmental conditions that favor metastatic colonization.[48] TDEs also enhance the invasive potential of tumor cells by inducing epithelial–mesenchymal transition (EMT) and stemness-associated phenotypes through activation of the NF-κB/SOX2 axis, accompanied by upregulation of EMT-related markers such as VIM, CDH2, ZEB2, TWIST, and SOX family genes.[47] In LC-specific regulatory contexts, EV-associated miRNAs and non-coding RNAs further amplify metastatic progression. For instance, EV-enriched miR-499a promotes EMT through modulation of the mTOR pathway, while miR-31 facilitates metastasis by targeting and silencing *SATB2* and activating MEK/ERK signaling.[49] Similarly, non-coding RNAs including LINC00963, circ_0001715, miR-19b-3p, and miR-770 contribute to metastatic niche remodeling by regulating macrophage polarization toward an M2 phenotype, reinforcing immunosuppression and enhancing LC invasion and metastasis.[50]

Immune Evasion

The programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) axis represents a central immune checkpoint pathway and a major focus in tumor immunotherapy. PD-1 is predominantly expressed on activated T lymphocytes and interacts with its ligands, PD-L1 and PD-L2, which are present on tumor cells as well as immune cells, including T and B cells. This interaction leads to functional exhaustion of T cells, impairing anti-tumor immune surveillance and cytotoxic activity.[51] PD-1 engagement triggers phosphorylation of the intracellular immunoreceptor tyrosine-based switch motif (ITSM), leading to recruitment and activation of SHP2 phosphatase. This event suppresses downstream signaling cascades, including PI3K/AKT, RAS/Raf, and PLCγ pathways, ultimately resulting in reduced T cell proliferation, diminished survival capacity, and decreased production of key anti-tumor cytokines.[52,53] Immune checkpoint inhibitors such as atezolizumab and durvalumab targeting the PD-1/PD-L1 pathway have demonstrated significant therapeutic efficacy across multiple malignancies.[54,55] Within the tumor microenvironment, TDEs have emerged as important regulators of immune evasion in LC, particularly through the transfer of functional PD-L1. Multiple experimental studies have confirmed that LC-derived EVs carry biologically active PD-L1 and contribute directly to immune suppression.[56] PD-L1$^+$ EVs inhibit CD8$^+$ T cell proliferation and activation, reduce the secretion of anti-tumor cytokines such as IL-2 and IFN-γ, and suppress NF-κB signaling activity. They promote PD-L1 expression in macrophages through glycolysis-associated metabolic reprogramming, reinforcing an immunosuppressive tumor microenvironment through coordinated cellular crosstalk.[57] In addition to local effects, PD-L1$^+$ EVs can disseminate systemically via lymphatic drainage and circulation, leading to widespread suppression of anti-tumor immune responses and facilitating immune evasion as well as metastatic progression in LC.[57] Furthermore, LC-derived EVs exhibit elevated expression of the transmembrane protein CD47, which interacts with signal regulatory protein alpha (SIRPα) on macrophages to deliver a "don't eat me" signal, inhibiting phagocytic clearance and further enabling tumor immune escape.[58]

Drug Resistance

Therapeutic resistance represents a major clinical barrier in oncology and is responsible for a substantial proportion of cancer-related mortality, estimated at approximately 90%.[59] Increasing evidence indicates that EVs play a central role in the development and propagation of drug-resistant tumor phenotypes.[60] One key mechanism involves intercellular transfer of resistance traits from drug-resistant to drug-sensitive tumor cells via EV-mediated delivery of functional biomolecules, including proteins and non-coding RNAs. For instance, tumor cells can secrete EVs enriched with the anti-apoptotic protein Survivin, which suppresses caspase activity in recipient cells and promotes resistance to apoptosis.[61] In LC, chemotherapy-induced stress has been shown to modulate EV cargo composition. Cisplatin exposure downregulates miR-100 in EVs derived from A549 cells, and these EVs regulate mTOR signaling by targeting its 3′-UTR, altering

cisplatin sensitivity and influencing downstream processes such as proliferation, apoptosis, and invasion in recipient cells.[62] Similarly, treatment with chidamide induces upregulation of the long non-coding RNA SNHG7 in lung adenocarcinoma-derived EVs, which promotes doxorubicin resistance by enhancing autophagy-related gene expression, including *ATG5* and *ATG12*, in recipient cells.[63] Moreover, mesenchymal-like LC cells can transmit ZEB1 mRNA via EVs, inducing epithelial–mesenchymal transition-associated drug resistance and conferring reduced sensitivity to cisplatin, gemcitabine, and combination chemotherapy regimens.[64] In addition to cargo-mediated reprogramming, TDEs also contribute to drug resistance through efflux-based mechanisms. These EVs frequently exhibit elevated expression of ATP-binding cassette (ABC) transporters, including *ABCG2* (BCRP, breast cancer resistance protein) and *ABCC1* (MRP1, multidrug resistance-associated protein 1), which actively export chemotherapeutic agents from tumor cells, reducing intracellular drug accumulation and promoting multidrug resistance (Figure 2).[65]

Application of EVs in the Diagnosis and Prognosis of LC

The lack of effective early detection strategies and the frequently asymptomatic nature of early-stage disease result in a substantial proportion of LC cases being diagnosed at advanced stages, contributing to a 5-year survival rate of approximately 19.7%.[66] Imaging modalities remain central to clinical diagnosis. Chest radiography is commonly used as

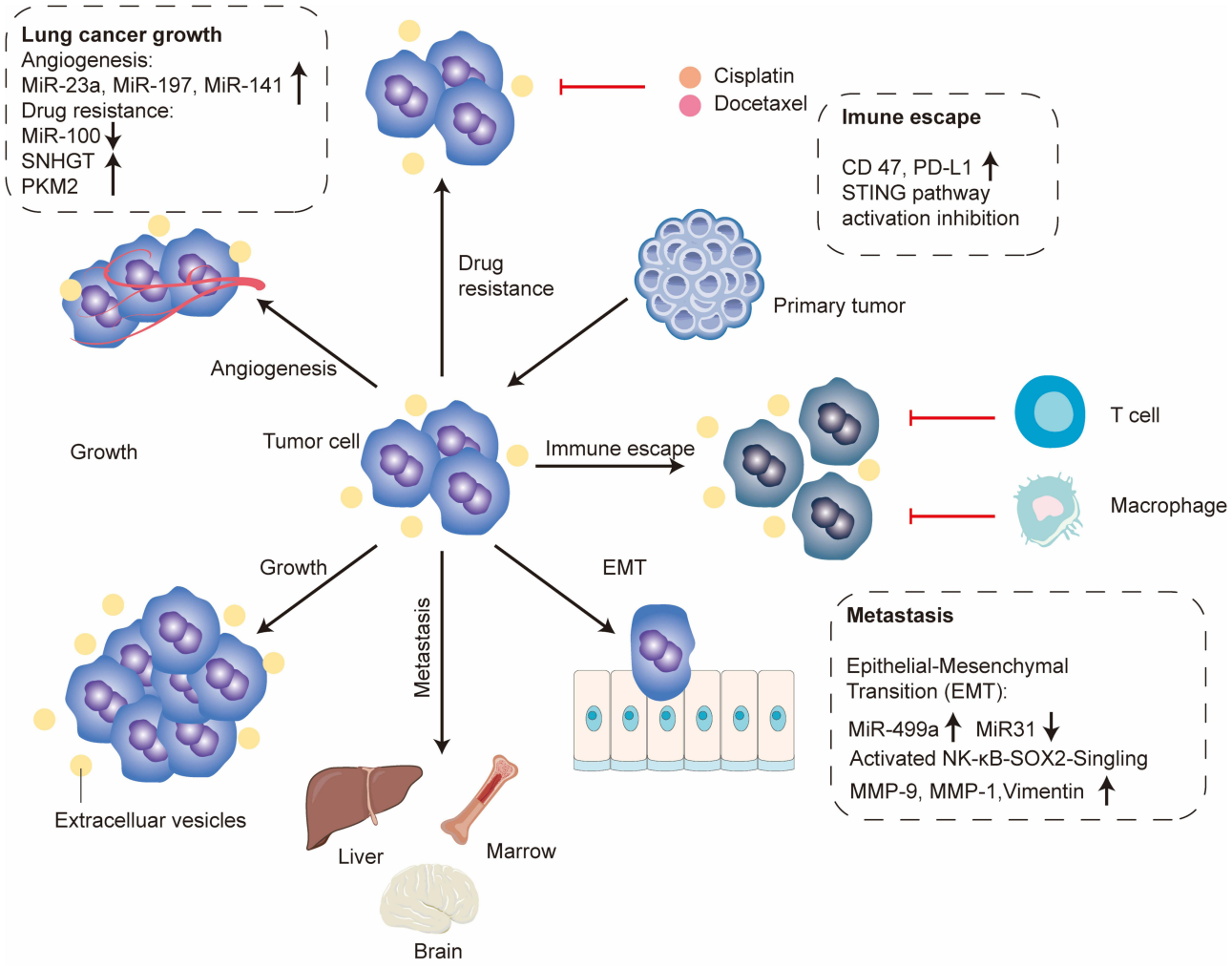

Figure 2 Components such as miR-23a, miR-197, miR-141, miR-100, SNHGT and PKM2 carried by extracellular vesicles regulate tumor angiogenesis and mediate cisplatin and docetaxel resistance in lung cancer. Surface molecules CD47 and PD-L1 on extracellular vesicles facilitate tumor immune escape, while miR-499a and miR-31 induce tumor epithelial-mesenchymal transition. These multiple mechanisms synergistically drive tumor progression and ultimately lead to distant organ metastasis.↑: upregulated expression; ↓: downregulated expression.

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an initial diagnostic tool, with findings such as irregular pulmonary margins, lobulated masses, pleural retraction, and patchy opacities raising suspicion of malignancy. However, its diagnostic specificity remains limited.[67] When radiographic findings suggest a malignant lesion, contrast-enhanced computed tomography (CT) provides a more detailed assessment of tumor morphology, extent, and anatomical relationships with adjacent structures.[1] Despite these advances, current diagnostic approaches remain insufficient for reliable early detection. Therefore, the development of accurate, minimally invasive, and reproducible diagnostic strategies remains a clinical priority. Liquid biopsy has emerged as a promising alternative owing to its minimal invasiveness and ability to facilitate longitudinal disease monitoring. Among the major liquid biopsy analytes, circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), and EVs have received considerable attention as potential biomarkers for LC diagnosis and prognosis.[68] The following sections summarize their diagnostic applications and associated limitations.

CTCs

CTCs are malignant cells that detach from primary tumors or metastatic lesions and enter the peripheral circulation either spontaneously or during clinical interventions. Because they provide direct evidence of tumor dissemination, CTCs have attracted considerable interest as biomarkers for early cancer detection, prognostic evaluation, and risk stratification.[69] Current CTC enrichment strategies primarily include density-gradient centrifugation and antigen-dependent immunomagnetic separation, while detection approaches include the CellSearch system, fiber-optic array scanning technology (FAST), flow cytometry, and immunofluorescence-based assays.[70] Early clinical investigations demonstrated the feasibility of CTC detection in LC. For example, Tanaka et al identified CTCs in 17 of 88 patients with suspected or confirmed primary LC using the CellSearch platform, corresponding to a detection rate of 19.3%.[71] Similarly, a study involving 101 untreated patients with stage III–IV NSCLC reported detectable CTCs in 21 individuals.[72] Despite their clinical potential, several challenges limit the routine application of CTCs. Their extremely low abundance in peripheral blood complicates reliable detection, and a large proportion of CTCs are rapidly eliminated from circulation following tumor cell dissemination.[73] Currently employed detection technologies display important technical limitations. The CellSearch system relies largely on EpCAM expression and may fail to identify EpCAM-negative tumor cells, whereas FAST requires relatively large sample volumes. Flow cytometry and immunofluorescence-based approaches may also be affected by suboptimal specificity. These limitations continue to hinder the widespread clinical implementation of CTC-based diagnostics in LC.[74]

ctDNA

cfDNA comprises extracellular DNA fragments released into the circulation primarily through apoptosis and necrosis. The fraction of cfDNA derived specifically from tumor cells is termed ctDNA, which can be detected in peripheral blood and other body fluids, including cerebrospinal fluid and urine.[75,76] Because ctDNA retains tumor-specific molecular alterations, including somatic mutations, DNA methylation patterns, and microsatellite instability, it provides a valuable non-invasive biomarker for monitoring tumor evolution and disease progression in real time.[77] Furthermore, ctDNA levels gradually increase with tumor burden and disease progression.[78] Advances in molecular detection technologies, including amplification refractory mutation system (ARMS), quantitative PCR (qPCR), digital PCR, and next-generation sequencing (NGS), have substantially improved the sensitivity and reliability of ctDNA analysis since 2016. Thus, ctDNA has become an important tool for assessing tumor heterogeneity, monitoring disease dynamics, and evaluating treatment responses across multiple malignancies, including LC, breast cancer, and colorectal cancer.[79] Clinical studies have demonstrated the diagnostic value of ctDNA in LC. In one investigation involving 68 patients with NSCLC, ctDNA was detected in more than 80% of serum samples, with *TP53, KRAS*, and *EGFR* being the most frequently mutated genes.[80] A subsequent meta-analysis reported a pooled sensitivity of 68% and a specificity of 98% for ctDNA-based *EGFR* mutation detection.[81] Aberrant methylation of genes including *MGMT, p16INK4a, RASSF1A, DAPK*, and RAR-β has been proposed as a potential biomarker panel for LC detection and risk assessment.[82] Although ctDNA offers high specificity and clinically meaningful sensitivity, its application remains constrained by biological and technical challenges. ctDNA typically constitutes only a small fraction of total circulating nucleic acids and exhibits a short half-life, often less than two hours, making its detection susceptible to pre-analytical and analytical variability. These limitations continue to affect its accessibility and routine clinical implementation.[83]

EVs

EVs have emerged as promising liquid biopsy biomarkers for LC because they encapsulate diverse molecular cargo that reflects the biological characteristics of their cells of origin. Among these cargo molecules, miRNAs, long non-coding RNAs (lncRNAs), and proteins have demonstrated considerable potential for early detection and disease monitoring.[84] As early as 2013, Harvey I. Pass et al employed the ExoQuick precipitation method to isolate peripheral blood-derived EVs from 10 patients with lung adenocarcinoma, 10 patients with lung granulomas, and 10 age- and sex-matched healthy smokers, followed by miRNA expression profiling using RT-PCR. Expression profiling identified significant upregulation of miR-151a-5p, miR-30a-3p, miR-200b-5p, miR-629, miR-100, and miR-154-3p in TDEs. The resulting biomarker panel achieved high diagnostic performance, with reported sensitivities exceeding 95% and an area under the receiver operating characteristic (ROC) curve greater than 0.90.[85] Subsequent investigations further supported the diagnostic value of EV-associated miRNAs in NSCLC. In a cohort comprising 100 patients with NSCLC and 90 healthy controls, elevated expression of several EV-derived miRNAs was observed. Among these candidates, miR-17-5p demonstrated an area under the ROC curve (AUC) of 0.746, with a sensitivity of 70.0% and specificity of 82.2% for NSCLC detection. Its diagnostic performance exceeded that of several routinely used serum biomarkers, including carcinoembryonic antigen (CEA), cytokeratin 19 fragment (CYFRA21-1), and squamous cell carcinoma antigen (SCCA), highlighting the potential clinical value of EV-associated biomarkers in LC detection.[86] Beyond individual miRNA candidates, numerous studies have identified additional EV-derived non-coding RNAs with potential diagnostic relevance, including miR-23a, miR-30b/30c, let-7 family members, miR-23a-3p, miR-486-5p, PITPNA-AS1, Mir100hg, TBILA, and AGAP2-AS1.[87–89] Protein cargoes carried by TDEs have also attracted attention as candidate biomarkers, with PD-L1, CD317, EGFR, A-kinase anchoring protein 4, and CD9 among the most frequently reported markers associated with LC.[90] Although peripheral blood remains the most commonly investigated source of EVs, other biological fluids may provide additional diagnostic opportunities. Bronchoalveolar lavage fluid (BALF) and sputum are enriched in tumor-associated EVs and may offer greater proximity to the primary tumor site. For example, comparative RNA sequencing of EVs isolated from BALF revealed significantly elevated miR-1246 expression in patients with LC compared with individuals with benign pulmonary nodules. Diagnostic analysis yielded an AUC of 0.743, with a sensitivity of 80% and specificity of 60%. Functional studies further demonstrated that miR-1246 promotes tumor cell proliferation, migration, and invasion, whereas its inhibition suppresses these malignant phenotypes.[91] Other biomarkers identified in BALF- and sputum-derived EVs, including miR-126, Let-7a, miR-21, miR-155, miR-210, and E-cadherin, have similarly shown potential utility for LC detection[92,93] (Figure 3).

CTCs, ctDNA, and EVs each provide unique advantages for liquid biopsy applications. However, the low abundance of CTCs and the limited stability of ctDNA present important analytical challenges. EVs are released in large quantities, possess a protective lipid bilayer that preserves their molecular cargo, and are widely distributed across biological fluids. These characteristics facilitate the detection of diverse nucleic acid and protein biomarkers and support applications in early diagnosis, disease monitoring, therapeutic response assessment, and drug resistance surveillance. As methodologies for EV isolation and characterization continue to improve, EV-based liquid biopsy approaches are expected to play an increasingly important role in precision diagnostics and personalized management of LC (Table 1).

Application of EVs in LC Treatment

Current EV-based therapeutic strategies in LC can be broadly categorized into two complementary approaches. The first approach focuses on mitigating the pathological effects of tumor-derived EVs by reducing their production, release, or functional interaction with recipient cells. This includes pharmacological inhibition of EV biogenesis, such as using sphingomyelinase inhibitors like GW4869, which suppress the production of pro-tumorigenic EVs. Other strategies involve blocking EV uptake or neutralizing circulating EVs to limit their ability to transfer oncogenic cargo and promote malignant progression.[94] The second approach involves the therapeutic engineering of EVs as natural nanocarriers for drug delivery. In this context, EVs can be loaded with diverse therapeutic agents, including small-molecule chemotherapeutic drugs, nucleic acid-based therapeutics, and immune checkpoint inhibitors. Owing to their intrinsic biocompatibility and targeting capability, engineered EVs can preferentially accumulate within the tumor microenvironment, where

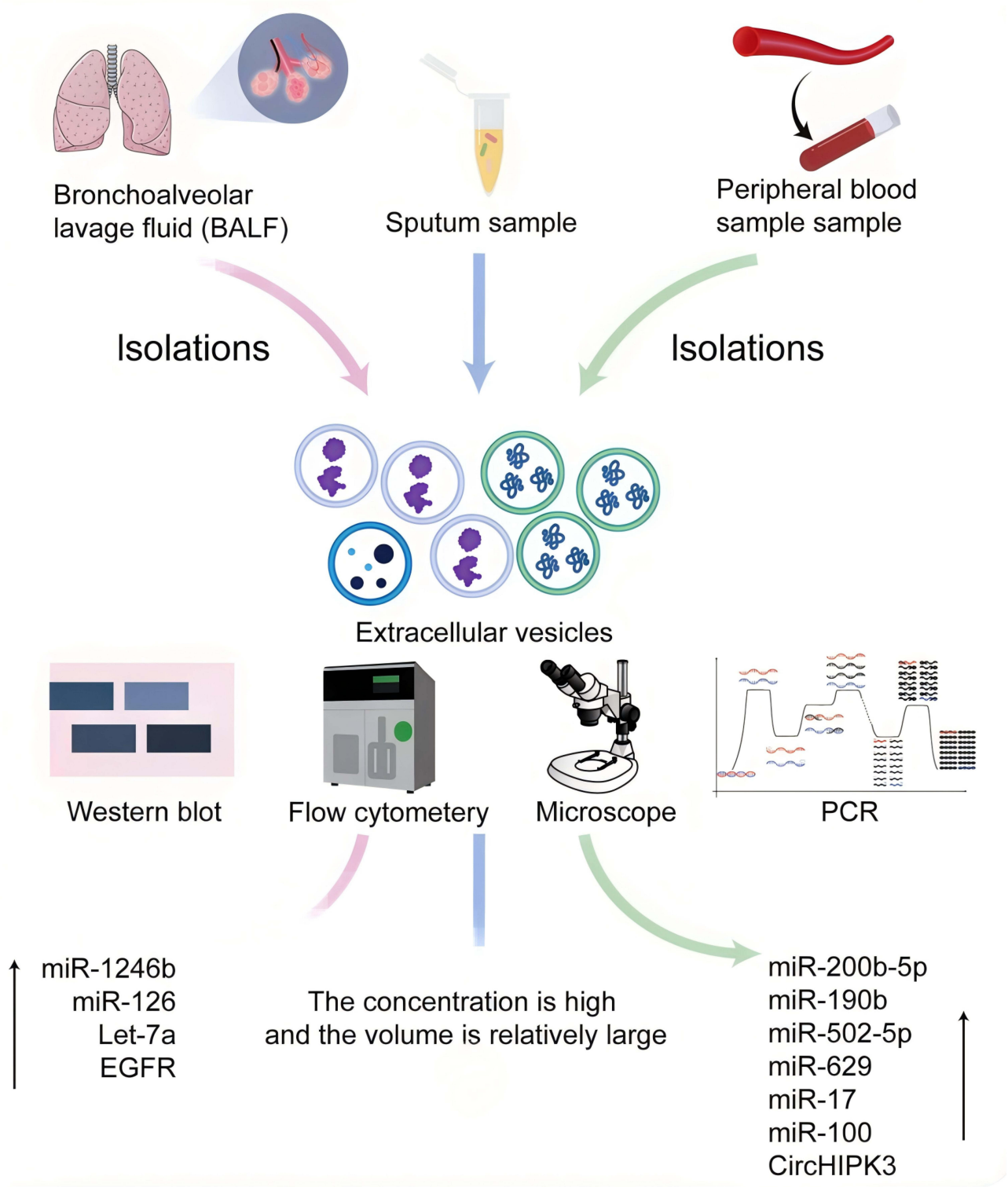

Figure 3 EVs as diagnostic biomarkers in lung cancer. EV-based diagnostic strategies employ multiple analytical platforms, including Western blot (WB), flow cytometry, PCR-based assays, nanoparticle tracking analysis, and morphological characterization, to evaluate EVs cargo derived from clinical specimens. EVs isolated from sputum, peripheral blood, and BALF of lung cancer patients exhibit distinct molecular signatures. BALF-derived EVs show elevated expression of miR-1246b, miR-190b, and miR-126, while blood-derived EVs are enriched in miR-502-5p, miR-629, and miR-17. Sputum-derived EVs display comparatively larger particle size distributions relative to non-malignant controls, highlighting their diagnostic heterogeneity and clinical utility. ↑: upregulated expression.

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Table 1 Comparison of Advantages and Disadvantages Between Traditional Lung Cancer Diagnosis and Liquid Biopsy Diagnostic Techniques

Technical Classification	Detection Method	Advantage	Disadvantage	References
Traditional diagnosis	Chest X-ray, enhanced CT, routine tumor markers (CEA, CYFRA21-1, SCCA, etc).	1. Technically mature, widely used in clinical practice 2. Can intuitively display the morphology and location of lesions, accurately completing tumor localization and staging 3. Moderate detection cost	1. Difficult to detect early small lesions, most diagnoses are at mid to late stages 2. Imaging lacks specificity and can be easily confused with benign lung lesions 3. Involves ionizing radiation, making repeated testing impossible 4. Traditional biomarkers have low diagnostic efficacy and cannot dynamically monitor tumor progression, drug resistance, and recurrence	[67]
Liquid Biopsy - CTCs	Peripheral blood circulating tumor cell detection	Can directly capture tumor cells, used for lung cancer screening, prognosis assessment, and risk stratification	1. Extremely low quantity in peripheral blood, easily cleared by the body, making detection difficult 2. Limited recognition ability of existing technology, insufficient specificity 3. Some tests require large samples, limiting clinical promotion	[70]
Liquid Biopsy - ctDNA	Peripheral blood circulating tumor DNA testing	1. Carries tumor-specific genetic information, with high diagnostic specificity 2. Can dynamically monitor tumor evolution and targeted drug resistance, with relatively mature clinical translation	1. Short half-life and easily degradable, with strict requirements for sample storage 2. Low abundance in the body, requiring high-precision detection equipment and relatively high cost	[74]
Liquid Biopsy - EVs	EVs detection in samples such as peripheral blood, sputum, and bronchoalveolar lavage fluid	1. Rich in content, with strong stability of lipid structure 2. Carries multiple types of bioactive substances, with diagnostic performance superior to traditional markers 3. Minimally invasive sampling, diverse sample types, supporting continuous dynamic monitoring	1. Complex sources, high heterogeneity, interfering with detection results 2. Lack of unified separation and quality control standards, poor reproducibility 3. Mostly in the basic research stage, with few mature clinical applications	[85]

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they exert anti-tumor effects through inhibition of tumor cell proliferation and induction of cancer cell death.[95] These strategies highlight the dual role of EVs in LC therapy, encompassing both inhibition of pathogenic EV signaling and exploitation of ECs as precision drug delivery systems.

Inhibition of Pathogenic EV Secretion

EVs serve as essential mediators of intercellular communication by facilitating the transfer of bioactive molecules between donor and recipient cells.[96] EV biogenesis and function depend on two key processes: the regulated formation and release of vesicles from donor cells and subsequent uptake of EVs by recipient cells through receptor-mediated binding, membrane fusion, or endocytosis, enabling intercellular signal transmission.[97] Accordingly, therapeutic strategies aimed at disrupting EV-mediated communication in LC primarily focus on interfering with vesicle biogenesis, secretion, or cellular uptake. One major pharmacological approach involves inhibition of EVs biogenesis and release. GW4869, a neutral sphingomyelinase inhibitor, suppresses ceramide-dependent EVs formation and has been widely used to attenuate the secretion of TDEs within the tumor microenvironment.[98] In LC models, GW4869 has been shown to reduce the release of EVs carrying oncogenic miRNAs, suppress aberrant signaling pathways, and reverse drug-resistant phenotypes. For example, inhibition of miR-7-containing EVs has been associated with restoration of Hippo pathway regulation and reversal of gefitinib resistance in LC cells.[99] EV suppression via GW4869 enhances the cytotoxic effects of chemotherapeutic agents such as cisplatin and etoposide in vitro.[100] Other small molecules, including pantethine and imipramine, have also been reported to modulate EV secretion indirectly through the regulation of lipid metabolic pathways. A second strategy targets intracellular trafficking and cytoskeletal regulation involved in EV release. Inhibition of Rho-associated coiled-coil-containing protein kinase (ROCK) using agents such as Y27632 disrupts actin cytoskeleton dynamics, impairing membrane budding and reducing EV secretion.[101] Similarly, Manumycin A, a Ras pathway inhibitor, interferes with ESCRT-associated EV biogenesis and has been reported to reduce tumor-derived EV release by approximately 50%–60% under experimental conditions.[102] In addition to pharmacological approaches, genetic modulation of Rab GTPases represents a key regulatory strategy for suppressing EV production and trafficking. Proteins such as Rab4, Rab11, Rab15, Rab35, and Rab27A regulate multiple stages of EV biogenesis, intracellular transport, and vesicle secretion. Their inhibition has been shown to significantly impair EV release in tumor systems.[103] In LC models, Rab27A knockdown reduces EV-mediated systemic effects, including adipocyte reprogramming and cancer-associated cachexia, by limiting EV uptake and downstream PKA signaling activation in adipose tissue.[104] These results indicate that pharmacological and genetic targeting of EVs biogenesis and secretion pathways may provide a promising strategy to attenuate tumor-promoting intercellular communication in LC. However, further studies are required to determine the translational feasibility, specificity, and systemic safety of these approaches in clinical settings.

Using EVs Directly as Therapeutic Agents

Because EVs selectively incorporate proteins, lipids, nucleic acids, and other bioactive molecules from their parent cells during biogenesis, they often retain important functional characteristics of their cellular origin. Consequently, in addition to strategies aimed at suppressing pathogenic EV signaling, increasing attention has been directed toward the therapeutic use of EVs as cell-free biological agents capable of modulating tumor progression in LC. Among the most extensively studied candidates are mesenchymal stem cell-derived EVs (MSC-EVs). Mesenchymal stem cells (MSCs) possess immunomodulatory, anti-inflammatory, and tissue repair properties while exhibiting relatively low immunogenicity due to limited expression of major histocompatibility complex class II (MHC-II) molecules and co-stimulatory proteins.[105] These biological characteristics are partially retained by MSC-EVs, which offer further advantages including improved stability, lower toxicity, and reduced risk of immune rejection compared with cell-based therapies. As a result, MSC-EVs have emerged as promising cell-free therapeutic platforms for cancer treatment. Experimental studies have demonstrated that MSC-EVs can suppress malignant behaviors in LC through the delivery of functional regulatory molecules. For example, human umbilical cord mesenchymal stem cell-derived EVs (hUCMSC-EVs) enriched in miR-320a inhibit LC progression through negative regulation of the SOX4/Wnt/β-catenin signaling pathway.[106] Similarly, bone marrow MSC-derived EVs carrying let-7i suppress LC cell proliferation, migration, and invasion through down-regulation of lysine demethylase 3A (KDM3A), whereas inhibition of let-7i largely abolishes these anti-tumor effects.[107]

However, the biological activity of MSC-EVs is not uniformly tumor suppressive. Emerging evidence indicates that certain MSC-derived populations may facilitate tumor progression depending on their molecular cargo. EVs carrying miR-21-5p, miR-410, and other regulatory molecules have been reported to enhance LC cell proliferation, promote EMT, increase migratory capacity, and induce macrophage polarization toward a pro-tumorigenic M2 phenotype.[108] These observations highlight the importance of rigorous cargo characterization, functional validation, and quality control when developing MSC-EV-based therapeutic strategies.

Beyond stem cell-derived vesicles, EVs released by immune cells have also attracted considerable interest as therapeutic agents capable of reversing the immunosuppressive tumor microenvironment characteristic of LC.[109] Dendritic cell-derived EVs contain functional MHC-peptide complexes, co-stimulatory molecules, cytokines, and chemokines that support antigen presentation and activation of both innate and adaptive immune responses.[110] Natural killer (NK) cell-derived EVs carry cytotoxic effector molecules, including perforin, granzymes, Fas ligand, and NKp46, enabling them to induce oxidative stress, DNA damage, and apoptosis in tumor cells.[111] T cell-derived EVs contribute to antitumor immunity through multiple mechanisms. EVs released from activated $CD8^+$ T cells can suppress tumor invasion and metastatic dissemination, whereas EVs secreted by IL-2-stimulated $CD4^+$ T cells are enriched in regulatory miRNAs, including miR-25-3p, miR-155-5p, miR-215-5p, and miR-375. Through paracrine transfer of these molecules, $CD4^+$ T cell-derived EVs enhance $CD8^+$ T cell proliferation and cytotoxic activity, strengthening anti-tumor immune responses.[109]

Using EVs as Drug Delivery Systems

Conventional therapeutic modalities for LC, including chemotherapy, radiotherapy, immunotherapy, and gene-based therapies, are frequently limited by inefficient drug accumulation at tumor sites and dose-limiting systemic toxicity. Although increasing drug dose may enhance local therapeutic exposure, it often results in undesirable off-target effects in healthy tissues and organs. Consequently, the development of safe and efficient drug delivery platforms has become a major focus of contemporary cancer research. Among emerging delivery systems, EVs have attracted considerable attention because of their intrinsic biocompatibility, low immunogenicity, prolonged circulation stability, and natural ability to mediate intercellular cargo transfer. Compared with conventional nanocarriers, including liposomes, polymeric nanoparticles, mesoporous materials, and metallic nanoparticles, EVs possess unique biological properties that facilitate cellular uptake and improve delivery efficiency within complex tumor microenvironments. As a result, EV-based drug delivery platforms have become an increasingly active area of investigation in LC therapy (Figure 4).

Chemotherapy

LC is broadly classified into SCLC and NSCLC based on histopathological characteristics.[112] Surgical resection remains the preferred treatment for early-stage disease, whereas chemotherapy and radiotherapy constitute the principal therapeutic approaches for advanced-stage tumors.[113] Platinum-based regimens, particularly combinations of etoposide with cisplatin or carboplatin, continue to serve as standard treatment options for many patients with LC.[114] However, their clinical efficacy is frequently constrained by poor tumor selectivity, limited bioavailability, systemic toxicity, and the emergence of drug resistance.[115] EV-based delivery systems have shown potential for overcoming several of these limitations. In an animal study conducted by Cui et al, cisplatin-loaded EVs derived from the breast cancer cell line MDA-MB-231 were preferentially internalized by A549 lung cancer cells following co-culture. No significant cytotoxicity was observed in non-malignant 293T or dendritic cells (DCs). In vivo administration of drug-loaded EVs significantly reduced tumor burden and prolonged survival in tumor-bearing mice, supporting the feasibility of EVs as targeted chemotherapy carriers.[116] In addition to enhancing drug delivery, EVs may contribute to overcoming chemotherapy resistance. Jin et al reported that airway administration of paclitaxel-loaded macrophage-derived exosomes significantly increased the sensitivity of Lewis lung carcinoma cells expressing high levels of P-glycoprotein (Pgp) to paclitaxel treatment. Furthermore, cellular uptake of exosome-loaded paclitaxel was approximately 30-fold higher than that observed with paclitaxel-loaded liposomes and polystyrene nanoparticles, highlighting the superior delivery efficiency of EV-based systems.[117]

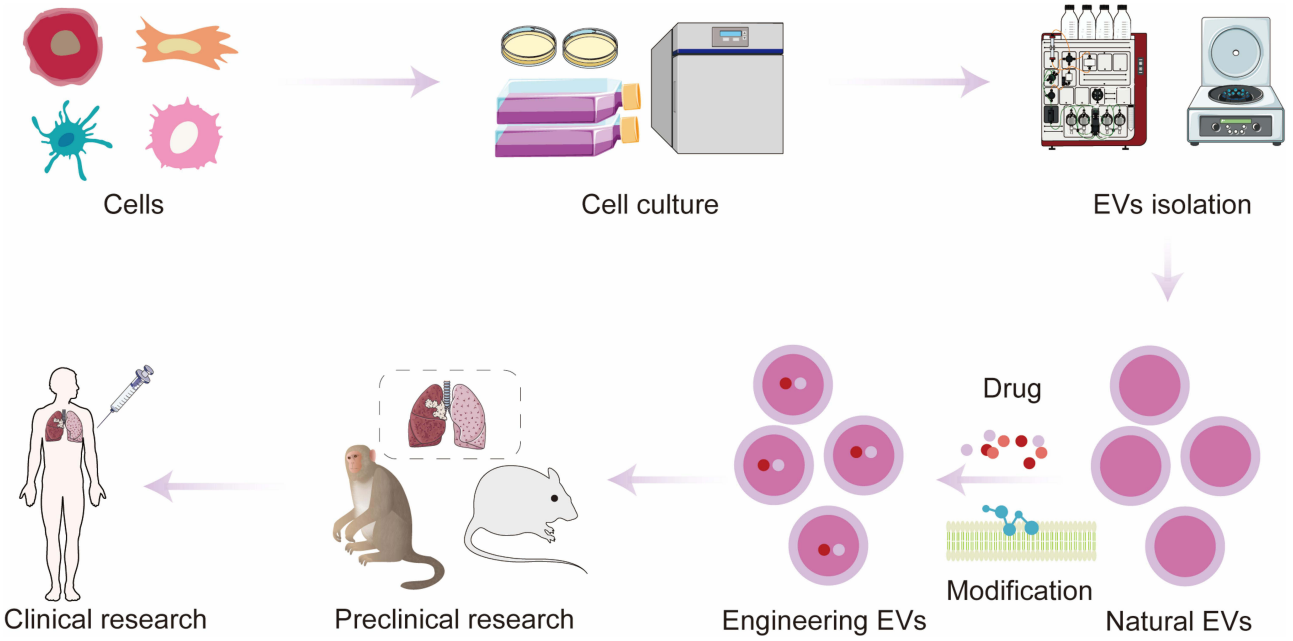

Figure 4 EVs have emerged as promising nanocarriers for targeted therapeutic delivery against lung cancer, with substantial efforts advancing their clinical translation. To generate clinical-grade EVs, these nanoparticles are initially purified from the conditioned culture medium of donor cells via three mainstream isolation approaches: ultracentrifugation, size-exclusion chromatography, and microfluidic separation. Following isolation, therapeutic cargoes encompassing chemotherapeutics, nucleic acids, and functional proteins are loaded into EVs using either endogenous biogenesis-mediated packaging or exogenous post-isolation loading techniques. Genetic manipulation of parent cells and chemical bioconjugation (eg, click chemistry) are two primary strategies to engineer EVs surface moieties, which drastically improves their specific tropism toward lung tumor lesions. Accumulating preclinical investigations across mouse, rabbit, and non-human primate animal models have validated the potent anti-tumor performance of EV-based therapeutics, laying robust preclinical evidence to support the clinical advancement of EVs delivery systems for lung cancer intervention.

Radiation Therapy

Radiotherapy represents a fundamental component of LC management, with a substantial proportion of patients receiving radiation treatment during the course of their disease.[118] Stereotactic ablative radiotherapy (SABR) has demonstrated favorable outcomes in patients with medically inoperable early-stage disease, while integration of radiotherapy with systemic treatment has improved progression-free survival (PFS) in selected patients with advanced-stage LC.[119,120] Despite these clinical benefits, several factors limit the effectiveness of radiotherapy. Tumor heterogeneity, hypoxia, physiological respiratory motion, and the proximity of radiosensitive organs increase the difficulty of delivering therapeutic radiation while minimizing damage to surrounding tissues.[121] Prolonged radiation exposure can induce adaptive resistance mechanisms involving altered DNA damage responses, cell-cycle regulation, signaling pathway activation, and metabolic reprogramming.[122] Emerging evidence suggests that EVs participate in radiotherapy resistance and may serve as vehicles for radiosensitizing agents. For example, irradiation of A549 cells has been shown to increase the expression of miR-1246, which promotes radioresistance through suppression of death receptor 5 (DR5) and facilitates resistance transfer to neighboring cells.[123] Radiation exposure has also been reported to stimulate EV release and influence immune-cell polarization within the tumor microenvironment.[124] To improve radiosensitivity, investigators have explored EV-mediated delivery of therapeutic molecules. Microvesicles derived from bone marrow MSCs carrying miR-101-3p were shown to enhance the radiosensitivity of NSCLC cells through suppression of enhancer of zeste homolog 2 (EZH2) and subsequent inhibition of the PI3K/AKT/mTOR signaling pathway.[125] Similarly, Liang et al developed M1 macrophage-derived EVs loaded with catalase (CAT), anti-PD-L1 (programmed death ligand-1) nanobodies, and DNA damage repair inhibitors (DDRi). This multifunctional platform alleviated tumor hypoxia, impaired DNA repair, relieved immune suppression, and enhanced radiotherapeutic efficacy through coordinated modulation of multiple resistance mechanisms.[126]

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Immunotherapy

Immunotherapy has transformed the therapeutic landscape of LC by harnessing host immune responses to recognize and eliminate malignant cells.[12] Immune checkpoint inhibitors targeting PD-1, PD-L1, and anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) have demonstrated substantial clinical benefits and are now incorporated into standard treatment strategies for multiple LC subtypes.[127] The PD-1/PD-L1 axis plays a central role in tumor immune evasion. Binding of PD-L1 expressed on tumor cells to PD-1 receptors on T lymphocytes suppresses T-cell activation, proliferation, effector function, and survival, weakening anti-tumor immunity.[128] Therapeutic blockade of this pathway can restore immune activity and inhibit tumor progression.[129] However, systemic administration of immune checkpoint inhibitors may be associated with immune-related adverse events (irAEs), including autoimmune complications. To improve therapeutic specificity and reduce systemic toxicity, EVs have increasingly been investigated as carriers for immunotherapeutic agents and cancer vaccines. A Phase II clinical study demonstrated that EVs derived from IFN-γ-stimulated DCs enhanced NK cell and T cell responses and were associated with improvements in PFS and overall survival (OS) among patients with LC.[130] Similarly, EVs derived from $CD4^+$ T cells and enriched with miR-25-3p, miR-155-5p, miR-215-5p, and miR-375 have been shown to stimulate $CD8^+$ T-cell activity without promoting regulatory T-cell expansion, supporting their potential application in cancer immunotherapy.[131] Advanced EV engineering strategies have further expanded the therapeutic potential of these vesicles. Huang et al developed chimeric antigen receptor T-cell-derived exosomes co-expressing mesothelin (MSLN) and PD-L1 targeting single-chain variable fragments. In preclinical models, these engineered EVs enhanced anti-tumor activity and prolonged survival. To address limitations associated with drug loading and delivery efficiency, the investigators subsequently integrated lung-targeting liposomes with engineered exosomes to create the Lip-CExo platform, combining the advantages of both delivery systems.[132] In another study, Ning et al developed an EV-based multifunctional therapeutic platform by encapsulating indocyanine green-loaded manganese dioxide nanoparticles within anti-PD-L1-modified exosomes. Within the acidic tumor microenvironment, controlled release of anti-PD-L1 enhanced T-cell activation, while manganese dioxide relieved hypoxia through catalytic oxygen generation. Upon near-infrared (NIR) irradiation, indocyanine green generated reactive oxygen species, resulting in synergistic integration of immunotherapy and photodynamic therapy for LC treatment.[133] These studies demonstrate the versatility of EVs as drug delivery platforms capable of integrating chemotherapy, radiotherapy sensitization, immunotherapy, and combination treatment strategies. Continued development of multifunctional EV-based systems that incorporate targeted delivery, stimulus-responsive release mechanisms, and complementary therapeutic modalities is expected to further advance precision treatment approaches for LC (Table 2).

Existing Bottlenecks in Clinical Translation

Despite the considerable promise of EVs in LC diagnosis and therapy, their clinical translation remains largely confined to experimental and preclinical settings.[135] Several scientific, technological, and manufacturing challenges continue to impede the development of standardized, scalable, and clinically applicable EV-based platforms.[136] Major obstacles include EV heterogeneity, limitations in large-scale production and purification, storage instability, suboptimal drug-loading methodologies, the absence of standardized quality-control frameworks, and insufficient in vivo tracking technologies.[137] One of the most significant challenges is the intrinsic heterogeneity of EVs populations. The molecular composition, biological activity, and functional properties of EVs are strongly influenced by the cellular origin, physiological state, and microenvironmental conditions of donor cells. Consequently, EVs derived from different sources often exhibit substantial variability in cargo composition and biological behavior. TDEs, while possessing inherent tumor-targeting capabilities, may also carry oncogenic proteins, pro-angiogenic factors, and immunosuppressive molecules that could potentially promote tumor progression and immune escape.[138] Similarly, EVs derived from stem cells and immune cells may exert either tumor-suppressive or tumor-promoting effects depending on the biological status of the parent cells, raising important concerns regarding therapeutic consistency and safety.[13,139] Additional complexity arises from the diversity of EVs subtypes. Exosomes, microvesicles, and apoptotic bodies differ considerably in their biogenesis, physicochemical characteristics, biodistribution, and clearance kinetics. For example, phosphatidylserine exposure on the surface of microvesicles and apoptotic bodies facilitates rapid recognition and elimination by the

Table 2 EVs as Drug Delivery Systems in the Treatment of LC

Combination Therapy Strategies	Sources of EVs	Level of Evidence	Loaded Goods	EV Carrier Role	Therapeutic Effect	Ref.
Chemotherapy	Breast cancer cell line MDA-MB-231	In vivo mouse experiment	Cisplatinum (chemistry)	Improved targeting and reduced drug toxicity	Significantly reduces tumour volume and increases survival in hormonal mice	[116]
Chemotherapy	Macrophage	In vivo mouse experiment	Paclitaxel	Reducing drug resistance and increasing drug sensitivity	Significantly inhibited the growth of LLC cancer cells	[134]
Radiotherapy	Bone marrow mesenchymal stem cells (MSC)	In vivo mouse experiment	miR-101-3p	Targeting Zeste enhancer homologue 2 (EZH2) to inhibit activation of the PI3K/AKT/mTOR signalling pathway	Modulation of DNA damage repair and autophagy levels to promote radiotherapy sensitivity in non-small cell lung cancer	[125]
Radiotherapy	M1 Macrophages	Cell experiments / in vivo mouse experiments	Catalase (CAT), anti-PD-L1 (programmed death ligand-1), DNA damage repair inhibitor (DDRi)	Improving drug tumour targeting	Remodelling of the tumour suppressor microenvironment to inhibit tumour survival	[126]
Immunotherapy	Antigen Receptor-T (CAR-T) Cells	Cell experiments / in vivo mouse experiments	Mesothelin (MSLN) and PD-L1	Improving drug tumour targeting	Prolonged survival of hormonal mice in the CT-26 metastatic lung cancer model	[132]
Immunotherapy and photodynamic therapy	Dendritic cell	In vivo mouse experiments	PD-L1 monoclonal antibody, hollow manganese dioxide (MnO_2) nanospheres (NPs)	Improve drug tumour targeting and reduce drug toxicity and side effects	Controlled release of anti-PD-L1 from acidic TME activates T-cell responses and O_2 generation of monoclinic oxygen ($^1O_2$) to kill tumour cells under 808 nm near-infrared (NIR) irradiation	[133]

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mononuclear phagocyte system, whereas exosomes generally demonstrate greater suitability for drug delivery applications.[140] However, universally accepted criteria for selecting specific EV subtypes for clinical applications remain unavailable. Large-scale production of clinical-grade EVs also presents a major challenge. Differential ultracentrifugation remains the most widely employed isolation method, yet it often yields preparations contaminated with protein aggregates and cellular debris and may compromise vesicle integrity through exposure to high centrifugal forces.[141] Size-exclusion chromatography preserves EVs structure more effectively but is limited by relatively low throughput.[142] Immunoaffinity-based approaches offer high specificity but are associated with elevated costs, restricted marker availability, and potential alterations in EVs biological activity.[143] Current isolation strategies suffer from limitations in yield, purity, reproducibility, and scalability, restricting their suitability for industrial and clinical implementation. Storage and long-term preservation represent another critical bottleneck. Current consensus recommends storage at 4 °C for short-term use and −80 °C for long-term preservation.[144] However, prolonged storage under either condition can adversely affect EVs quality. Reductions in particle concentration, RNA content, protein stability, and surface marker integrity have been reported, together with alterations in physicochemical properties such as zeta potential.[145] Furthermore, ice-crystal formation during freezing may disrupt membrane integrity and compromise biological function.[145] Although cryoprotectants such as trehalose and dimethyl sulfoxide have shown potential in mitigating cryoinjury,[134] and lyophilization has been explored as an alternative preservation strategy, long-term stability remains a significant unresolved issue.[146] Drug loading technologies also require substantial optimization. Existing approaches, including passive incubation, electroporation, sonication, and lipid-based transfection, frequently exhibit limited loading efficiency and may compromise EVs integrity or biological activity.[34,147] Physical loading procedures can induce membrane disruption and cargo leakage, whereas chemical modifications may alter surface proteins and impair natural targeting properties. Moreover, the diverse physicochemical characteristics of therapeutic cargoes, including small molecules, nucleic acids, and proteins, make the development of universally applicable loading strategies particularly challenging.[148–150] Variability introduced during loading procedures further complicates batch standardization and large-scale manufacturing.[151] Another major limitation is the absence of standardized quality-control systems. Current characterization approaches largely focus on particle size, concentration, morphology, surface markers, and loading efficiency. While informative, these parameters do not adequately assess critical attributes such as structural integrity, cargo stability, biodistribution, pharmacokinetics, targeting efficiency, and biological activity.[152] The establishment of comprehensive and universally accepted quality standards remains essential for regulatory approval and clinical implementation. Finally, technologies for monitoring EVs biodistribution and fate in vivo remain relatively limited. Current strategies primarily rely on direct labeling methods using lipophilic fluorescent dyes such as KH26, DiD, DiR, or radioactive isotopes or bioluminescent reporter systems.[153] Although these techniques have advanced understanding of EVs trafficking, challenges related to signal persistence, labeling specificity, imaging sensitivity, and quantitative interpretation continue to hinder accurate assessment of EV behavior in vivo. EVs heterogeneity, manufacturing challenges, preservation instability, inefficient drug-loading technologies, inadequate quality-control frameworks, and limitations in in vivo tracking collectively represent the principal barriers to clinical translation. Addressing these issues will be critical for the successful integration of EV-based technologies into future LC diagnostic and therapeutic strategies (Table 3).

Conclusion

EVs have emerged as pivotal regulators of LC initiation, progression, and therapeutic response. Through the transfer of diverse bioactive cargoes, EVs mediate extensive intercellular communication within the tumor microenvironment and influence multiple hallmarks of malignancy, including angiogenesis, EMT, immune evasion, metastatic dissemination, and therapeutic resistance. Consequently, EVs occupy a central position in the molecular networks that drive LC progression. From a diagnostic perspective, EVs offer several advantages over liquid biopsy components, including CTCs and ctDNA. Their abundance in biological fluids, structural stability conferred by a lipid bilayer membrane, and capacity to protect nucleic acids and proteins from degradation make them attractive candidates for non-invasive disease detection and monitoring. The molecular cargo carried by EVs provides valuable information regarding tumor biology and has demonstrated considerable potential as a source of biomarkers for early diagnosis, prognostic evaluation, treatment monitoring, and assessment of therapeutic resistance. EVs represent a versatile platform with multiple potential applications. Strategies aimed at suppressing the production or activity of

Table 3 Clinical Translation Constraints of EV-Mediated Drug Delivery Strategies for LC Treatment

Transformation Constraint Stage	Current Conventional Strategies and Principles	Current Strategy Flaws	Optimization Strategy	References
EV large-scale purification	Ultracentrifugation method: The sedimentation rates of nanoparticles differ under different centrifugal forces	Centrifugal force destroys the complete structure of the EV, takes a long time, and the equipment is expensive	Reduce the centrifuge speed, shorten the duration of each centrifugation, and avoid high-pressure shear from damaging the vesicle membrane; operate at 4°C throughout to reduce EVs protein degradation.	[13]
	Immunoaffinity separation: Principle of antigen-antibody specific binding	High cost, low throughput, antibody contamination	Use multiple labeled antibodies; improve antigen-antibody binding efficiency	[140]
	Size exclusion chromatography: Using differences in molecular sieve pore size to fractionally elute and separate EVs based on molecular size	Low yield, time-consuming, sample dilution	Control the sample loading volume and flow rate, reduce EV dilution and retention, operate at low temperature and away from light throughout, and maintain vesicle integrity	[141]
	Ultrafiltration: Screening different nanoparticles through a membrane	Shear force-damaged EVs and protein co-contamination	Select a 100–300 kDa ultrafiltration membrane to retain EVs and allow free small molecule proteins to pass through the membrane, maintaining a low temperature of 4°C throughout to inhibit EV membrane protein degradation and aggregation.	[140]
	Microfluidic technology (separating EVs through microchannel structures and external forces (hydrodynamic/ electric fields))	The sample processing volume is low, the cost of equipment consumables is high, and microchannel blockage is prone to occur.	Optimize microchannel design; integrate multiple forces; develop large-scale equipment; simplify chip manufacturing	[144]
EVs Cargo Loading	Co-incubation: EV passive adsorption / penetration	Drug loading has low loading efficiency and long incubation time	Choose donor cells in the logarithmic growth phase, control temperature duration, and adjust drug concentration.	[146]
	Transfection: Carrier-mediated drug gene entry into parent cells,	Secreting drug-loaded EVs is complex and potentially cytotoxic.	Optimize plasmid ratios and choose low-toxicity transfection reagents.	[147]
	Electroporation: The electric field-induced poration causes gaps in the vesicle membrane, allowing drugs to diffuse inside.	The electric field causes damage to the EVs membrane, leading to drug inactivation.	Optimizing the adaptation of voltage pulses and hypotonic buffers, and controlling the physical and chemical properties of the buffer, can reduce electroporation-induced membrane damage and improve loading efficiency.	[148]
	Ultrasound treatment: ultrasonic waves break membranes, drugs penetrate and then membranes self-repair.	Ultrasound can cause damage to the intact structure of EVs.	Optimize ultrasound amplitude, frequency, and treatment duration.	[149]

(*Continued*)

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Table 3 (Continued).

Transformation Constraint Stage	Current Conventional Strategies and Principles	Current Strategy Flaws	Optimization Strategy	References
EVs drug-loading characterization strategy	Characterization of structural integrity: Transmission Electron Microscopy (TEM)	Drying and gold sputtering deformation, unable to observe internal structure	Cryo-preparation, low vacuum mode, shorten drying time	[150]
	Characterization of structural integrity: Cryo-EM	Cryo-electron microscopy equipment is expensive, has low throughput, and sample preparation is difficult.	Thin ice layer sample preparation, low-dose imaging, batch rapid sample preparation.	[151]
	Evaluation of Protein Cargo Loading Efficiency: Western Blotting (WB), Enzyme-Linked Immunosorbent Assay (ELISA), and Flow Cytometry	WB is only semi-quantitative and has a complicated procedure; ELISA is easily affected by contaminating proteins and cannot distinguish protein localization; flow cytometry has a low detection rate for small-sized EVs. None of the three methods can simultaneously quantify and distinguish proteins inside and outside vesicles.	Complementary verification using three combined methods: WB with internal reference correction to achieve relative quantification, ELISA with blank controls to reduce interference from contaminant proteins and for membrane/intracellular component detection, and flow cytometry using specific fluorescent labeling to enhance the detection signal of small EVs, allowing multi-dimensional synchronous evaluation of protein loading and distribution.	[152]
EVs storage	−80 °C ultra-low temperature storage: Low temperatures inhibit enzymatic digestion, slow down vesicle membrane degradation, and maintain the long-term stability of EV structure and protein activity.	Ice crystals pierce the vesicles, repeated freeze-thaw damages activity, and the concentration, purity, and Zeta potential of EVs decrease	Add cryoprotectants such as trehalose and dimethyl sulfoxide (DMSO) during freezing, aliquot into single-use portions, and avoid repeated freeze-thaw cycles	[144]
	Vacuum freeze-drying: removing free water through low-temperature vacuum sublimation to maintain the stability of the EV membrane structure	Integrity of lyophilized stress-damaged EVs, protein inactivation, easy aggregation	Add lyophilization protectant, gradual cooling, low-temperature vacuum freeze-drying	[134]

pathogenic EVs may help disrupt tumor-promoting communication networks within the tumor microenvironment. Functional EVs derived from stem cells or immune cells offer opportunities for cell-free therapeutic interventions. Moreover, the development of EV-based drug delivery systems has created new possibilities for improving the precision, efficacy, and safety of radiotherapy, chemotherapy, immunotherapy, and nucleic acid-based treatments. Despite these advances, substantial challenges continue to impede clinical translation. Key limitations include intrinsic EVs heterogeneity, difficulties in standardized large-scale production, instability during long-term storage, limited drug-loading efficiency, the absence of universally accepted quality-control standards, and insufficient understanding of EVs biodistribution and targeting behavior in vivo. Overcoming these challenges will require coordinated efforts across basic research, translational medicine, and regulatory science. Future investigations should focus on elucidating the molecular mechanisms governing EVs biogenesis and function in LC, identifying robust multi-omics biomarker signatures, and establishing standardized workflows for EV isolation, characterization, and clinical application. Advances in EVs engineering, targeted cargo delivery, and multimodal therapeutic design may further enhance their clinical utility. Equally important will be the development of rigorous quality-control frameworks and safety assessment

standards to support regulatory approval and clinical adoption. With continued technological and methodological progress, EV-based approaches have the potential to become an integral component of precision oncology and to contribute substantially to improved outcomes for patients with LC.

Abbreviation

SCLC, Small cell lung cancer; NSCLC, Non-small cell lung cancer; LUAD, Lung adenocarcinoma; PI3K, Phosphatidylinositol 3-kinase; EVs, Extracellular vesicles; MVB, Multivesicular bodies; MVs, Microvesicles; ESCRT, Endosomal sorting complex required for transport; TME, Tumor microenvironment; ECs, Endothelial cells; HUVECs, Human umbilical vein endothelial cells; KLF, Krüppel-like factor; EMT, Epithelial-mesenchymal transition; ALDH, Aldehyde dehydrogenase; CSCs, Cancer stem cells; APCs, Antigen-presenting cells; TAMs, Tumor-associated macrophages; DCs, Dendritic cells; CAFs, Cancer-associated fibroblasts; CT, Computed tomography; ctDNA, Circulating tumor DNA; cfDNA, Cell-free DNA; TEM, Transmission electron microscopy; SEM, Scanning electron microscopy; HIPK3, Homologous domain interaction protein kinase 3; BALF, Bronchoalveolar lavage fluid; EGFR, Epidermal growth factor receptor; COPD, Chronic obstructive pulmonary disease; LLC, Lewis lung cancer; NSMase, Neutral sphingomyelinase; DCLK1, Dual corticotrophin-like kinase 1; NK, Natural killer; PD-1, Programmed cell death 1; PD-L1, Programmed cell death ligand 1; TDEs, Tumor-derived EVs.

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Disclosure

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