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REVIEW

Engineered MSC-Exosomes Delivering miRNAs for Respiratory Disease Diagnostics and Therapy: Opportunities and Challenges

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Abstract: Respiratory diseases pose a severe threat to global health, with notable limitations in current diagnosis and treatment, such as insufficient sensitivity of diagnostic tools and a lack of effective targeted therapies. Due to their highly efficient information transmission capabilities and excellent safety profile, exosomes carrying non-coding RNA, particularly microRNA (miRNA), are increasingly attracting attention. Compared with free miRNAs, exosomes can protect miRNAs from nuclease degradation, prolong their circulation time in the body, thereby improving the stability and bioavailability of miRNAs. At the same time, they can also address the major bottleneck in the clinical application of miRNAs, including low in vivo delivery efficiency, poor stability, lack of targeting specificity, and off-target effects. Increasing evidence indicate that miRNAs play a significant role in respiratory diseases, including targeting multiple signaling pathways, regulating inflammation and oxidative stress, influencing tumor growth and apoptosis, and participating in tissue damage and repair, thus holding promising prospects for diagnosis and treatment in respiratory diseases. MSC-derived exosomes exhibit low tumorigenic risk because they originate from adult stem cells with limited differentiation ability, have low immunogenicity, and do not highly express major histocompatibility complex class II (MHC-II) molecules, making them suitable for allogeneic use. To enhance the therapeutic efficacy and specificity of exosomes in respiratory diseases, engineering modifications of MSC-exosomes (MSC-exos) are crucial. Current methods for engineering MSC-exos primarily include cargo loading and surface modification to improve therapeutic efficacy and targeting specificity. Through these engineering methods, more precise miRNA delivery can be achieved, reducing the side effects of traditional treatments and improving treatment efficacy. Although MSC-exos demonstrate significant potential in treating respiratory diseases, their clinical translation is hindered by critical hurdles, including individual differences in therapeutic efficacy, insufficient miRNA targeting specificity, challenges in large-scale production, and potential immunogenicity risks. To accelerate clinical application, future research should prioritize optimizing engineered targeting strategies (eg, precision surface modification), enhancing large-scale preparation efficiency of functional MSC-exos, and validating their long-term safety and efficacy in multi-center studies. At present, the good manufacturing practice (GMP) production process of MSC-exos has been established. Early clinical trials (Phase I/II) have shown its potential in respiratory diseases such as pulmonary fibrosis without serious adverse reactions. However, it has not yet been approved for clinical transformation and still faces challenges such as large-scale targeting and safety. Overall, MSC-exos carrying miRNAs show great promise in the treatment of respiratory diseases, but their true clinical application still requires more systematic research and validation.

Keywords: lung disease, extracellular vesicles, exosome, microRNA, engineering, diagnosis, therapy

Introduction

Respiratory diseases, including asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, and lung cancer, continue to have high incidence and mortality rates.[1] The diagnosis and treatment of respiratory diseases face two

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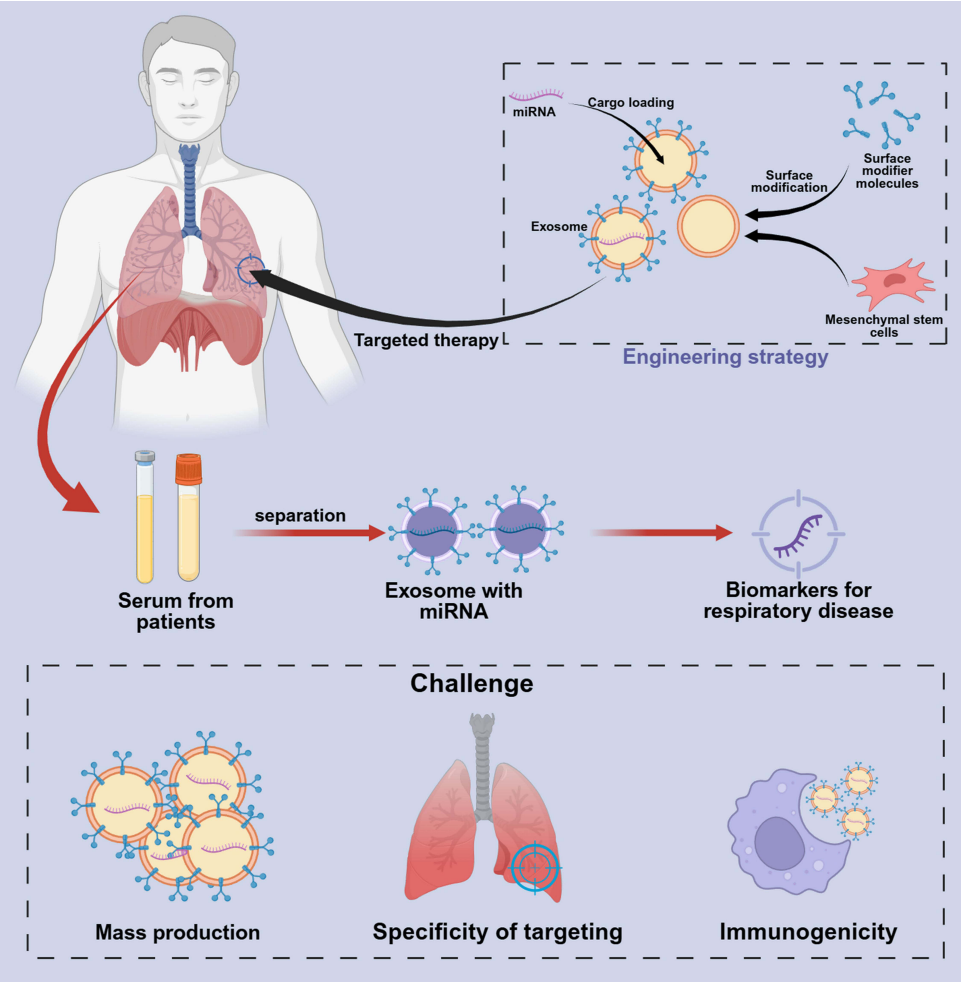
Graphical Abstract



major challenges: first, the lack of sensitive and specific early diagnostic biomarkers, leading to diseases often being identified only in advanced stages;[2] second, current treatment methods primarily focus on symptom relief and are unable to reverse the pathological process, lacking effective strategies for precisely regulating key disease pathways, particularly inflammation regulation, tissue repair and regeneration, and immune microenvironment modulation, which urgently require precise methods.

Stem cells and their vesicles, due to their ability to act through mechanisms such as regeneration, immune regulation, and multi-target synergistic effects, hold promise to overcome the limitations of traditional therapies that can only delay disease progression. They particularly demonstrate potential for reversing damage in fibrotic and chronic inflammatory diseases such as interstitial lung disease and COPD.[3,4] The most extensively studied are exosomes derived from mesenchymal stem cells (MSCs). MSCs are a type of adult stem cell with self-renewal and multi-lineage differentiation potential, primarily found in tissues such as bone marrow, fat, and umbilical cord.[5–8] MSCs primarily release active components such as extracellular vesicles (EVs) through paracrine action rather than direct differentiation.[9] EVs are nanoscale membrane-bound vesicles actively secreted by cells, widely involved in intercellular communication and possessing important physiological and pathological functions. Based on their diameter, EVs are classified into apoptotic bodies, macrovesicles, and exosomes.[7,10] Among these, MSC-exosomes (MSC-exos) are widely studied for their regenerative and immunomodulatory effects.[11] In addition, since MSCs are adult stem cells with limited differentiation ability, the risk of tumorigenesis is low.[12] Moreover,

MSCs do not highly express MHC-II molecules and have low immunogenicity, making them suitable for allogeneic use.[13] Therefore, MSC-exosomes (MSC-exos) have a small molecular weight, low immunogenicity, and can carry active substances such as proteins and Non-coding RNA (ncRNA),[14,15] thereby playing important roles in inflammation inhibition, tissue repair, immune regulation, and angiogenesis.[16,17]

ncRNA is a group of RNA molecules that have garnered significant attention in recent years for their involvement in cellular basic functions, transcribed from non-coding regions of the genome.[18] Although they do not directly participate in protein synthesis, they play a crucial role in gene expression regulation, cellular physiology, tissue repair, and pathological processes.[19] Based on their length and function, ncRNA can be classified into microRNA (miRNA), long non-coding RNA (lncRNA), ribosomal RNA (rRNA), and others. One of the key components of exosomes is ncRNA. Among these, the role of miRNA in respiratory diseases is the most well-defined.[20] MiRNA is a class of small non-coding RNA molecules approximately 18–25 nucleotides in length that regulate gene expression and influence cellular function. In MSC-exos, miRNA is encapsulated within exosomes and acts as an important biological messenger, participating in intercellular communication and disease regulation via exosomes.[21] MSC-exos-derived miRNAs undergo three stages from production to secretion: biogenesis, miRNA sorting and packaging, and release and transport.[22] Once released and entering target cells, they can broadly participate in gene expression regulation through post-transcriptional regulatory mechanisms.[23] Their mechanism of action is complex, primarily regulating gene expression by binding to the messenger RNA (mRNA) of target genes, thereby achieving gene silencing (inhibition) or activation. Additionally, miRNAs can target signaling pathways to regulate inflammation and oxidative stress, promoting or inhibiting tumor growth and metastasis,[24] and participating in tissue repair processes.[25] Specifically, miRNAs bind to the 3'-untranslated region of target mRNAs, leading to translation inhibition or mRNA degradation, thereby affecting cellular functions (Figure 1).[26] Notably, miRNAs can be precisely packaged and stably transported by exosomes into the body fluid circulation, enabling these nanoscale "messengers" to exert long-range signaling effects both within and outside the respiratory system. Numerous studies have shown that miRNA derived from exosomes can regulate molecular pathways and cellular pathological processes in respiratory diseases, including inflammation, apoptosis, fibrosis, and oxidative stress, through signal transduction, and thus can serve as early diagnostic markers for disease onset.[27] Exosomes are not only natural carriers of miRNA but also play dual diagnostic and therapeutic roles in respiratory diseases.

Engineered MSC-exos represent a major breakthrough from cell therapy to cell-free therapy.[28] They not only perfectly inherit the therapeutic characteristics of MSC but also avoid the risks associated with cell transplantation.[29] They possess the advantages of targeting, stability, and multifunctionality.[30] To enable its application to achieve tissue repair, immune regulation, and disease treatment in a safer, more precise, and more controllable way, providing brand-new solutions for a variety of difficult-to-treat diseases, it is expected to lead the future development direction of regenerative medicine and precision medicine.[31] The engineering methods of exosomes include large-scale production optimization (such as enhancing secretion through genetic engineering regulation of molecules like the RAB family and optimizing 3D culture),[32] specific modification (through gene modification of donor cells, etc),[33] and loading engineering (such as miRNA encapsulation technology, and electroporation).[34] Engineering can increase the yield and purity of MSC-exos to meet clinical needs, precisely deliver therapeutic miRNAs and prevent their degradation, enhance targeting to specific tissues and cells of the respiratory system, and optimize the functional properties of exos (such as enhancing anti-inflammatory, anti-fibrotic, and promoting tissue repair).[35] This will further provide support for the accurate diagnosis and effective treatment of respiratory diseases. Their core advantage lies in combining the biocompatibility of natural exosomes with the flexibility of engineered design, offering a novel cell-free therapeutic strategy for pulmonary diseases.[36,37] By engineering modifications to MSC-exos and mass-producing exosomes enriched with specific miRNAs, clinical translation can be achieved. In this review, we will provide a detailed overview of existing engineering methods for MSC-exos, the miRNAs carried by MSC-exos, and their latest advances in the diagnosis and treatment of pulmonary diseases. We will also summarize existing achievements and future challenges, and present our perspectives and future expectations.

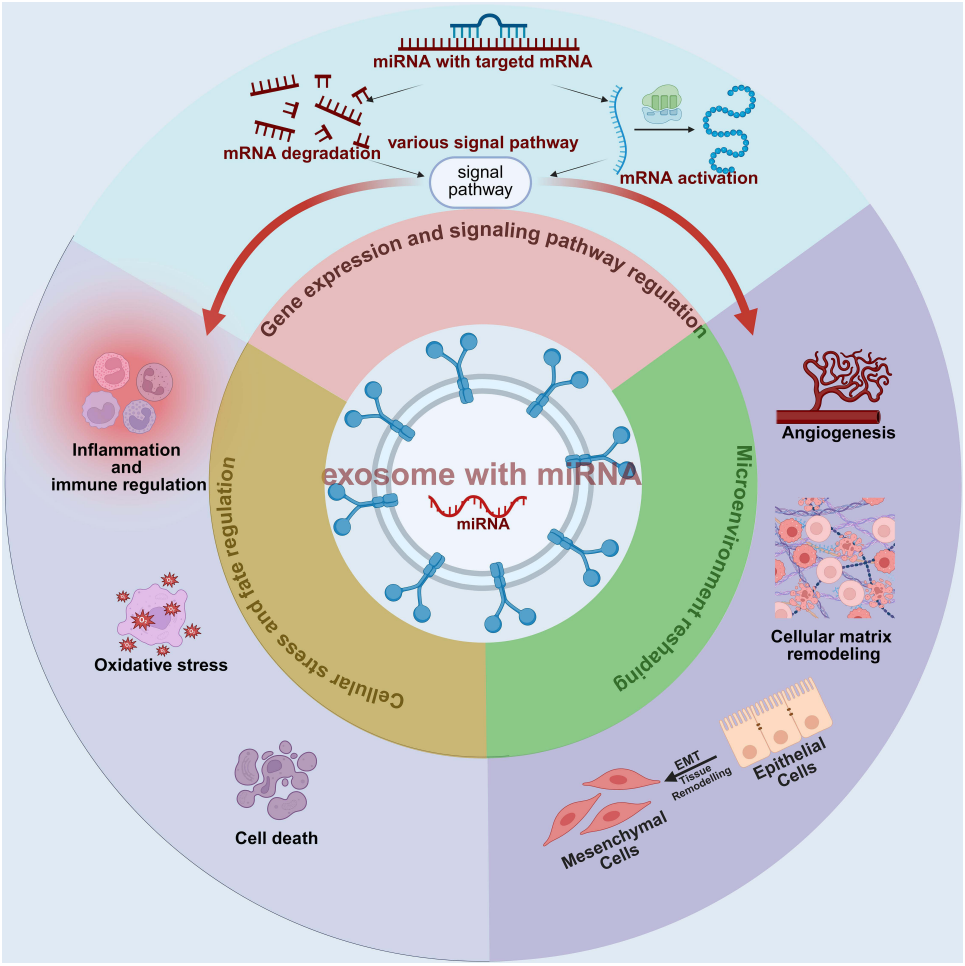


Figure 1 The mechanism of action of miRNA derived from exosomes. miRNAs carried by exosomes can participate in processes such as gene expression and signaling pathways, cell stress and fate regulation, and microenvironment regulation. Specifically, miRNAs can target and bind to mRNAs, thereby causing mRNA activation or degradation, affecting signaling pathways, and subsequently influencing cell immunity and immune regulation, oxidative stress and cell death, as well as vascular remodeling, interstitial remodeling, and epithelial-mesenchymal transition.

Research on the Functional Mechanisms of miRNA in the Respiratory System and Diagnostic Markers in Exosomes

miRNAs have emerged as pivotal regulators in the pathogenesis of respiratory diseases, while exosome-encapsulated miRNAs (exo-miRNAs) further stand out as promising diagnostic tools[38]—these two roles are inherently interconnected, with mechanistic studies laying the foundation for clinical diagnostic applications. On the one hand, miRNAs mediate a spectrum of pathological processes in the respiratory system: they regulate key signaling pathways involved in inflammation (eg, NF-κB, NLRP3), oxidative stress (eg, NFE2L2), tissue remodeling (eg, TGF-β/Smad), and cell fate (eg, apoptosis, epithelial-mesenchymal transition).[39,40] These mechanistic roles determine that miRNAs exhibit disease-specific expression patterns—for instance, a miRNA that promotes airway epithelial barrier disruption in chronic inflammation will be abnormally upregulated in diseases characterized by epithelial damage.[41] Such specificity is the core premise for miRNAs to serve as diagnostic biomarkers. On the other hand, exosomes act as "natural carriers" that protect miRNAs from degradation in bodily fluids, a property that is critical for their diagnostic applicability.[42] Specifically, miRNAs derived from exosomes not only regulate interactions between bronchial epithelial cells, immune cells, and interstitial cells but also exist in detectable forms in blood, sputum, or exhaled condensate.[43] With their specific expression profiles and high stability, these exosome-derived miRNAs offer new non-invasive diagnostic opportunities

for early identification of various respiratory diseases such as asthma, COPD, pulmonary fibrosis, and lung cancer, and lay the molecular foundation for precise classification and treatment monitoring.[44]

In respiratory diseases, currently, commonly used biomarkers (such as exhaled breath biomarkers and serum markers like IgE) are easily influenced by various factors and struggle to distinguish between different disease phenotypes. Ideal biomarkers should be easy to measure, non-invasive, and highly specific and sensitive. Given the low immunogenicity of exosomes, miRNAs within exosomes are highly stable and easily accessible due to the protection provided by the exosome membrane, making them promising candidates for early, non-invasive diagnostic markers for respiratory diseases. Based on a comprehensive literature review, we have compiled a list of exosomal miRNAs that include their mechanisms of action and diagnostic potential in respiratory diseases (Table 1 and S1).

Furthermore, the aforementioned characteristics—namely specificity conferred by mechanisms and detectability guaranteed by exosomes—collectively constitute the core advantage of exo-miRNAs as diagnostic biomarkers. However, screening diagnostic biomarkers without combining mechanism studies often lacks causal support (for example, it is impossible to distinguish whether changes in miRNA expression are a driving factor or a secondary consequence of the disease), and mechanism studies that ignore the characteristics of exosome carriers make it difficult to transform them into clinically applicable diagnostic tools. Based on this shared logic, the following sections will take common respiratory diseases (eg, asthma, COPD, pulmonary fibrosis, lung cancer) as entry points, respectively elaborating on the specific evidence for exo-miRNAs as diagnostic biomarkers. Emphasis will be placed on analyzing "how mechanistic research provides direction for validating diagnostic value", thereby offering theoretical and data support for the clinical translation of exo-miRNAs.

The Diagnostic Value of Exosome-Derived miRNAs as Biomarkers in Asthma

Asthma is a common chronic inflammatory airway disease with a complex clinical phenotype and high heterogeneity.[57,58] Diagnostic biomarkers for asthma help us understand and identify phenotypes, and assist in determining alternative treatment methods that may be effective for individual asthma patients who are unlikely to respond adequately to first-line drug therapy, as well as evaluating treatment responses.[59] However, due to the need to validate novel damage mechanisms in asthma and the limited predictive value of existing biomarkers for treatment responses, identifying rapid and specific diagnostic biomarkers is a crucial prerequisite for asthma treatment. The changes in miRNA expression caused by various reasons can enable miRNA to become a core participant in the onset of asthma by regulating inflammation, immune response, tissue remodeling, and cell communication.[60,61] For example, the key pathogenic factor PM2.5 induces exosomal miR-129-2-3p to target and inhibit the TIAM1/RAC1/PAK1 signaling pathway, disrupting airway epithelial barrier function and exacerbating asthma.[62] And based on these mechanisms of action, especially the changing trends of miRNA during these processes, miRNA has the potential to serve as a typing marker.

Exosome-derived miRNAs participate in intracellular exchange under both physiological and pathological conditions, regulating immune and inflammatory responses, and play a significant role in the pathogenesis of asthma.[63] Vázquez-Mera et al validated the value of serum exosomal miRNA expression profiles in the stratified diagnosis of asthma. They collected serum exosomes from 30 healthy controls and 119 asthma patients, separated and identified them, and measured their miRNA content.[45] They found that compared to mild asthma patients, miR-21-5p, miR-126-3p, miR-146a-5p, and miR-215-5p were significantly upregulated in moderate-to-severe patients, and miR-21-5p and miR-126-3p were significantly elevated in type 2 high (T2-high) asthma patients. These miRNAs may play multiple roles in the pathophysiological processes of asthma, with miR-215-5p influencing asthma susceptibility by silencing the transcription of RUNX1.[45] Other studies have found that serum exosome-derived miR-21, miR-223, let-7a, and miR-125b can serve as indicators for predicting asthma severity;[46,64,65] miR-155 and miR-221 demonstrate excellent efficacy in predicting severe asthma patients.[66] However, many of these findings still require validation in larger, multicenter clinical studies.

The Diagnostic Value of Exosome-Derived miRNAs as Biomarkers in COPD

COPD is a chronic inflammatory lung disease characterized by irreversible, persistent airflow limitation and poses a serious threat to the health of patients.[67] There is no cure for COPD, and the focus is on prevention and rehabilitation therapy. There is an issue

Table 1 Exosomal miRNAs as Biomarkers in Respiratory Diseases

Name of Disease	Exosomal miRNA	Expression Trend	Sources of Sample and Detection Technology	Mechanism and Clinical Meaning of miRNA	Reference
Asthma	miR-21-5p, miR-126-3p and miR-146a-5p from serum-derived exosomes	In MSA (VS MA) in MA (VS HC)	Serum from healthy people and asthma patients; RT-qPCR	Functionally involved in asthma pathogenesis, and thus can be part of the miRNA panel for assessing asthma severity risk	[45]
	miR-215-5p from serum-derived exosomes	↑ in MSA (VS MA) ↓ in MA (VS HC)	Serum from healthy people and asthma patients; RT-qPCR	Silences RUNX1 transcription to affect asthma susceptibility, and thus may serve as a potential biomarker for severe asthma	[45]
	miR-125b from serum-derived exosomes	↑ in Asthma patients (VS HC)	Serum from healthy people and asthma patients; RT-qPCR	Inhibits the expression of the p53 gene, and thus serves as a noninvasive diagnostic marker for asthma severity	[46]
COPD	miR-21 from serum-derived exosomes	↑ in smoking COPD patients and smokers (VS HC)	Serum from smoking patients with COPD, smokers without COPD and never-smoking volunteers; RT-qPCR	Targets the pVHL/HIF-1α signaling pathway to promote the myofibroblast differentiation phenotype of MRC-5 cells, and thus serves as a potential diagnostic biomarker and therapeutic target for COPD	[47]
	miR-23a, miR-221 and miR-574 from serum derived exosomes	↑ in patients with COPD (VS healthy non-smoking individuals)	Whole blood from patients with COPD and healthy non-smoking individuals; high-throughput sequencing RT-qPCR	May be involved in many pathways and biological processes, and thus may serve as novel diagnostic biomarkers of COPD	[48]
PF	miR-142-3p, miR-33a-5p and let-7d-5p in exosomes from serum	↑ (miR-142-3p, miR-33a-5p) in and ↓ (let-7d-5p) in patients with pulmonary fibrosis	Serum from healthy people and pulmonary patients; RT-qPCR	The mechanism remains unknown, but it has the potential to serve as a biomarker for diagnosing and assessing the severity of diseases	[49]
	miR-1343 in exosomes from cell line	↓ in patients with pulmonary fibrosis	Sample from cultured cell line; RT-qPCR	Targets the TGF-β pathway, and thus serves as a therapeutic target and diagnostic marker for pulmonary fibrosis	[50]
LUAD (NSCLC)	miR-16-5p in exosomes from serum	↓ in patients with LUAD	Serum from healthy people and adenocarcinoma patients; RT-qPCR	The mechanism remains unknown, but it has the potential to serve as a tumor inhibitor and as a biomarker in PD-L1 inhibitor-dependent immunotherapy	[51]
	miR-342-5p and miR-574-5p in exosomes from serum	↑ in patients with LUAD	Serum from LUAD patients; RT-qPCR	miR-574-5p can promote tumor invasion and metastasis by targeting protein tyrosine phosphatase receptor type U, and promote proliferation by affecting TLR9 signaling, while the related mechanism of miR-342-5p remains unclear, and thus serves as a promising diagnostic biomarker for patients with early-stage LUAD	[52]
SCLC	miR-1228-5p from serum-derived exosomes	↑ in patients with SCLC (VS HC)	Serum from 18 SCLC patients (pre-treatment) and 12 healthy volunteers. SCLC cells (H1048, H1688, H446); RT-qPCR and exosomal miRNA sequencing	Downregulates DUSP22 (a tumor suppressor) to promote proliferation, migration, and metastasis, and thus serves as a potential marker for SCLC diagnosis and prognosis	[53]

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	miR-92b-3p from serum-derived exosomes	↑ in patients with SCLC in post-chemoresistant stage (VS SCLC patients in pre-chemoresistant stage)	Serum from SCLC patients in the post-chemoresistant stage and pre-chemoresistant stage); NGS and RT-qPCR	Promotes SCLC chemoresistance through the PTEN/AKT pathway via exosomes, and thus might serve as a potential dynamic biomarker to monitor the chemoresistance, chemotherapy response, and prognosis of SCLC patients	[54]
ARDS	Let-7a-5p from serum-derived exosomes	↓ in patients with ARDS (VS HC)	Serum from patients with ARDS and healthy subjects; small RNA-seq principal component analysis	Involves in the development of inflammatory diseases, and thus serves as potential markers to discriminate ARDS patients from HC	[55]
Pneumonia	miR-450a-5p, miR-103a-3p, miR-103b-5p and miR-98-5p	↑ (miR-103b-5p and miR-450a-5p) and ↓ (miR-103a-3p and miR-98-5p) in patients with adenovirus infection	Serum from healthy people and patients with adenovirus infection; RT-qPCR	May be related to the regulation of immune function, and thus serves as a potential diagnostic biomarker for identifying adenovirus-induced pneumonia in children	[56]

Notes: ↑: The expression level of exosomal miRNA is upregulated in the target disease group (compared with the control group, as indicated by "VS" in the table); ↓: The expression level of exosomal miRNA is downregulated in the target disease group (compared with the control group, as indicated by "VS" in the table).

Abbreviations: miR, microRNA; VS, Versus; HC, Healthy Control(s); MSA, Mild to Severe Asthma; MA, Mild Asthma; COPD, Chronic Obstructive Pulmonary Disease; PF, Pulmonary Fibrosis; LUAD, Lung Adenocarcinoma; NSCLC, Non-Small Cell Lung Cancer; SCLC, Small Cell Lung Cancer; ARDS, Acute Respiratory Distress Syndrome; RT-qPCR, Reverse Transcription Quantitative Polymerase Chain Reaction; NGS, Next-Generation Sequencing; pVHL, von Hippel-Lindau Protein; HIF-1α, Hypoxia-Inducible Factor 1-Alpha; TGF-β, Transforming Growth Factor Beta; RUNX1, Runt-Related Transcription Factor 1; DUSP22, Dual Specificity Phosphatase 22; PTEN, Phosphatase and Tensin Homolog; AKT, Protein Kinase B; TLR9, Toll-Like Receptor 9; PD-L, Programmed Cell Death Ligand 1.

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of underdiagnosis of COPD.[68] Therefore, research into early diagnostic biomarkers for COPD holds significant clinical importance, especially as exosome-derived miRNAs have shown links between their pathogenic roles and diagnostic potential.

Although the exact mechanisms underlying COPD pathogenesis remain unclear, exosomes and the miRNAs they carry have been widely recognized for their role in disease development.[69–71] These exosome-carried miRNAs mediate COPD pathogenesis by regulating core pathological processes such as airway remodeling. A study put forward a complete pathogenic cascade: it found in a cigarette smoke-induced mouse model, miR-21 was upregulated in bronchial epithelial cell-derived exosomes.[47] These exosomes were shown to promote the differentiation of fibroblasts into myofibroblasts by targeting the pVHL/HIF-1α pathway, thereby directly contributing to airway remodeling. Crucially, the clinical relevance of this mechanism was corroborated by elevated levels of miR-21 in serum exosomes from COPD patients, which correlated negatively with lung function (FEV1/FVC).[47]

Building upon such mechanistic understanding, the diagnostic potential of exosomal miRNAs in COPD has been extensively explored. The advent of high-throughput technologies, particularly next-generation sequencing (NGS), has been pivotal in uncovering exosomal miRNA signatures. NGS is a powerful methodology that enables massive parallel sequencing of millions of DNA or RNA molecules,[72] allowing for comprehensive and unbiased profiling of entire transcriptomes, including miRNAs. Utilizing this approach, Sundar et al conducted the first comprehensive NGS analysis of miRNAs in plasma exosomes from non-smokers, smokers, and COPD patients, finding significant differences in exosome characteristics and miRNA expression profiles between groups, thereby proposing that miRNAs in exosomes could serve as biomarkers for COPD.[73] Subsequently, Wang et al found that miR-1258 in serum exosomes was upregulated in COPD patients, potentially associated with inflammatory and immune responses,[74] while Shen et al reported that miR-23a, miR-221, and miR-574 levels were elevated in serum exosomes from COPD patients.[48] MiRNAs derived from serum exosomes can be obtained through routine blood tests, which are simple to perform and suitable for dynamic monitoring of disease progression. In addition to being elevated in serum exosomes, miR-320b and miR-22-3p were found to be upregulated in exosomes derived from bronchoalveolar lavage fluid (BALF) in COPD patients, while miR-423-5p and miR-100-5p were also upregulated. In patient lung tissue-derived exosomes, miR-122-5p was downregulated,[75] and miR-185-5p and miR-182-5p were also downregulated.[76]

This compartmentalization of miRNA signatures highlights a crucial point: the exosomal miRNA profile is highly dependent on its tissue of origin. Serum exosomes may reflect systemic inflammatory responses, while BALF and tissue-derived exosomes offer a more direct window into the pulmonary microenvironment. Thus, future efforts must identify the most relevant miRNA panels for specific diagnostic purposes.

The Diagnostic Value of Exosome-Derived miRNAs as Biomarkers in Pulmonary Fibrosis

Pulmonary fibrosis is an end-stage manifestation of interstitial lung disease. Idiopathic pulmonary fibrosis (IPF), the most common and severe form, is a chronic, progressive, and irreversible fibrotic lung disease of unknown cause characterized by a poor prognosis and high mortality rate,[77] with an increasing trend in incidence in recent years. The diagnosis of IPF is based on high-resolution computed tomography and lung biopsy,[78] but in clinical practice, accurate and timely diagnosis of IPF remains a challenge. Exosome-derived miRNAs, which participate in fibrotic pathogenesis through specific regulatory mechanisms, have emerged as promising diagnostic and prognostic tools to address this challenge.

The diagnostic potential of miRNAs from their direct involvement in core fibrotic processes. For example, miR-21 has been shown to have a pro-fibrotic effect, while miR-92a has an anti-fibrotic function.[79–81] Studies have shown miR-143-5p and miR-342-5p are significantly upregulated in exosomes and AT2 cells from IPF patients, inhibiting fatty acid synthesis in ATII cells, inducing cellular senescence, and activating fibroblast fibrosis.[82] Although this study had a small sample size, it suggests the potential of exosomal miRNA for diagnosing IPF.

Serum exosomal miR-142-3p, miR-33a-5p, and let-7d-5p were found to be potential biomarkers for diagnosing pulmonary fibrosis and grading its severity;[49] while cellular experiments revealed that miR-1343 in exosomes targets the TGF-β pathway, potentially serving as a target for pulmonary fibrosis diagnosis and treatment.[50] Another study focused on the miRNA profile and functional roles of exosomes in the urine of IPF patients, identifying dysregulated expression

of miR-let-7D, miR-199a-3p, miR-29a-5p, and miR-181b-3p in patient urine, and demonstrated that the relative expression of these miRNAs in lung tissue and serum of IPF individuals was not significantly different, suggesting that urine could replace serum as a detection sample.[83]

Exosome-derived miRNAs can also serve as a prognostic biomarker for pulmonary fibrosis. Researchers identified significant upregulation of miR-21-5p-a known pro-fibrotic miRNA, in a bleomycin-induced mouse model of pulmonary fibrosis.[84] Notably, they subsequently conducted a prospective cohort study in 41 IPF patients and found that baseline levels of serum exosomal miR-21-5p were predictive biomarkers for IPF prognosis after 30 months of follow-up analysis.[84]

While these studies provide compelling mechanistic insights, their small sample sizes necessitate validation in larger, multi-center cohorts to assess true diagnostic robustness and generalizability.

The Diagnostic Value of Exosome-Derived miRNAs as Biomarkers in Lung Cancer

Lung cancer is a malignant tumor originating from respiratory epithelial cells, accounting for a high proportion of all malignant tumors. Its mortality rate remains persistently high, making it one of the leading causes of cancer-related deaths.[85] The lack of effective early detection methods is one of the primary reasons for the poor prognosis of lung cancer. Currently, the commonly used clinical screening methods for lung cancer all have certain limitations. For example, the low-dose radiation from imaging examinations may increase the risk of patients developing other cancers; sputum cytology has low accuracy; and bronchoscopy has limited ability to detect precancerous lesions.[86] Exosome-derived miRNAs, which mediate lung cancer progression through regulating key signaling pathways and the tumor microenvironment, have garnered significant attention in recent years as an emerging non-invasive diagnostic marker and tool for monitoring lung cancer progression.[87]

Multiple studies have shown that exosome-derived miRNAs participate in the occurrence and development of lung cancer by regulating key signaling pathways: serum exosomal miR-96 in lung cancer patients promotes lung cancer progression by targeting LMO7;[88] miR-106b targets PTEN to promote lung cancer cell migration and invasion, and is associated with TNM staging and lymph node metastasis.[89] Zhang et al found that exosomes secreted by hypoxia-pretreated BMSCs, rich in miR-193a-3p, miR-210-3p, and miR-5100, can be taken up by neighboring cancer cells and promote tumor cell invasion and EMT by activating the STAT3 signaling pathway.[90] Notably, these Exosome-derived miRNAs are significantly elevated in the plasma of lung cancer patients and have the potential to distinguish metastatic lung cancer patients from non-metastatic lung cancer patients, suggesting their potential as diagnostic and staging biomarkers for lung cancer.[90] Recent studies have also found that serum exosomal miR-151a-3p and miR-877-5p can effectively predict bone metastasis in lung cancer,[91] further highlighting their clinical translational value. Lung cancer can be further classified into non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). Below, we will describe NSCLC and SCLC, respectively.

The Diagnostic Value of Exosome-Derived miRNAs as Biomarkers in NSCLC

NSCLC is the primary type of lung cancer, accounting for approximately 85% of all lung cancer cases, according to estimates by the World Health Organization (WHO).[92] Its molecular heterogeneity determines the diversity of diagnostic and therapeutic strategies. Despite significant advances in the treatment and management of NSCLC in recent years,[93] early diagnosis remains a crucial means of improving prognosis. Exosome-derived microRNAs, as a novel liquid biopsy tool, have garnered significant attention for their dual role as functional regulators of non-small cell lung cancer progression and disease-specific biomarkers.

Studies have found that the expression profile of serum exosomal miRNA in lung adenocarcinoma patients has undergone significant changes, with reduced levels of miR-16-5p and miR-195-5p, and the latter can target APLN to inhibit the Wnt/β-catenin pathway and suppress tumor malignant behavior.[51,94] Elevated miR-31-5p reduces SATB2 expression and increases MEK/ERK pathway activity;[95] elevated miR-574-5p promotes tumor invasion and metastasis by targeting protein tyrosine phosphatase receptor U and enhances cell proliferation by influencing the TLR9 signaling pathway.[52] Additionally, Chang et al found that miR-197-3p was significantly upregulated in serum exosomes from patients with metastatic lung adenocarcinoma and confirmed that it indirectly activates MMPs by inhibiting metalloproteinase inhibitors TIMP2/3, thereby promoting angiogenesis.[96] This finding reveals a new mechanism of Exosome-

derived miRNAs in tumor microenvironment remodeling. What's more, exosomic-miR-222-3p functions as a principal regulator of gemcitabine resistance and malignant characteristics by targeting SOCS3.[97]

In summary, Exosome-derived miRNAs not only serve as potential diagnostic markers for NSCLC but are also associated with tumor staging and prognosis and serve as biomarkers for drug resistance. In patients with lung squamous cell carcinoma, miR-126 and Let-7a in BALF exosomes and miR-369 in serum exosomes are elevated.[98,99] miR-369 interacts with NF1 to activate the mitogen-activated protein kinase signaling pathway, thereby enhancing the growth of lung squamous cell carcinoma cells,[98,99] linking its pathogenic role to its value as a disease-specific biomarker.

The Diagnostic Value of Exosome-Derived miRNAs as Biomarkers in SCLC.

SCLC is a high-grade neuroendocrine carcinoma, accounting for approximately 13% of lung cancers,[100] with smoking being the primary risk factor. Low-dose CT has low sensitivity for early-stage SCLC,[101] making early diagnosis challenging. Approximately two-thirds of patients are found to have distant metastases at the time of initial diagnosis, resulting in a poor prognosis.[102] Exosome-derived miRNAs—which drive SCLC progression and exhibit disease-specific expression—hold promise as early molecular biomarkers for SCLC.

Kim et al proposed an exosomal miRNA-based liquid biopsy protocol for SCLC.[103] They found that miR-200b-3p and miR-3124-5p were elevated in serum exosomes from patients, while miR-92b-5p was decreased, and these could serve as diagnostic and prognostic markers for SCLC. The combination of the three miRNAs was identified as the ideal diagnostic model. Mu et al identified upregulation of circulating miR-1228-5p in SCLC patients through exosome miRNA sequencing and experimentally demonstrated that it promotes SCLC proliferation, migration, and metastasis by downregulating the tumor suppressor DUSP22,[53] confirming that its diagnostic utility stems from its mechanistic role in tumor progression. Exosomes also play an important role in tumor resistance. Li et al first demonstrated that miR-92b-3p in serum exosomes from SCLC patients promotes the PTEN/AKT pathway, leading to resistance in small cell lung cancer.[54] This extends the value of exosomal microRNAs from diagnosis to monitoring therapeutic response.

Exosomal miRNAs in Respiratory Diseases: From Diagnostic Biomarkers to Theranostic Platforms

In addition to serving as biomarkers for the aforementioned diseases, Exosome-derived miRNAs also hold potential diagnostic value in other respiratory system diseases. For instance, the known inflammatory signaling modulator let-7a-5p exhibits significantly reduced levels in serum exosomes from patients with acute respiratory distress syndrome (ARDS), this downregulation correlates with the severity of alveolar epithelial injury, thereby enabling reliable differentiation between ARDS patients and healthy individuals;[55] In sepsis-induced acute lung injury (SALI), miR-92a-3p secreted by alveolar epithelial cell (AEC) exosomes is elevated, mechanistically, it promotes neutrophil activation by targeting anti-inflammatory genes, and its serum level correlates with disease progression, making it both an important biomarker and a potential therapeutic target for acute lung injury (ALI);[104] for pediatric adenovirus-induced pneumonia, miR-450a-5p, miR-103a-3p, miR-103b-5p, and miR-98-5p are dysregulated—these miRNAs are involved in regulating innate immune responses (eg, TLR signaling and cytokine secretion)—and their combined expression profile shows promise for distinguishing adenoviral pneumonia from other infectious etiologies.[56]

Despite this promise, current research faces considerable limitations. Firstly, most mechanistic insights are derived from in vitro models, which fail to fully recapitulate the complexity of the in vivo microenvironment. Secondly, the pathological roles of miRNAs often lack disease specificity due to their involvement in multiple, overlapping signaling pathways. Perhaps the most critical challenge is the inherent ambiguity of correlation-based biomarker studies: cross-sectional designs cannot distinguish whether aberrant miRNA expression is a primary driver of pathology or merely a secondary consequence of the disease state.

The concept of theranostics—integrating diagnostic monitoring with targeted therapy—offers a framework to directly test miRNA function in vivo.[105] By employing engineered exosomes to therapeutically modulate a candidate miRNA and simultaneously tracking its levels as a diagnostic and pharmacodynamic biomarker, researchers can directly assess its causal role in disease progression and treatment response. This approach effectively turns the primary limitation of correlation-based

studies into a definitive tool for functional validation. In this context, engineered MSC-exosomes (MSC-exos) emerge as an ideal platform to implement this theranostic strategy—a strategy that integrates therapy and diagnostics.

In summary, exosomal miRNAs have emerged as promising non-invasive diagnostic biomarkers for respiratory diseases due to their disease-specific expression profiles, high stability, and easy accessibility from bodily fluids (as summarized in Table 1).[27] The clinical significance of these miRNA signatures, however, extends far beyond their role as mere indicators of disease. Crucially, their aberrant expression is often not an epiphenomenon but a direct reflection of their active involvement in core disease mechanisms, such as regulating inflammation, fibrosis, apoptosis, and cellular proliferation.[60,79,106,107] This functional involvement provides the foundation for a theranostic strategy. Rather than relying on the same miRNA molecule for both diagnosis and therapy, theranostics in this context exploits the functional connectivity within miRNA regulatory networks. The diagnostic profile of a set of miRNAs reveals the activity of a specific pathological pathway. This diagnostic insight then rationally informs the choice of a therapeutic miRNA designed to counteract that same pathway. Engineered MSC-exos are uniquely suited to realize this principle. They can be loaded with these therapeutic miRNAs to correct pathogenic imbalances. The efficacy of this intervention can be monitored by tracking the subsequent normalization of the original diagnostic miRNA signatures or key downstream biomarkers, thereby creating an integrated theranostic feedback loop. This synergy supports a new paradigm in targeted, feedback-informed personalized medicine for respiratory diseases. The following sections detail engineering strategies for enhancing MSC-exos' efficacy within this broader theranostic framework.

Large-Scale Production and Engineering Strategy of MSC-Exos

Large-Scale Production of MSC-Exos

The production of MSC-exos is a multi-step complex process involving cell culture, exosome separation and purification, and quality control. Exosomes are generated through multiple endocytosis and membrane fusion processes, which are complex and have limited efficiency.[108] Multiple studies have shown that MSCs secrete a low baseline number of exosomes under conventional culture conditions, insufficient to meet large-scale clinical demands.[108–110] Therefore, achieving large-scale production of MSC-exos is a crucial step in exosome engineering. We will now introduce strategies for achieving large-scale production of MSC-exosomes, with a focus on improving yield and purity, Figure 2 and compare the advantages and disadvantages of various techniques (Table 2).

Large-Scale Production of MSC-Exos Through Genetic Engineering

Genetic engineering is a powerful tool that manipulates the biosynthesis and secretion of exosomes by altering the expression of molecules related to the formation of multivesicular bodies (MVBs), vesicle transport, and membrane fusion. The RAB family has been confirmed to be involved in the precise control of vesicle transport, maturation, and the fusion process of MVB with the cell membrane.[32,120,121] RAB27A/B is in MVB and cell membranes, promoting the transport and anchoring of MVB to the cell membrane and regulating the fusion of MVB with the plasma membrane. RAB11 and RAB35 are directly associated with the early secretion of MVB,[122] while RAB7 promotes the fusion of MVB with lysosomes, leading to the degradation of exosomes rather than their release.[123] Overexpression of RAB27A/B through genetic engineering technology increases the fusion efficiency of MVBs with the cell membrane, thereby increasing the release of exosomes. Or lowering RAB7 to reduce the degradation of MVBs might be an effective method to increase exosome production, and it is currently applied in the treatment of triple-negative breast cancer (TNBC) and pancreatic ductal carcinoma.[111,124] Furthermore, the exosome secretion capacity of cells can also be enhanced by overexpressing tetracaine CD9 or TSPAN6.[108]

Large-Scale Production of MSC-Exos Through Optimizing the Culture Protocol

Traditional static two-dimensional (2D) culture conditions confine adherent cells to 2D boundaries, which, to some extent, disrupts intercellular communication and may result in insufficient rates and total amounts of exosome secretion.[125] In contrast, three-dimensional (3D) culture allows for affinity interactions between cells and provides more diffusion space. Cells cultured in this microenvironment exhibit higher survival rates.[113,126] By combining 3D culture technology with microcarriers and stirred bioreactor systems, large-scale production of human amniotic membrane-derived mesenchymal

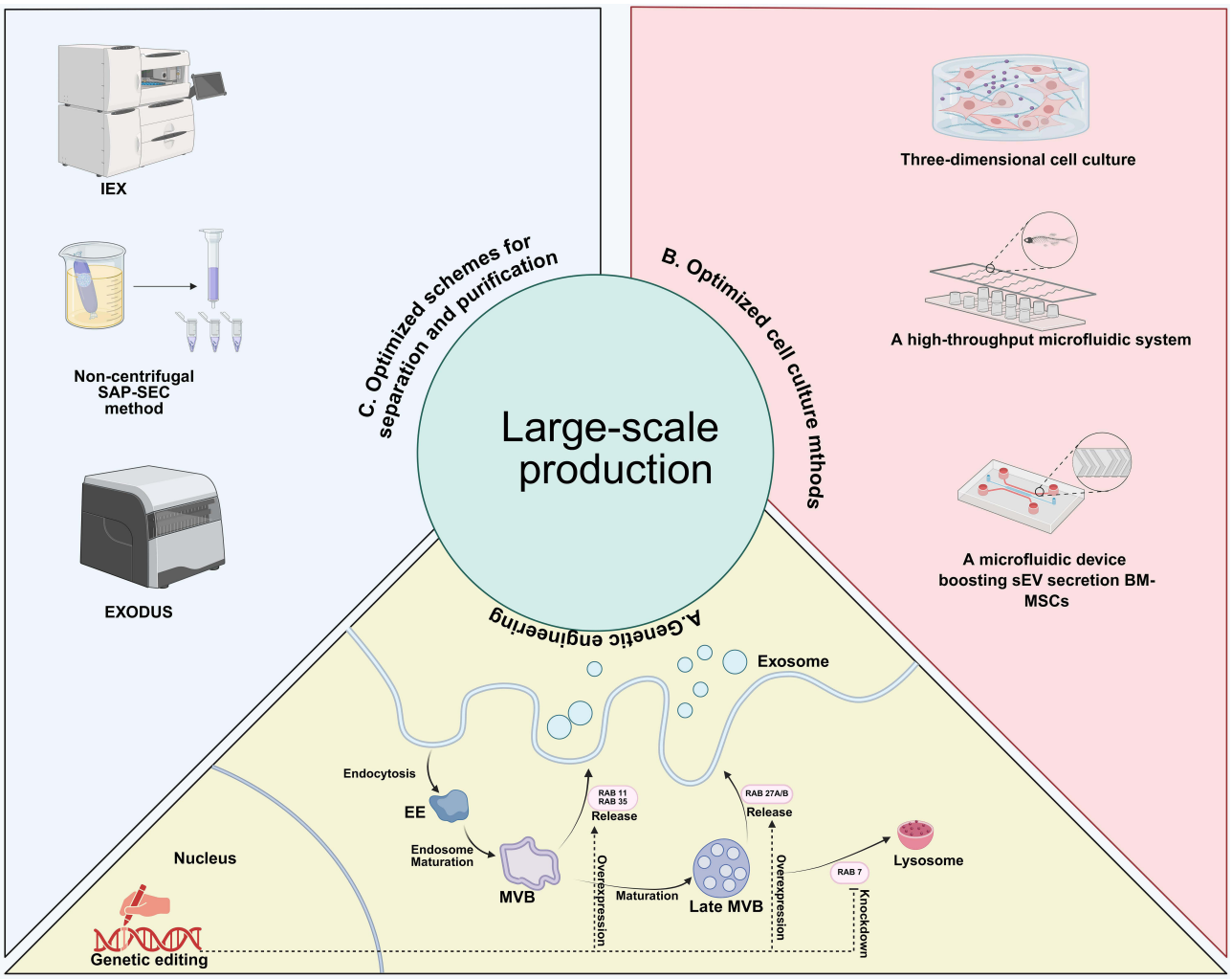

Figure 2 Novel large-scale production method. (**A**) Genetic engineering technology modifies the expression of the RAB family involved in exosome secretion, thereby increasing exosome yield. (**B**) Optimized cell culture protocols, including 3D culture technology; utilization of microfluidic chips with fishbone channels and microcolumn arrays to enhance nutrient delivery efficiency; and narrow channels to enhance MSC secretion efficiency. (**C**) Optimized separation and purification protocols, including ion exchange chromatography to enhance purity via charge interactions; non-centrifugal SAP-SEC method to highly concentrate and purify exosomes via SAP permeation; and exosome detection via the ultrafast-isolation system to reduce separation time.

Abbreviations: MSC, mesenchymal stem cells; EE, early endosome; MVB, multivesicular body; RAB, Ras-related protein in brain; BM-MSCs, Bone Marrow-derived Mesenchymal Stem Cells; IEX, Ion exchange chromatography; UC, ultracentrifugation; IDC, Ion Exclusion Chromatography; EXODUS, exosome detection via the ultrafast-isolation system.

stem cells (hMSCs) is achievable (more than 1×10^9 cells).[127] Furthermore, hMSCs and their exosomes produced by this method effectively reduced the levels of pulmonary inflammatory factors, decreased protein leakage, and improved pathological features of lung tissue in a mouse model of LPS-induced ALI.[127] Huang et al utilized a microfluidic chip integrated with herringbone grooves and micropillar arrays to achieve sufficient hydrodynamic stimulation and efficient nutrient delivery via turbulent vortices, thereby significantly enhancing exosome production.[114] Hao et al discovered that rapid passage of cells through a microfluidic channel with constriction ridges increases membrane permeability via mechanical stimulation, thereby promoting EV secretion with an approximate 4-fold yield enhancement.[115] Using perfusion bioreactors combined with 3D-printed scaffolds to culture MSCs at a flow rate of 5 milliliters per minute, the production of MSC EVs was 83 times higher than in traditional cell culture, and the produced MSC EVs demonstrated significant vasculogenic-promoting and wound-healing capabilities in both in vitro and in vivo experiments.[116]

Table 2 Techniques for Enhancing Exosome Production

Technology	Mechanism	Suitable Applications	Strengths	Limitations	Reference
Knock out the Rasal2 gene.	Knockout of Rasal2 enhances Rab27a activity, thereby promoting exosome secretion.	Production of exosomes enriched in autophagy-related proteins for studying autophagy in cancer.	High yield and unique composition (contain autophagy-related proteins).	Off-target risks, unknown safety	[111]
CD9 overexpression.	CD9 facilitates the biogenesis of MVBs and their subsequent membrane fusion.	Scalable production of exosomes from common human cell lines (HEK293, HeLa, etc.) for various downstream applications.	CD9 overexpression significantly boosts exosome yield and, unlike TSG101 or Alix, induces no overt cytotoxicity.	The underlying mechanism remains incompletely understood, and exosome functionality may be altered.	[112]
Dynamic 3D-culture	3D spheroid formation mimics the in vivo microenvironment. Orbital shaking enhances nutrient/waste exchange.	Treatment of ischemic vascular diseases and neurological injuries/degeneration.	Scalable, efficient, reproducible, and enhanced therapeutic cargo.	No cell proliferation, and dependence on culture conditions	[113]
High-Throughput Herringbone Microfluidics	The herringbone structure in microfluidic chips generates vortices that enhance the cell environment, while a specialised surface coating promotes exosome secretion by controlling cell morphology.	Production of exosomes enriched in active proteins (eg, HGF) for diabetic wound healing.	High yield, strong controllability, and high content of active proteins such as HGF in exosomes.	The equipment is complex and relatively costly, with its long-term stability yet to be verified.	[114]
SEED	Mechanical stress applied to cells via extrusion ridges within microchannels induces transient membrane permeabilisation, thereby promoting exosome secretion.	Healing of corneal epithelial wounds.	High cell viability without altering EV function, compatible with other delivery technologies.	Applicable only to cells larger than the gap; custom equipment is required	[115]
3D-printed scaffold-perfusion bioreactor system	Perfusion flow with low shear stress enhances extracellular vesicle secretion and improves metabolite exchange.	Diabetic wound healing and adaptable for other tissue engineering applications.	High yield, maintains or enhances biological activity, highly adaptable	The equipment is complex, and parameter optimisation is intricate. The purity and subtype composition of EVs may vary with changes in culture conditions.	[116]
IEX	Separates vesicles based on surface charge using an anion-exchange resin. Exosomes are eluted with a high-salt buffer.	IEX-EVs show enhanced anti-inflammatory activity, indicating greater therapeutic potential for inflammatory and autoimmune conditions.	High Purity, high Yield, and functional activity.	Potential for shear stress damage at high pressure, higher equipment and resin costs, and more complex process optimisation	[117]
Combining SAP in a dialysis membrane with SEC	The sample is first concentrated using water-absorbing SAP (isolated by a dialysis membrane) to prevent contamination, and is then efficiently separated from protein contaminants by size using SEC.	Possesses wound-healing potential.	No need for ultracentrifugation, preserving exosome functionality, and suitable for serum-free media.	Longer concentration time, lower purity than UC, and applicability to complex biological fluids (such as plasma) remain to be validated.	[118]
EXODUS	Using nanoporous membrane resonators, membrane fouling is reduced and efficient separation is achieved under negative pressure oscillation and dual-coupled resonators.	Production of high-purity exosomes containing specific wound-healing proteins (M-CSF1, COL2A1, COL5A1, PTX3, FN1) for tissue repair and regeneration.	High yield, high purity, fast processing, preserves exosome integrity and RNA integrity, and automation.	Highly dependent on equipment, limited sample throughput, and relatively high cost.	[119]

Abbreviations: MVBs, multivesicular bodies; HGF, hepatocyte growth factor; SEED, Small Extracellular Vesicles Developer; EV, extracellular vesicles; IEX, ion exchange chromatography; SAP, superabsorbent polymers; SEC, size exclusion chromatography; UC, Ultracentrifugation; EXODUS, Exosome detection via the ultrafast-isolation system.

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Large-Scale Production of MSC-Exos Through Optimizing the Separation and Purification Scheme

Due to their small size (30–150 nm), MSC-exos separated using traditional ultracentrifugation (UC) methods often contain other extracellular vesicles or non-vesicular components, and there are also issues of low efficiency and low quality.[128,129] Other conventional separation and purification methods include density gradient centrifugation, ultrafiltration (UF), and polyethylene glycol (PEG) precipitation. In comparison, some novel separation and purification methods demonstrate unique advantages.

Ion exchange chromatography (IEX) uses ion exchange resins to purify EVs through charge interactions. EVs separated by IEX exhibit significantly higher protein and RNA yields than UF and demonstrate stronger immunomodulatory functions.[117] A novel strategy for purifying EVs combines superabsorbent polymer (SAP)-dialysis membrane concentration with size exclusion chromatography (SEC). Through SAP-mediated osmotic concentration technology, EVs in culture supernatants can be concentrated over 300-fold without ultracentrifugation, significantly improving sample processing efficiency. Subsequent SEC purification efficiently removes contaminants such as protein aggregates, achieving EV purity comparable to that of ultracentrifugation combined with density gradient centrifugation, while preserving the biological activity of the separated EVs.[118] A novel separation and purification technology named Exosome detection via the ultrafast-isolation system (EXODUS) can rapidly process 10–20 mL of MSC conditioned medium, with the entire process completed in less than one hour. This is significantly faster than the PEG method and the UC method. The number of EVs isolated from 10 mL of conditioned medium using EXODUS is five times that of the PEG and UC methods. Additionally, EVs isolated using EXODUS have higher RNA content, including long non-coding RNA and mRNA.[119,130]

Engineering Strategies for MSC-Exos-Derived miRNA

Increasing research indicates that the therapeutic benefits of stem cells, including anti-inflammatory, immunomodulatory, tissue repair, and neuroprotective effects, are largely mediated by the exosomes they release.[131,132] MSC-derived exosomes (MSC-exos) offer distinct advantages over other exosome sources and synthetic nanoparticles. Unlike synthetic nanoparticles, they exhibit superior biocompatibility and low immunogenicity. Unlike exosomes from other cell sources, MSC-exos are derived from clinically relevant, immunoprivileged cells, avoiding critical safety and scalability issues, thereby making them a promising cell-free therapeutic platform,[133] enabling them to circumvent safety and ethical issues associated with stem cell therapy, thereby making them an ideal cell-free therapy. Their intrinsic cargo of miRNAs enables them to modulate protein synthesis in recipient cells, underpinning their therapeutic effects in various disease models.[134–137]

However, natural exosomes present three major limitations for clinical translation: insufficient yield in vitro, low targeting efficiency, and endosomal escape, resulting in ineffective exosomal delivery of miRNAs and a failure to achieve the intended therapeutic outcome.[138] Engineering strategies are therefore employed to overcome these hurdles. These efforts primarily focus on three complementary objectives: (1) Cargo-centric engineering, which aims to enhance the loading efficiency and stability of therapeutic molecules into exosomes. (2) Membrane-centric engineering is designed to improve the targeting specificity and delivery efficiency of exosomes. (3) Cellular preconditioning to naturally engineer exosomes with enhanced therapeutic potency by modulating the parent MSCs' microenvironment.

In the following sections, we systematically describe the engineering methods for loading cargo, modifying the surfaces of exosomes, and preconditioning parent cells (Figure 3 and Table 3).

Direct Engineering Strategies for Cargo Loading

The efficient loading of therapeutic miRNAs into exosomes is a prerequisite for their function. Currently, various methods have been employed for MSC-exos cargo loading.[152] The choice of strategy is often dictated by the nature of the cargo. Delivering large macromolecules presents a significant challenge due to the limited loading capacity of exosomes. To address this, Lin et al designed an exosome-liposome hybrid nanoparticle, leveraging the liposome's superior capacity for macromolecular encapsulation.[153] However, the introduction of liposomes potentially triggers immune responses or cytotoxicity, which may affect the natural biocompatibility and low immunogenicity of exosomes. In contrast, therapeutic miRNAs, being relatively small nucleic acids, are much more amenable to loading into exosomes

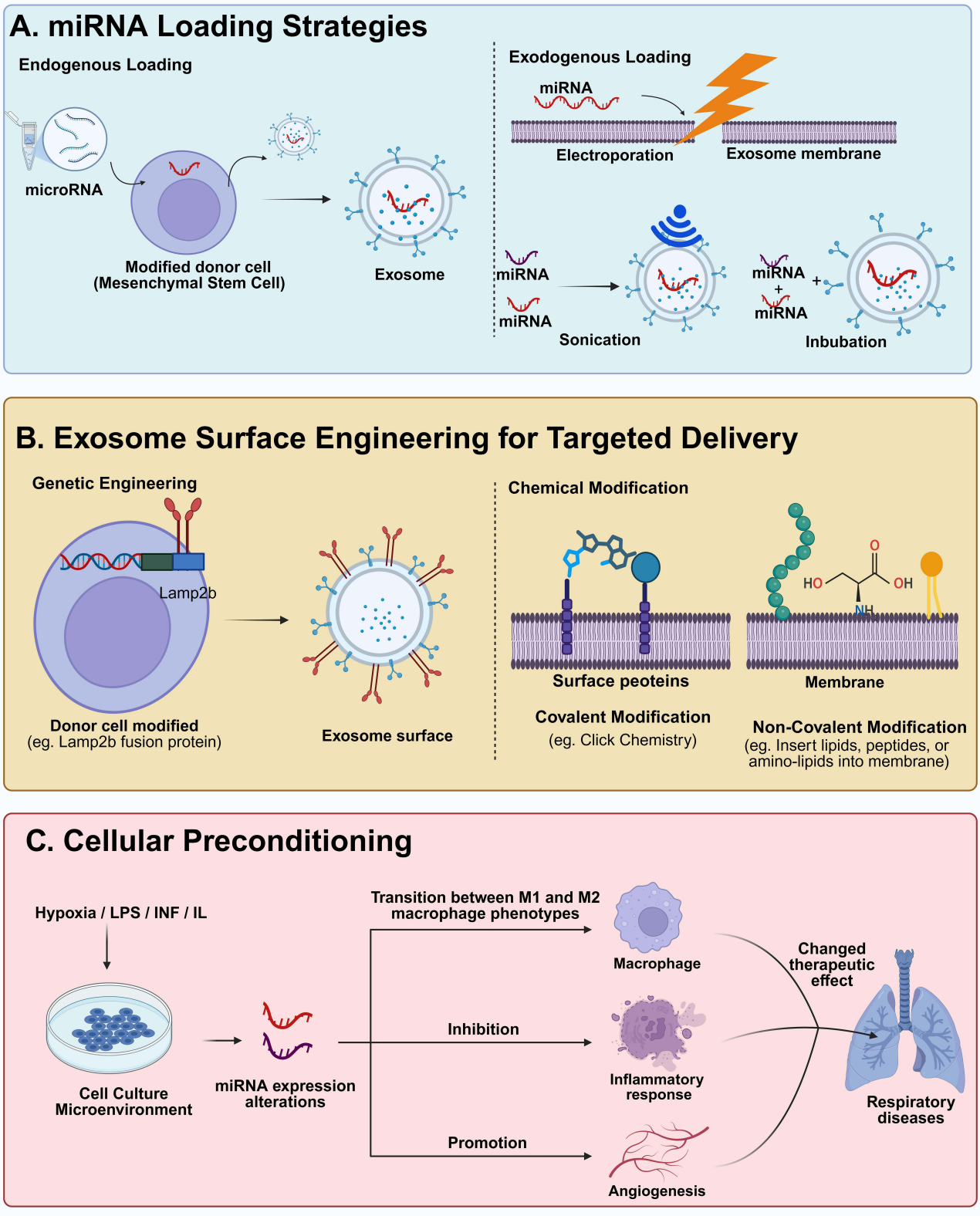

Figure 3 Exosome engineering methods. (**A**) miRNA Loading Strategies. Depicts methods for encapsulating therapeutic miRNAs into exosomes, including endogenous loading via genetic modification of donor cells and exogenous loading techniques like electroporation, sonication, and incubation. (**B**) Surface Modification for Targeted Delivery. Shows strategies to functionalize the exosome membrane for improved targeting, such as genetic engineering (eg, Lamp2b fusion proteins) and chemical modifications (covalent and non-covalent). (**C**) Pre-treatment Strategies. Illustrates strategies to modulate the parental cell microenvironment (hypoxia, LPS, or cytokines) can alter the exosomal miRNA cargo and enhance therapeutic functions.
Abbreviation: LPS, lipopolysaccharide.

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Table 3 Categorization and Comparison of Major Engineering Strategies for Mesenchymal Stem Cell-Derived Exosomes

Engineering Strategy	Advantages	Limitations	Representative Application	Reference
Cargo Loading: **Improve miRNA loading efficiency**				
Endogenous Loading (eg Donor cell transfection)	Preserves exosome integrity and natural membrane composition	Technically complex, difficult to regulate	Transducing MSCs with lentivirus to overexpress miR-132-3p, generating EVs that exhibited protective effects in a myocardial infarction model	[139]
	Suitable for large-scale, stable production	Biosafety concerns	Engineered BM-MSCs by lipofectamine-based transfection with an miR-146a mimic, generating modified exo-146a that ameliorated diabetic neuropathy in db/db mice	[140]
Exogenous Loading (eg, electroporation)	Operational simplicity and flexibility do not require cell manipulation	Risk of damaging EV membrane integrity, leading to aggregation	Loading miR-381-3p mimics into MSC-EVs via electroporation effectively inhibits the migratory capacity of triple-negative breast cancer cells.	[141]
	Rapid loading of various miRNAs into exosomes, ideal for proof-of-concept studies, high-throughput screening, and personalized medicine approaches	Unstable loading efficiency and potential cargo leakage	Loading MSC- exos with miR-21 agomir via electroporation, generating Agomir21-Exo to enhance the loading of this therapeutic miRNA	[142]
Targeting Strategies: **Enhance targeting efficiency**				
Genetic Surface Modification (eg Donor cell modification)	Homogeneous and stable modification, representing a native component of the EV	High technical barriers	Using Lamp2b-RVG fusion protein to engineer EVs for targeted delivery of siRNA to neurons in the brain	[143]
	Excellent biocompatibility	May interfere with natural EV biogenesis or function	Pioneering a versatile GPI-anchoring strategy to engineer EVs displaying anti-EGFR nanobodies, thereby programming them for specific homing to tumor cells	[144]
Chemical Surface Modification (eg, click chemistry)	Potent functionality enabling precise delivery	Coupling efficiency varies	Employing a biocompatible copper-free azide-alkyne cycloaddition reaction for rapid covalent surface modification, engineering exosomes that effectively targeted the ischemic region of the brain	[145]
	High flexibility and modularity	May lead to aggregation	Functionalizing exosomes with the RGE peptide via a two-step click chemistry approach: first introducing alkyne groups onto the membrane using EDC/NHS chemistry, followed by a CuAAC reaction to conjugate the azide-containing RGE peptide, thereby generating glioma-targeting exosomes.	[146,147]

Preconditioning strategies: Alter the miRNA expression profile				
Hypoxic Preconditioning	Simulating the pathophysiological environment of ischemic diseases significantly enhances the pro-angiogenic capacity of exosomes.	The composition of exosomes is not yet fully defined, and stringent quality control is required.	Hypoxic olfactory mucosal MSC-exos upregulate miR-612 and use it as a functional messenger to promote angiogenesis, providing a new therapeutic strategy for ischemic diseases.	[148]
Inflammatory factor preconditioning	Modifying miRNA expression and enhancing the anti-inflammatory capacity of exosomes	Overactivation of immune regulatory pathways may lead to excessive immunosuppression, increasing the risk of infection.	hUC-MSCs pretreated with IFN-γ inhibit the NF-κB signaling pathway by activating the miR-199b-5p/AFTPH axis, demonstrating a synergistic in vitro/in vivo therapeutic effect in ALI models	[149]
Systematic Strategies	Therapeutic outcomes are significantly superior to single strategies	The difficulty of operation is significantly greater than that of a single method	Transducing human hUC-MSCs with lentivirus to overexpress miR-486-5p and engineer the expression of SARS-CoV-2 Spike RBD on the exosome surface, generating lung-targeting, miR-486-RBD-Exos that exhibited protective effects by suppressing ferroptosis in lung epithelial cells and alleviating RILI and long-term pulmonary fibrosis in mouse models.	[150]
			Functionalizing BMSC-derived exosomes with a cardiomyocyte-homing peptide via chemical conjugation and loading miR-302 mimics via electroporation, generating engineered EVs that mitigated myocardial ischemia/reperfusion injury.	[151]

Notes: The bolded terms (Cargo Loading: improve miRNA loading efficiency; Targeting Strategies: enhance targeting efficiency; Preconditioning strategies; Systematic Strategies: the different engineered therapeutic strategies).
Abbreviations: MSC, mesenchymal stem cell; EVs, extracellular vesicles; MSC-EVs, mesenchymal stem cell derived extracellular vesicles; BM-MSCs, Bone Marrow-derived Mesenchymal Stem Cells; hUC-MSCs, human umbilical cord mesenchymal stem cells RVG, Rabies Virus Glycoprotein; GPI, GlycosylPhosphatidylinositol; EGFR, Epidermal growth factor receptor; IFN-γ, Interferon–gamma; CuAAC, copper-catalyzed azide-alkyne cycloaddition; RILI, radiation-induced lung injury; ALI, acute lung injury; NF-κB, Nuclear Factor Kappa-light-chain-enhancer of activated B cells; RGE, Arg-Gly-Glu (A peptide sequence); EDC, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; NHS, -Hydroxysuccinimide; SARS-CoV-2, Severe Acute Respiratory Syndrome Coronavirus 2; S-RBD, Spike protein's Receptor-Binding Domain; miR, microRNA.

without the need for complex hybridization. Their smaller molecular weight facilitates efficient encapsulation, thereby effectively preserving the natural advantages of exosomes. This inherent ease of loading, coupled with a more favorable safety profile, makes miRNAs ideal cargo for exosome-based therapeutics. MiRNA loading methods can be broadly classified into endogenous loading and exogenous loading, each with distinct strategic advantages.[154]

Endogenous Loading

Endogenous loading involves genetically engineering the donor cells to produce exosomes preloaded with desired miRNA.[33] The specific method involves first transfecting MSCs with miRNA using biological tools, then utilizing the biological molecular mechanisms of exosome synthesis to encapsulate it into exosomes. Common biological tools used for transfection include adenoviruses and lentiviruses. This approach leverages the cell's own machinery to package the desired miRNA into exosomes during their biogenesis. For instance, Pan et al successfully loaded miR-132-3p into exosomes by transducing MSCs with a lentiviral vector.[139] A key advantage of this method is that it avoids post-isolation damage to the exosomes and preserves their quality and function. Since the miRNA is loaded during the natural formation process, the resulting exosomes have intact membranes and native surface characteristics. This makes endogenous loading particularly suitable for applications demanding high vesicle integrity and scalable, reproducible production, such as developing off-the-shelf therapeutic candidates. The main drawbacks are the technical complexity, variable transfection efficiency, and potential biosafety considerations associated with viral vectors. Therefore, the search for alternative artificial nonviral carriers for small RNA remains a hot topic in gene therapy.[155]

Exogenous Loading

In contrast, exogenous loading methods manipulate purified exosomes directly. A commonly used technique is electroporation.[156] This method has high efficiency, as it uses an external electric field to create reparable pores in the phospholipid bilayer, allowing various small molecules to enter the exosomes through the pores under the influence of the electric field, thereby achieving encapsulation.[157] Shojaei et al used electroporation to encapsulate miRNA-381-3p mimics into MSC-exos and delivered them to TNBC cells, thereby inhibiting their migratory capacity.[141] Alvarez-Erviti, Chang, and others also used electroporation to load exosomes with exogenous small molecules.[142,143,158] The key advantage here is operational simplicity and flexibility. This method allows for the rapid loading of various miRNAs into pre-formed exosomes without the need for genetic manipulation of cells, making it ideal for proof-of-concept studies, high-throughput screening, and personalized medicine approaches where rapid iteration is key. However, the harsh physical conditions can compromise exosome integrity, leading to aggregation, membrane damage, inconsistent loading efficiency and potential cargo leakage. Other exogenous methods include co-incubation, sonication, and so on.[159]

In summary, the choice of loading strategy is a critical trade-off. Endogenous loading is the gold standard for clinical translation, producing exosomes with native integrity and is ideal for scalable, off-the-shelf therapeutics, despite its technical complexity. In contrast, exogenous loading offers unparalleled speed and flexibility, making it the method of choice for proof-of-concept studies and high-throughput screening, albeit at the cost of potential vesicle damage and inconsistent loading. The selection should be guided by the primary goal.

Direct Engineering Strategies for Surface Modification

The binding of exosomes to recipient cells is not random. Some molecules contained in exosomes can be delivered to target recipient cells,[15] but their ability to target specific regions and actively transport within the body is insufficient to meet the needs of precision medicine. To direct exosomes to diseased tissues and avoid off-target effects, surface engineering is paramount. The two primary philosophies, genetic engineering and chemical modification, offer complementary pathways to achieve this goal by installing targeting moieties.[160,161] Surface modification of exosomes can confer targeting specificity, enabling exosomes carrying therapeutic miRNAs to exert their therapeutic effects more effectively. The two primary engineering philosophies offer different pathways to achieve this goal.[162]

Genetic Engineering

Like cargo loading, genetic engineering involves modifying the parent MSCs to express a fusion protein that displays a

targeting ligand on the surface of the secreted exosomes. This is commonly achieved by fusing the ligand to exosome-enriched membrane proteins such as Lamp2b, CD9, CD63, or CD38.[163] By transfecting donor cells with plasmids encoding these fusion proteins, engineered exosomes displaying targeting ligands on their surface can be secreted. This strategy significantly improves the specificity and delivery efficiency of exosome-targeted cell interactions.[160] Targeting peptides such as RGD, rabies virus glycoprotein RVG, cardiac muscle cell-specific peptides, TAT, designed anchor protein repeat proteins G3, and IL-3 fragments can be genetically integrated into these membrane protein sequences. For example, Alvarez-Erviti et al fused the RVG peptide with Lamp2b.[143] This fusion protein was incorporated into EVs, enabling EVs to target neurons, oligodendrocytes, and microglia. Lamp-2b has also been used as a base for other targeting peptides, such as integrin-binding peptides or cell-penetrating peptides. Although this genetic approach is effective, the targeting peptide may be degraded in some cases. A glycosylation motif can be added to the N-terminus of the peptide-LAMP-2b fusion to enhance its stability.[164]

To address the stability issues of Lamp-2b more effectively, Kooijimans et al proposed using glycosylphosphatidylinositol (GPI) as an alternative.[144] They constructed a GPI-anchored sequence fused to an anti-epidermal growth factor receptor (EGFR) antibody and used genetic engineering to transfect cells with the fusion protein, causing the cells to secrete exosomes expressing anti-EGFR nanobodies. Similar methods have also been used to integrate fluorescent proteins into exosomes, enabling the tracking of exosome biogenesis, intercellular transfer, and systemic distribution.[165]

Chemical Modification

Chemical modification involves displaying various natural or synthetic targeting ligands on the surface of exosomes through a range of techniques. As a direct post-isolation manipulation strategy, it can be broadly classified into covalent and non-covalent modifications.[160,161,166] Covalent modification refers to the formation of stable chemical bonds between exogenous molecules and functional groups present on the exosomal membrane—such as amino groups and terminal carboxyl residues on phospholipids—through specific chemical reactions. Commonly employed covalent strategies include bio-coupling, amination, aldehyde-amine condensation, and click chemistry, with the latter being the most widely used.[167] A classic example is the copper-catalyzed azide-alkyne cycloaddition (CuAAC),[168] a ring-closing reaction widely applied in exosome engineering. For instance, Jia et al and Fan et al conjugated neuropilin-1-targeting peptides to exosome membranes via click chemistry, generating glioblastoma-targeted exosomes capable of imaging and therapy.[146,147] The Michael addition reaction between maleimide and thiol groups is also commonly used for efficient and selective modification of protein sites. Xia et al utilized azide-alkyne cycloaddition and Michael addition reactions to attach polyethyleneglycol (PEG) chains to the exosome surface.[169] The primary role of PEGylation is to significantly extend their circulation half-life by reducing immune clearance and renal filtration. This prolonged circulation time increases the probability of exosomes reaching the target tissue, thereby indirectly enhancing the overall targeting efficiency when combined with specific targeting ligands.[169] Additionally, Tian et al designed a more convenient and rapid covalent modification method, using a copper-free azide-alkyne cycloaddition method to obtain exosomes targeting the brain ischemic region.[145]

Non-covalent modification strategies mainly include hydrophobic insertion, ligand-receptor binding, and multivalent electrostatic interactions.[170] Regarding ligand-receptor binding, exosomes carry certain surface molecules that can specifically interact with corresponding ligands. For example, mixing RGD peptides with exosomes enables targeted modification through the binding of RGD to integrins. Although this method is simple to operate and highly specific, the weak non-covalent bond strength may cause the modification to detach during exosome circulation within the body, and it requires high specificity for both ligands and receptors, thus limiting its application. In a more sophisticated application, engineering the SARS-CoV-2 spike receptor-binding domain (RBD) onto exosomes allows it to bind the ACE2 receptor, which is highly expressed on lung epithelial cells, vascular endothelial cells, and pulmonary mesenchymal cells, thereby improving lung-targeting specificity. This results in a significant increase in the distribution ratio and retention efficiency of exosomes carrying therapeutic agents in the lungs, making them suitable for treating radiation-induced lung damage and long-term pulmonary fibrosis.[150] However, due to changes in temperature, solution, and ionic strength, non-covalently bound substances are prone to dissociation, which may limit the materials and applications they can modify.[171]

The fundamental goal of surface engineering is to overcome the nonspecific distribution of native exosomes by leveraging specific ligand-receptor interactions. Both genetic engineering and chemical modification have their respective advantages and limitations. Therefore, the choice between them should be strategic rather than merely technical. Genetically encoded targeting integrates the targeting motif during exosome biogenesis, resulting in homogeneous, stable display and superior biocompatibility, making it ideal for building scalable, consistent therapeutic platforms against well-defined targets. Chemically installed targeting, while potentially less stable, offers greater flexibility for using non-biological ligands and is excellent for exploratory research, personalized approaches, and rapid iteration of targeting motifs.

Indirect Functional Engineering Modification via Cellular Preconditioning

Unlike direct genetic or chemical engineering, cellular preconditioning offers a distinct bio-inspired engineering approach. It essentially induces MSCs to release exosomes that are inherently loaded with specific therapeutic miRNA cargos by mimicking pathological cues. Manipulating the cellular environment or applying physical stimuli can induce specific changes in the miRNA spectra carried by MSC-exos, thereby optimizing their regenerative and immunomodulatory functions.[172] The study found that hypoxia (1–5% O2) altered the expression of miRNAs in MSC-EVs and identified four differentially expressed miRNAs: hsa-miR-181c-5p, hsa-miR-18a-3p, hsa-miR-376a-5p, and hsa-miR-337-5p[173] can also up-regulate the expression levels of miR-210 and miR-21-5p.[174,175] Hypoxic olfactory mucosal MSC-exos upregulate miR-612 and use it as a functional messenger to promote angiogenesis, providing a new therapeutic strategy for ischemic diseases.[148]

In LPS-treated MSCs and their exosomes, the expressions of inflammatory factors TNF-α and IL-1β, lncRNA-p21, and Toll-like receptor 4 (TLR4) were upregulated, and lncRNA-p21 interacted with miR-181, miR-181 targets silent information regulator 1(SIRT1) to regulate LPS-induced inflammatory responses, thereby inhibiting the progression of SALI.[176] Compared with untreated MSC-derived extracellular vesicles, LPS-pre-treated extracellular vesicles have a better ability to regulate macrophage balance, which is attributed to their upregulation of anti-inflammatory cytokine expression and promotion of M2 macrophage activation.[177]

Inflammatory factor pretreatment can significantly enhance the therapeutic potential of MSC-exos by regulating their miRNA expression profiles. Studies have shown that human umbilical cord mesenchymal stem cells (hUC-MSCs) pretreated with Interferon-γ (IFN-γ) inhibit the NF-κB signaling pathway by activating the miR-199b-5p/AFTPH axis, demonstrating a synergistic in vitro/in vivo therapeutic effect in ALI models.[149] Further research has found that immunomodulation-related miRNAs such as miR-492, miR-133b, miR-188-3p, and miR-139-5p are significantly enriched in IFN-γ-induced exosomes. These molecules expand the therapeutic window by regulating mechanisms such as apoptosis and tissue repair.[178] It is worth noting that the dose effect of IFN-γ exhibits dual characteristics: a low dose (5 ng/mL) can inhibit the expression of pulmonary fibrosis markers, while a high dose (20 ng/mL) can simultaneously improve tissue remodeling and anti-inflammatory responses.[179] Similarly, stimulation of hUC-MSCs with a low concentration of TNF-α (10 ng/mL) can specifically increase the content of miR-146a in exosomes. When the concentration is increased to 20 ng/mL to treat human adipocyte mesenchymal stem cells, exosomes not only maintain a high expression of miR-146a but also show a significant enrichment of miR-34.[180] In addition to IFN-γ, IL-1β pretreatment has also been confirmed to reshape the miRNA composition of MSC-exos. Among them, pro-repair miRNAs such as miR-205-5p and miR-149-5p enhance tissue regeneration ability by inhibiting inflammatory signals and promoting cartilage matrix synthesis.[181] This treatment strategy can also specifically upregulate the expression of miR-21, significantly alleviating the symptoms of mice and improving the survival rate in the sepsis model.[182] Recent studies have also found that IL-23 pretreatment can achieve precise immune regulation by delivering miR-493-3p and miR-130b-3p through exosomes: The former inhibits macrophage chemotaxis by targeting MIF, while the latter induces M2-type macrophage polarization through the PTEN/PI3K/Akt pathway, providing a new target for the treatment of inflammatory diseases.[183]

Engineering the Homing Cascade: From Systemic Circulation to Targeted Delivery

While cargo loading and surface targeting are foundational, the ultimate therapeutic efficacy of engineered exosomes is dictated by their in vivo journey, which is a multi-step homing cascade.[184] This section delves into the functional outcome: how these tools are strategically deployed to master the multi-step biological journey—the homing cascade—of exosomes

in vivo. We will dissect this cascade into its key stages and explore the biological mechanisms through which specific engineering interventions overcome barriers at each step, thereby collectively enhancing or altering the homing capability.

Endogenous stem cell homing refers to the trafficking of endogenous MSCs to injured tissues, where they participate in immunodulation and tissue repair.[185] It is a natural self-healing response, encompasses systemic circulation, tissue extravasation, and specific cellular uptake. Sophisticated engineering strategies are required to overcome the biological barriers at each stage. The initial hurdle is achieving sufficient circulation time. Exosomes have a short half-life and are rapidly taken up by the mononuclear-phagocyte system.[186,187] Engineering surface properties, for instance via PEGylation, enables exosomes to extend circulatory half-life and increase the probability of exosomes reaching the target tissue.[169] The underlying mechanism involves the formation of a hydrophilic "stealth" corona that sterically hinders opsonin adsorption, thereby reducing immune clearance.[187,188] Upon reaching the target vasculature, exosomes must extravasate and specifically bind to recipient cells. This is achieved by engineering surface ligands (eg, RGD, RVG) that co-opt natural ligand-receptor systems.[143,150] This strategy hijacks highly specific cell communication pathways. For instance, RGD-integrin binding can trigger outside-in signaling that promotes clathrin-dependent endocytosis.[189]

In summary, by engineering exosomes to evade immune clearance, achieve tissue-specific arrest, and promote cellular uptake, we can enhance the natural homing capabilities of stem cells. This multi-faceted engineering approach transforms exosomes from passively distributed vesicles into active, targeted therapeutic systems, thereby paving the way for a new era of precision medicine for respiratory diseases.

The Potential of MSC-Exos-Derived miRNAs in the Treatment of Respiratory Diseases

The pathology of respiratory diseases is highly heterogeneous,[190] necessitating therapeutic strategies that are precisely tailored to the underlying mechanisms of each condition. Building upon the diagnostic miRNA signatures established in Chapter 2, which are not merely biomarkers but active functional participants in pathogenesis, we now explore how these miRNAs can be employed in therapy. MSC-exos serve as ideal natural nanocarriers for this purpose. Critically, as detailed in Chapter 3, the efficacy of these natural carriers is vastly enhanced through engineering. Engineered MSC-exos, by delivering therapeutic miRNAs to correct disease-specific dysregulations, enable a paradigm where the diagnostic biomarker simultaneously serves as a pharmacodynamic readout to monitor the efficacy of the therapeutic intervention. Through a comprehensive analysis of recent studies, we identified research indicating that engineered MSC-exos carrying miRNAs have potential therapeutic effects in lung ischemia-reperfusion injury (LIRI), pulmonary arterial hypertension (PAH), lung cancer, primarily NSCLC, ALI, pulmonary fibrosis, asthma, and other respiratory diseases (Table 4, Table S2). They can exert anti-inflammatory, antioxidant, immunomodulatory, and gene delivery effects through multiple molecular pathways by influencing target cells in lung tissue, including immune cells such as macrophages, tracheal endothelial cells, vascular endothelial cells, and epithelial cells (Figure 4).[191]

MSC-Exos-Derived miRNAs in Asthma

Current therapies, such as corticosteroids and steroid-like substances, have achieved some efficacy in asthma, but they only alleviate respiratory tract inflammatory responses and have limited efficacy in severe asthma patients.[202] Building on the diagnostic miRNA signatures discussed in Chapter 2, which reveal critical roles in asthma pathogenesis, therapeutic strategies can now be precisely designed to correct these specific dysregulations, often employing engineering techniques from Chapter 3.

Previous studies have shown that MSCs and MSC-exos have different mechanisms of action in allergic asthma mouse models,[203] while MSC-exos, which are cell-free, non-tumorigenic, less likely to block blood vessels, and highly stable, exhibit greater advantages.[204] Shan et al[192] used a BALB/c mouse model to confirm that bone marrow-derived MSC exosomes (BMSC-exos) derived miR-188 inhibits bronchial smooth muscle cell proliferation by suppressing the JARID2/Wnt/β-catenin axis. Li et al[205] found that BMSC-derived exosome miR-223-3p alleviates inflammation and airway remodeling by targeting NLRP3-induced ASC/Caspase-1/GSDMD.

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Table 4 Therapeutic Applications of MSC-Derived Exosomal miRNAs in Respiratory Diseases

Target Lung Disease	Source of MSC-exo	Model of Experimental Animal	Model of Disease	Loaded microRNA	Mechanism of microRNA	Therapeutic Effect	Reference
Asthma	hBMSC-exos	In vitro: Human bronchial smooth muscle cells (BSMCs); In vivo: BALB/c mice (6–8 weeks, 20 ± 2 g)	In vitro: transforming growth factor (TGF) - β1 induced asthma; In vivo: ovalbumin-induced asthma	miR-188	Suppressing the JARID2/Wnt/β-catenin axis	Inhibit the proliferation of bronchial smooth muscle cells in asthma	[192]
	hucMSC-exos	In vitro: 16HBE cells; In vivo: Female C57BL/6 mice (six weeks old);	Dermatophagoides farina induced asthma	MiR-146a-5p	The targeting effect of miR-146a-5p leads to the down-regulation of the expression of irak1 and TRAF6, subsequently reducing the nuclear translocation of NF-κB, which in turn results in a reduction of NLRP3 inflammasome and a decrease in the release of inflammatory cytokines.	Ameliorates inflammation	[193]
COPD	BMSC-exos	In vitro: pulmonary microvascular endothelial cells; In vivo: C57BL/6J male mice (6 weeks)	Cigarette smoke extract induced COPD	miR-30b	Targeting miR-30b/Wnt5a pathway	Inhibit the apoptosis of pulmonary microvascular endothelial cells in COPD	[106]
IPF	hESC-exo	In vitro: Beas-2b cells; In vivo: C57BL/6 male mice (8 weeks)	BLM induced IPF	miR-17-5p	Target thrombospondin-2	Anti-inflammatory and anti-fibrotic	[35]
	BMSC-exos	In vitro: not applicable; In vivo: C57BL/6 male mice (6–10 weeks, 16–21g)	BLM induced-IPF	miR-186	Targeting SOX4 and its downstream gene Dickkopf-1	Inhibiting the activation of fibroblasts and alleviating PF	[194]
NSCLC	hBMSC-exos	In vitro: A549 and H1299 cells; In vivo: Male BALB/c nude mice with a tumor xenograft lacking mature T cells in the thymus (6 weeks)	NSCLC model of tumor xenotransplantation	miR-145-5p	miR-145-5p functions by targeting SOX9.	Inhibit the progression of NSCLC	[195]
	BMSC-exos	In vitro: A549 cells and NCI-H1299 cells; In vivo: BALB/c male nude mice	Subcutaneously injected NSCLC cells	miR-30b-5p	Targeting the EZH2/PI3K/AKT axis	Promote apoptosis of NSCLC	[196]

LIRI	BMSC-exos	In vitro: MLE-12 cell line In vivo: C57BL/6 mice	In vitro: H/R-induced LIRI cell mo In vivo: Unilateral hilar occlusion-reperfusion induced LIRI	miR-202-5p	Suppressing pyroptosis to inhibit LIRI progression by targeting CMPK2	Inhibit LIRI progression	[197]
PAH	hASC-exos	In vitro: human pulmonary artery endothelial cells; In vivo: male Sprague-Dawley rats (240 ± 30 g)	Monocrotaline pyrrole induced PAH	miR-191	miR-191 affects the development of PAH by targeting and regulating BMPR2.	Alleviate the progress of PAH	[198]
	BMSC-exos	In vitro: pulmonary artery smooth muscle cells; In vivo: Sprague-Dawley mice (4 weeks, weighing 150–200 g).	Monocrotaline-induced PAH	miR-200b	miR-200b accelerates the transformation of macrophages to the M2 phenotype and targets PDE1A to induce PKA phosphorylation, accelerating macrophage polarization and thereby reversing PAH-related diseases	Inhibit the formation of PAH	[199]
ALI	hUC-MSCs-exos	In vitro: A549 and Human primary small airway epithelial cells; In vivo: Male C57BL/6 mice (18–22 g)	LPS-induced ALI model	miR-223-3p	miR-223-3p targets lung epithelial cells and then down-regulates PARP-1 to inhibit the inflammation of lung epithelial cells induced by LPS	Alleviate ALI	[200]
	BMSC-exos	In vitro: not applicable; In vivo: C57Bl/6J mice (six weeks old)	LPS-induced ALI model	miR-150	Targeting the MAPK pathway	Alleviate ALI	[201]

Abbreviations: MSC, Mesenchymal Stem Cell; MSC-exo, Mesenchymal Stem Cell-derived exosomes; hBMSC, human Bone Marrow-derived Mesenchymal Stem Cell; hUc-MSC-exos, human umbilical cord-derived mesenchymal stem cell exosomes; BMSC-exos, bone marrow mesenchymal stem cell-derived exosomes; HESC-exos, human embryonic stem cell-derived exosomes; hASC, human adipose stem cells; COPD, chronic obstructive pulmonary disease; IPF, Idiopathic Pulmonary Fibrosis; NSCLC, non-small cell lung cancer; LIRI, Lung Ischemia-Reperfusion Injury; PAH, pulmonary arterial hypertension; ALI, acute lung injury; BSMCs, Bronchial Smooth Muscle Cells; BLM, bleomycin; Irak1, Interleukin-1 receptor-associated kinase 1; TRAF6, TNF receptor-associated factor 6; NF-κB, Nuclear Factor Kappa-light-chain-enhancer of activated B cells; NLRP3, NLR Family Pyrin Domain Containing 3; SOX4, SRY-Box Transcription Factor 4; SOX9, SRY-Box Transcription Factor 9; EZH2, Enhancer of Zeste Homolog 2; PI3K, phosphoinositide-3 kinase; AKT, serine/threonine protein kinase b; CMPK2, Cytidine/uridine Monophosphate Kinase 2; BMPR2, Bone Morphogenetic Protein Receptor Type 2; PDE1A, Phosphodiesterase 1A; PKA, protein kinase A; PARP-1, Poly(ADP-ribose) polymerase 1; MAPKA, Mitogen-Activated Protein Kinase; LPS, lipopolysaccharide; BALB/c, A strain of laboratory mouse; C57BL/6, A strain of laboratory mouse.

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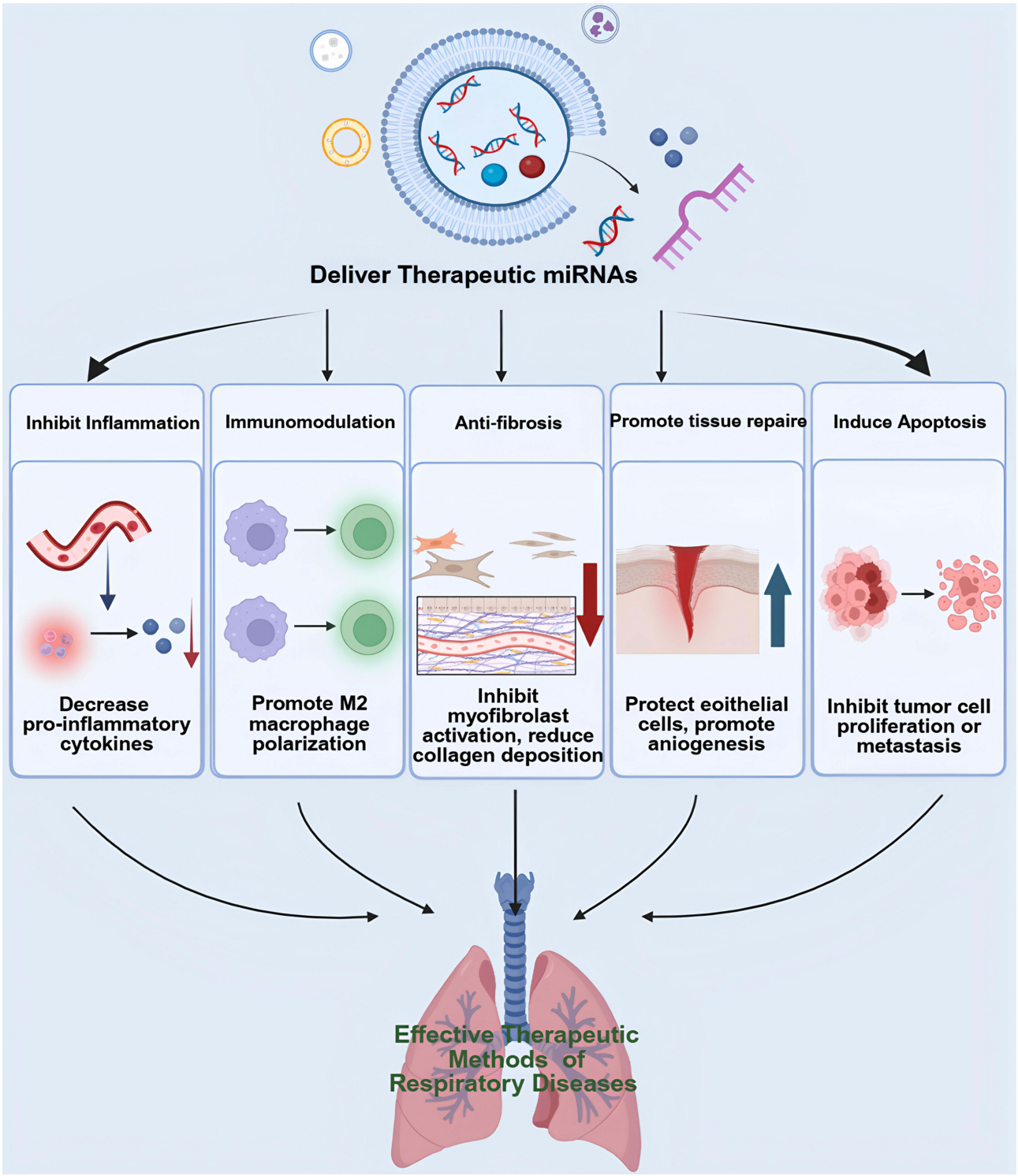

Figure 4 Therapeutic mechanisms of engineered exosome-derived miRNAs in respiratory diseases. Engineered exosomes deliver therapeutic miRNAs to the lung microenvironment, eliciting multifaceted therapeutic effects including inhibition of inflammation, immunomodulation, anti-fibrosis, promotion of tissue repair, and induction of apoptosis, collectively contributing to the treatment of various respiratory diseases.

In recent years, the underlying mechanisms linking macrophage polarization imbalance to asthma pathogenesis have become increasingly clear.[206] The reversal of M1 macrophages to the M2 phenotype has been shown to attenuate airway remodeling.[207] Tu et al found that miR-511-3p can reverse the conversion of macrophages to the M2 phenotype. They also developed mannosyl-modified exosomes (a chemical surface modification strategy) to achieve targeted delivery of

miR-511-3p to macrophages and identified complement C3 as the primary target of miR-511-3p.[208] This approach exemplifies theranostics, in that diagnostic evidence of miR-511-3p's role in macrophage polarization directs its therapeutic application.

MSC-Exos-Derived miRNAs in COPD

Conventional treatments for COPD (such as drug therapy, oxygen therapy, pulmonary rehabilitation, and surgery) can alleviate symptoms but are limited in efficacy and associated with severe adverse effects.[209,210] As we mentioned in Chapter 2, the pathological features of COPD demand therapeutic strategies focused on modulating the chronic inflammatory microenvironment and halting tissue remodeling. Reflecting this pathophysiology, MSC-exos exert beneficial effects by delivering miRNAs that target these core processes.

Studies have shown that human umbilical cord mesenchymal stem cell exosomes can prevent and protect against papain-induced pulmonary emphysema-induced apoptosis, with miRNAs potentially involved in this protective effect.[107] In another study, Song et al investigated the role and mechanism of BMSC-exos in COPD lung microvascular endothelial cell apoptosis. They found that BMSC-exos could attenuate cigarette smoke-induced lung microvascular endothelial cell apoptosis, a mechanism achieved through miR-30b targeting Wnt5a, which was validated in an animal model.[106] This study revealed a new mechanism for treating COPD, namely, using MSC-exos to deliver miR-30b to target Wnt5a.

MSC-Exos-Derived miRNAs in IPF

Current treatment options for IPF are limited, and there is an urgent need to overcome treatment challenges. First-line drugs for IPF include pirfenidone and nintedanib, as well as non-pharmacological treatments such as oxygen therapy, pulmonary rehabilitation, mechanical ventilation, and palliative care.[211,212] While these interventions can slow disease progression, they cannot reverse the fibrotic process, with patients having a median survival of only 2 to 3 years. Lung transplantation can improve survival rates but is limited by high costs and a shortage of donors.[213] The distinct pathology of IPF dictates that therapeutic exosomes must be engineered to directly counteract pro-fibrotic signaling.

MSCs and their derived exosomes have demonstrated anti-fibrotic potential in animal models, offering new hope for IPF treatment.[214,215] Zhou et al established a bleomycin-induced IPF mouse model and administered treatment via tail vein injection, finding that miR-186 carried by BMSC-exos targets SOX4 and its downstream gene DKK1, thereby inhibiting fibroblast activation and alleviating IPF.[194] More innovatively, Zhang et al used adenovirus infection of hUC-MSCs to obtain miR-486-5p-engineered MSCs (miR-486-MSCs) to enhance their anti-fibrotic effects, and anchored SARS-CoV-2-S-RBD on the surface to enhance the lung-targeting ability of exosomes.[150] In vivo experiments ultimately demonstrated that miR-486-RBD-MSC-exos improved survival rates in mice with pulmonary fibrosis, reduced collagen deposition, and showed a significant reduction in fibrotic areas on CT scans.[150]

The study by Zhang et al represents a sophisticated theranostic design. The diagnostic observation of miR-486-5p dysregulation informs the choice of therapeutic cargo.[216] The engineered exosome is both the drug (carrying miR-486-5p) and the delivery vehicle. The treatment's efficacy is monitored by tracking the delivered miRNA and the resultant improvement on CT scans, creating a seamless diagnostic-therapeutic-monitoring continuum.

MSC-Exos-Derived miRNAs in NSCLC

Clinical treatment of NSCLC requires the development of individualized strategies based on molecular typing.[217] Current treatment strategies primarily include surgical resection, radiotherapy, chemotherapy, targeted therapy, and immunotherapy.[218] However, chemotherapy and radiotherapy cause significant damage to normal tissues, impairing quality of life. The long-term efficacy of targeted therapy and chemotherapy is limited by tumor heterogeneity and microenvironmental regulation, and existing therapies struggle to target metastatic lesions or modulate the tumor microenvironment.[219,220] As a cell-free therapy, MSC-exos can avoid the tumorigenic risks associated with live cell transplantation, exhibit high stability, and target specific sites in NSCLC.[221] The focus is to halt uncontrolled proliferation, induce apoptosis, and reverse immune evasion.

Yan et al[195] used a BALB/c nude mouse model to demonstrate that BMSC-exos' miR-145-5p inhibits NSCLC progression by targeting SOX9. Wu et al[196] reported that BMSC-exos' miR-30b-5p promotes NSCLC cell apoptosis through the EZH2/PI3K/AKT axis. Liang et al[222] found that BMSC-exos' miR-144 inhibits NSCLC progression by

targeting CCNE1 and CCNE2. Liu et al[223] confirmed that miR-204 in BMSC-exos inhibits NSCLC cell EMT, migration, and invasion by targeting KLF7 and regulating the AKT/HIF-1α axis. Sun et al[224] reported that miR-101-3p in BMSC-exos enhances NSCLC sensitivity to radiotherapy by regulating EZH2.

In NSCLC, the theranostic approach is particularly powerful. For instance, if a patient's tumor is diagnosed with low miR-145-5p, therapy with miR-145-5p-enriched exosomes can be initiated. The subsequent increase in miR-145-5p levels in circulating exosomes confirms successful delivery, while tumor imaging assesses the functional response. This links a specific molecular diagnosis to a targeted treatment and a quantifiable monitoring plan.

MSC-Exos-Derived miRNAs in LIRI

LIRI primarily occurs in lung transplantation, which is the main reason for primary graft dysfunction (PGD), and is a major cause of mortality and morbidity in the postoperative period.[225] Its pathophysiological mechanisms involve ischemia-induced cell death and reperfusion-induced activation of inflammatory mediators and reactive oxygen species, leading to primary graft dysfunction, which is the primary cause of lung transplantation failure and mortality.[226] Various treatment modalities have been developed for LIRI, including ex vivo lung perfusion (EVLP), surfactants, inhaled β2-adrenergic receptor agonists, fibrinolytic therapy, and MSC therapy. However, EVLP itself may induce an inflammatory response; surfactants are costly and rarely used; corticosteroids only inhibit a single inflammatory pathway and cannot simultaneously regulate pyroptosis, oxidative stress, and repair mechanisms, and systemic administration may result in insufficient drug distribution in lung tissue, while local administration, such as inhalation, has technical limitations. Additionally, long-term use of immunosuppressive agents may increase the risk of infection, limiting the application of these therapies.[227,228]

MSC-exos offer greater safety and demonstrate advantages in LIRI treatment, including multi-target regulation and low side effects, particularly in inhibiting pyroptosis and promoting repair, outperforming traditional therapies.[3] Yang et al[229] established a LIRI model in Lewis mice and confirmed through in vitro studies that hUC-MSCs suppress NLRP3 inflammasome activation by delivering miR-146a, thereby reducing inflammation and LIRI. This demonstrates a clear theranostic loop. The diagnostic identification of NLRP3 inflammasome activation in LIRI guides a therapeutic strategy that delivers miR-146a to counteract this pathway. The reduction in inflammatory markers post-treatment validates the therapeutic effect, using the pathogenic pathway itself as the readout. What's more, Sun et al[197] found that miR-202-5p from BMSC-exos inhibits the expression of pyroptosis-related proteins (GSDMD-N, NLRP3) by targeting CMPK2. Gao et al[230] reported that miR-381 from BMSC-exos alleviates inflammatory responses by inhibiting YTHDF1 and activating Treg cell differentiation. Li et al[231] confirmed that miR-21-5p in BMSC-exos inhibits the apoptosis pathway by targeting PTEN and PDCD4.

MSC-Exos-Derived miRNAs in PAH

PAH is a fatal vasculopathy due to a progressive increase in pulmonary vascular resistance and eventual right ventricular failure.[232] Various treatment modalities for PAH exist, however, traditional PAH treatments primarily address symptoms rather than reversing disease progression. Emerging treatment approaches targeting the BMPR2 pathway, GF/TK signaling pathways, immunity, and inflammation are also under investigation.[233]

With a deeper understanding of the pathogenesis of PAH, the expression of various miRNAs is significantly altered in PAH, suggesting they may serve as promising potential therapeutic targets for PAH. Experimental findings indicate that MSC-exo-derived miRNAs can regulate pulmonary vascular remodeling through multi-target regulation, demonstrating potential advantages as a therapeutic approach. For example, Zhang et al[198] used a Sprague-Dawley rat model to confirm that hASC-exos deliver miR-191 to the target and regulate BMPR2 in the development of PAH. Wan et al[74] found that miR-200b from BMSC-exos accelerates the conversion of macrophages to the M2 phenotype, reversing PAH-related diseases. Other studies have found that KLF2 induces the production of miR-181a-5p and miR-324-5p by human pulmonary arterial endothelial cell exosomes, which target Notch4 and ETS-1, inhibiting abnormal proliferation and inflammatory responses in vascular endothelial cells and vascular smooth muscle cells. This confirms that therapeutic supplementation with miR-181a-5p and miR-324-5p reduces cell proliferation and vascularization responses, thereby alleviating the progression of PAH.[234]

MSC-Exos-Derived miRNAs in ALI

ALI represents a severe, diffuse alveolar insult with high mortality, often triggered by sepsis, pneumonia, or aspiration.[235] The pathology is characterized by uncontrolled inflammation, alveolar epithelial and endothelial damage, and pulmonary edema. Traditional treatment methods primarily include mechanical ventilation, fluid management, anti-inflammatory therapy, and symptomatic support.[236] However, positive pressure ventilation may improve oxygenation but may exacerbate lung injury; restricting fluid intake may reduce pulmonary oedema but requires balancing organ perfusion. Glucocorticoids are used to suppress inflammatory responses, but long-term use has significant side effects.

Research on the use of MSC-exos carrying miRNA for the treatment of ALI has been extensively studied.[237] It has been found that the miRNA carried by MSC-exos can achieve precise anti-inflammatory, anti-apoptotic, and reparative effects, as well as immune regulation, to alleviate ALI while avoiding secondary damage caused by immunogenicity.[238] For example, Lin et al[239] found that MSC-exos miR-7704 inhibits inflammation and improves ALI by driving macrophage M2 polarization. In another study,[240] it was reported that BMSC-exos miR-23b-3p inhibits M1φ activation through the Lpar1-NF-κB signaling pathway. Peng et al[241] found that iPSC-derived MSC-exos deliver miR-125b-5p to target TRAF6, thereby inhibiting inflammation and improving ALI. In terms of anti-apoptosis and repair, Wei et al[242] reported that BMSC-exos miR-377-3p induces autophagy by targeting RPTOR, thereby improving ALI. Tao et al[243] confirmed that BMSC-exos miR-125b-5p mediates macrophage pyroptosis by targeting STAT3. Qianfei et al[244] found that BMSC-exos miR-26a-3p regulates apoptosis, inflammation, and autophagy by targeting PTEN.

Summary of Therapeutic Effects

The therapeutic potential of MSC-exos-derived miRNAs, as detailed in this chapter, is not an independent endeavor but the direct translational continuation of the diagnostic foundation established in Chapter 2. The journey from biomarker discovery to effective therapy embodies the core principle of theranostics.

The identified dysregulation of specific miRNAs in patient samples does more than just signal disease presence; it pinpoints functional drivers of pathology. This diagnostic insight provides the critical rationale for therapeutic targeting. This vertical theranostic approach—where the same miRNA is both the diagnostic signal and the therapeutic target—is powerfully exemplified in studies where correction of a specific miRNA aberration (eg, miR-486-5p in IPF) directly translates to functional improvement, with the miRNA's level serving as a built-in pharmacodynamic biomarker. More commonly, a horizontal theranostic strategy emerges, reflecting the complexity of respiratory diseases. Here, the diagnostic miRNA profile of an entire pathological network informs a multi-targeted therapeutic intervention. Even when the specific therapeutic agent differs from the initial diagnostic marker, the efficacy of this intervention is then monitored by assessing the normalization of the broader pathological network.

In these studies, the most used engineering method is transfection to achieve overexpression or knockdown of miRNA to enhance therapeutic efficacy. The ultimate goal is to close the theranostic loop, using the diagnostic miRNA signature to select a therapy and then using the subsequent change in that signature, or in key downstream pathological indicators. However, how to optimise drug delivery efficiency through surface modification and drug encapsulation remains a focus for future research. Engineering may also raise safety concerns, such as the immunogenicity risk associated with S-RBD modification, which has not been assessed.[150] Additionally, MSC-exos have complex compositions and play diverse roles in various pathological processes, and some studies have failed to elucidate the specific mechanisms underlying their therapeutic effects.

In summary, the transition from the diagnostic biomarkers of Chapter 2 to the therapeutic applications of this chapter charts a clear path toward a true theranostic paradigm in respiratory medicine. MSC-exos, by providing a versatile platform to therapeutically modulate disease-associated miRNA signatures identified through diagnostic profiling, enable a future of feedback-informed, personalized treatment where diagnosis and therapy are inextricably linked.

Challenges and Solutions for MSC-Exos-Derived miRNAs in Clinical Translation

GMP-Compliant Production Standards and Clinical Trials

As previously mentioned, the diagnostic and therapeutic effects of MSC-exos and miRNAs have been fully demonstrated in preclinical studies. However, their clinical translation remains challenging due to issues in production and safety. The Good Manufacturing Practice (GMP) production standards for MSC-exos emphasize comprehensive quality control throughout the entire process—from the cell source to the final product—including cell bank verification, culture contamination control, and exosome characterization/functional activity testing.[245–247] The safety, efficacy, and batch-to-batch consistency of the product are ensured by these measures, establishing a reliable foundation for clinical application.

A Phase I clinical trial of MRX34 (a miR-34a mimic) delivered via a liposome-based system for NSCLC was terminated due to severe immune-related adverse events, although stable disease (indicating halted tumor growth) was observed.[248] While this trial demonstrated the efficacy of miRNA therapy, it also highlighted the challenges of immunogenic toxicity and lack of targeting specificity associated with the delivery system. The characteristically low immunotoxicity of MSC-exos may consequently enhance their therapeutic efficacy.[249] Figueroa Valdés et al[245] established a manufacturing process for h UC-MSC-EVs, whose product exhibited positive therapeutic effects in its first-in-human application for knee osteoarthritis (OA). In the respiratory system, Li et al[250] developed a GMP-compliant manufacturing process for hUC-MSC-EVs, which not only significantly increased the survival rate (from 20% to 80%) and alleviated fibrosis in a bleomycin-induced mouse pulmonary fibrosis model, but also demonstrated marked improvement in lung function in patients during Phase I clinical trials. Similarly, in Phase II clinical trials, both ExoFlo (BMSC-exos) and human placental MSC-exos significantly reduced mortality rates in COVID-19 patients with respiratory failure.[246,247] Despite early-stage trials of GMP-compliant MSC-exos, their clinical translation is hindered by heterogeneity and safety concerns, necessitating large-scale validation and standardized protocols.

Differences in Therapeutic Efficacy of MSC-Exos from Different Sources

Due to the diverse sources of MSC-exos, including bone marrow, adipose tissue, placenta, and umbilical cord,[251] the miRNA profiles carried by MSC-exos from different sources exhibit significant differences, leading to inconsistent therapeutic efficacy.[172] Existing studies have shown that BMSC-exos primarily carry miRNAs related to bone/ cartilage repair and protection-related miRNAs,[252–254] primarily applied in anti-inflammatory (eg, miR-199a-3p),[255] anti-fibrotic (eg, miR-204-5p),[256] and pro-angiogenic (eg, miR-21-5p[257]). Adipose-derived MSC exosomes (ADSC-exo) carry miRNAs that are more inclined toward repairing damage caused by metabolic or inflammatory conditions, such as promoting scar-free healing of diabetic wounds through the miR-204-5p/TGF-β1/Smad pathway,[258] or by delivering miR-181a-5p to target STAT3 signaling and alleviate lung damage induced by Klebsiella pneumoniae infection.[259] They also play roles in anti-EMT[260] and anti-fibrosis while promoting angiogenesis.[261] Placental/umbilical cord-derived MSC exosomes (PMSC/UC-MSC-exos) highly express miRNAs related to embryonic development, promoting cellular reprogramming and tissue regeneration.[262] Additionally, due to this heterogeneity, MSC-exos from different sources exhibit varying therapeutic effects. Wharton's Jelly-derived MSC-exos promote keratinocyte growth and migration more effectively than AD-MSC-exos, demonstrating higher wound healing potential.[263]

To address the heterogeneity of MSC-exos, proteomics systems can be used to comprehensively analyse human MSC-exos, revealing their potential applications in various fields.[264] Predicting miRNA target genes and determining their biological processes.[265] High-throughput sequencing technologies (Next-Generation Sequencing, NGS) can be used to identify expression differences in disease-associated miRNAs,[265,266] thereby identifying therapeutic targets. Based on the above processes, a source-disease matching database can be constructed to determine which disease benefits most from which type of MSC-exos carrying which miRNA.

Safety Concerns in Clinical Translation

Biodistribution

Enhancing the targeting capability of drugs and their delivery systems has long been a sustained objective in the field of biomedical engineering. The seed sequence at the miRNA 5' end enables binding with imperfect complementarity,[267]

facilitating a "one-to-many" regulatory mode and consequently triggering hybridization-dependent off-target effects.[268,269] Additionally, non-hybridization-dependent off-target effects may also occur due to: (1) non-specific uptake of the delivery system;[270] (2) immune activation preventing miRNA from reaching the therapeutic target;[270] (3) competition between therapeutic miRNAs and endogenous miRNAs for key processing proteins like Ago2, disrupting endogenous miRNA maturation/function and causing dysregulated gene expression.[271] Drug accumulation in off-target tissues or organs reduces the effective concentration at the target site, undermining therapeutic efficacy; conversely, it may also directly cause damage to non-target tissues and even induce hepatorenal toxicity.

To address the "one-to-many" regulatory mode of miRNAs, on the one hand, deep learning models like TEC-miTarget can be utilized to reduce their reliance on the seed region during target gene recognition, thereby mitigating the off-target effects caused by imperfect base pairing;[272] on the other hand, maintaining the GC content within 30–50% can ensure in vivo stability and enhance binding precision to the target genes.[273,274] Furthermore, by incorporating modifications such as 2'-O-methylation or locked nucleic acids into the seed region, the affinity and specificity of miRNAs for their target genes can be enhanced.[274–276] To address non-hybridization-dependent off-target effects, engineered delivery systems can be optimized to enhance targeting specificity (see Direct Engineering Strategies for Surface Modification for details).

Immunogenicity Assessment

Overall, it has lower immunogenicity and immunomodulatory properties compared to traditional delivery systems, which may be related to the lack of major histocompatibility complex molecules on the surface of exosomes.[277] In animal models, the EV delivery system delivers vascular endothelial growth factor A (VEGF-A) mRNA in a mouse model of ischemic injury without significant activation in both innate and adaptive immune systems compared to adeno-associated virus and Lipid nanoparticles (LNPs);[278] no significant adverse effects were observed in cynomolgus monkeys following the injection of 3.85×10^{12} hUC-MSC-exos.[249] Additionally, the first-in-human trial of MSC-EVs for knee osteoarthritis and its 12-month follow-up revealed no treatment-related adverse events.[245] These preclinical and clinical evidence together establish the excellent compatibility of MSC-exos as a low-immunogenicity delivery system, providing a safe basis for clinical translation.

Nevertheless, this does not imply that MSC-exos are completely non-immunogenic. Currently, therapeutic exosomes can be derived from xenogeneic or allogeneic sources, and their surface proteins may be recognized as "non-self" by the host immune system, potentially triggering immune responses.[33] Although allogeneic exosomes are less likely to cause an immune response, they are at higher risk after multiple injections.[279] Additionally, the introduction of exogenous proteins, nucleic acids, and other components during drug loading, as well as ligands and peptides introduced through surface modifications, may alter the inherent surface properties of exosomes and increase the risk of immune recognition.[280,281] When using loading methods such as electroporation that compromise the integrity of the exosome membrane, the leakage of its cargo may occur, thereby potentially triggering severe inflammatory responses.[33] Impurities introduced during exosome and miRNA isolation and purification can also affect overall immunogenicity.[282] Adverse reactions caused by this include acute immune reactions such as fever and chills, anaphylaxis, autoimmune reactions, and even cytokine storms. Given this, cell sources should be optimized during the production phase, and serum-free media should be used, with impurities removed through multi-step purification. In clinical applications, initial dosages must be carefully determined, immune responses closely monitored, and emergency intervention measures prepared.

Potential Toxicity of Engineered Exosomes

As previously mentioned, exosomes hold therapeutic potential but exhibit disease-promoting or resistance-inducing properties in pathological microenvironments (particularly tumors), limiting clinical application. In the respiratory system, PM2.5-induced exosomal miR-129-2-3p targets the TIAM1/RAC1/PAK1 pathway, disrupting the airway epithelial barrier and exacerbating asthma;[41] silicosis patient-derived exosomal miR-23a-3p mediates macrophage-epithelial cell communication, accelerating pulmonary fibrosis.[283] Multiple exosomal miRNAs critically contribute to lung cancer initiation, progression, drug resistance, and immune evasion.[284] Notably, miRNA functions are context-dependent: MiR-223-3p acts as a tumor suppressor in breast cancer, NSCLC, and glioblastoma, yet promotes malignancy in colorectal,

ovarian, pancreatic cancers, and acute lymphoblastic leukemia,[285] highlighting potential off-target organ damage and necessitating precise delivery systems for miRNA therapies. Regarding miRNA-mediated enhancement of drug resistance, exosomal miR-21 transmission confers methotrexate resistance to sensitive cells, correlating with poor prognosis in NSCLC leptomeningeal metastasis.[286] Similarly, miR-130a-3p in resistant lung adenocarcinoma cells downregulates RUNX3 to reduce osimertinib efficacy and propagates resistance via extracellular vesicles.[287] These studies not only provide new directions for targeted inhibition but also suggest the need to block the cancer-promoting and resistance-mediating functions of exosomes. Therefore, when advancing exosome therapies, we must remain vigilant about their inherent "double-edged sword" nature; any engineering modifications may, due to their unpredictability in complex in vivo microenvironments, lead to new safety risks or unintended consequences.

The aforementioned engineering methods can enhance drug accumulation in target organs to avoid damage to non-target organs. Furthermore, single-cell RNA sequencing (scRNA-seq) can be utilized to classify MSCs into subpopulations, identify subsets with distinct functional characteristics, and screen and characterize relevant miRNAs to avoid the use of pathogenic or resistance-promoting miRNAs.[288–290] Simultaneously, genetic engineering techniques can be employed to target and silence specific miRNAs in MSCs, preventing their mediated toxic responses.[291]

Long-Term Safety

The long-term safety of mesenchymal stem cell-derived exosomes for knee osteoarthritis therapy has been demonstrated. Following the first-in-human administration of hUC-MSC-exos for knee osteoarthritis, no adverse events were reported during 12-month follow-up;[245] another pre-clinical trial[292] involving 41 patients receiving this exosome therapy showed no treatment-related adverse events or significant safety concerns after 9-month long-term follow-up. Regarding respiratory disease therapeutics, a clinical trial on nebulized hUC-MSC-EVs for pulmonary fibrosis reported no adverse events throughout 12-month follow-up;[250] in a phase II trial using bone marrow mesenchymal stem cell-derived exosomes for COVID-19 respiratory failure, no drug-related adverse events or serious adverse events were observed during the 60-day monitoring period.[246] Overall, mesenchymal stem cell-derived exosomes exhibit favorable therapeutic potential and safety profiles. However, their clinical application remains at an early stage, requiring validation of long-term efficacy through Phase III or IV clinical trials alongside systematic assessment of their extended impact on patient survival rates and quality of life for comprehensive clinical translation.

Regulatory Hurdles

Despite their significant therapeutic potential, no exosome-based therapeutic had received food and drug administration (FDA) approval as of October 2025, underscoring the regulatory hurdles. Although the International Society for Extracellular Vesicles (ISEV) mandates quantitative characterization using particle-tracking techniques to define physical and biochemical integrity, its guidelines do not stipulate specific criteria for engineered exosomes co-loaded with therapeutic miRNAs.[293]

Regarding regulatory classification, the European Union designates them as Advanced Therapy Medicinal Products (ATMPs) under Regulation (EC) 1394/2007, subjecting them to corresponding approval and data requirements.[294] In contrast, other regions may regulate them by emphasizing their nature as "cell-derived products"[295] or focusing on their role as nanocarriers.[296] This inconsistent classification creates complexities in designing preclinical and clinical trials, particularly in safety assessment, potency determination, and batch-to-batch consistency. Manufacturing standardization remains a major challenge. This is due to the stringent controls required for GMP-compliant production across the entire process, from cell sourcing to final product, combined with a lack of universal quality standards for critical attributes like purity and function, which together impede reproducibility and scalability.

Therefore, establishing consensus guidelines for exosome characterization and potency assessment and developing standardized, scalable production platforms that ensure product consistency are imperative.

Conclusions and Future Prospects

Current drug delivery systems largely rely on LNPs and viral vectors, yet their drawbacks, such as strong immunogenicity and low targeting specificity, remain unavoidable.[297,298] MSC-exos possess inherent low immunogenicity, effectively

avoiding rapid clearance by the mononuclear phagocyte system, thereby prolonging in vivo circulation time and reducing the likelihood of immune reactions.[277] Simultaneously, inheriting the natural tropism of parent cells towards injured, inflamed, and tumor sites, MSC-exos preferentially accumulate in pathological tissues, achieving active targeting.[14,15] Consequently, MSC-exos represent an ideal nanocarrier. In clinical practice, detection technologies can identify significantly altered expression levels of specific miRNA sets in diseases compared to healthy states, suggesting these aberrant miRNAs can serve as potential biomarkers.[299] Bioinformatics analysis enables the association of these miRNAs with their regulated genes and signaling pathways, thereby providing therapeutic strategies for correcting such abnormal expression.[300] Integrating the carrier advantages of exosomes with the dual potential of miRNAs as biomarkers and therapeutic targets allows not only the development of diagnostic kits and therapeutic drugs but also dynamic monitoring of treatment efficacy and prognosis. Engineering this system can achieve the following objectives: (1) screening and loading of therapeutic miRNAs; (2) enhancing lung tissue or specific cell targeting through surface modifications; (3) improving exosome stability to extend their in vivo circulation time.[160,171]

However, translating these promising findings from the laboratory to clinical application still faces many challenges. Key issues requiring resolution include biodistribution, immunogenicity, potential toxicity, and long-term efficacy.[191] The main challenge involves establishing robust, scalable manufacturing processes compliant with GMP standards, alongside validating product quality, safety, and efficacy through clinical trials. Specific GMP requirements include rigorous donor cell screening and master cell bank establishment, standardized and scalable production processes, and comprehensive quality control (QC) systems to ensure critical parameters such as exosome purity, concentration, morphology, and bioactivity.[293] The current clinical trial landscape for mesenchymal stem cell-derived exosomes (MSC-exos) remains developing, demonstrating favorable therapeutic outcomes without reported adverse events, yet is confined to Phase I or II studies without any approved drugs; furthermore, there is a notable lack of research on engineered MSC-exosomes carrying specific miRNAs for respiratory diseases.[245–247]

Future research should not only focus on continuously enhancing the therapeutic efficacy of MSC-exos through engineering technologies, but also prioritize advancing and optimizing their clinical translation pathway. This requires systematically designing and conducting multicenter clinical trials to verify not only short-term safety but also assess the durability of therapeutic effects and potential adverse reactions through long-term follow-up. Concurrently, it is essential to determine the optimal administration routes (intravenous injection or nebulized inhalation) and establish scientifically sound dosing strategies, thereby enabling in-depth investigation of how individual patient differences influence treatment outcomes. Collection of clinical application data will inform personalized treatment approaches, while constructing individualized therapeutic frameworks based on miRNA signature profiles will enhance intervention specificity. In addition, the establishment of standardized manufacturing procedures and safety evaluation criteria will be essential for the clinical application of MSC exosomes. Ultimately, by developing a multidimensional diagnostic and therapeutic system centered on exosomal miRNAs, we can achieve precise, comprehensive management of respiratory diseases—encompassing early intervention, dynamic monitoring, and personalized treatment throughout the entire clinical workflow.

Abbreviation

MSC, mesenchymal stem cells; Exos, exosomes; miRNA, microRNA; ncRNA, non-coding RNA; COPD, chronic obstructive pulmonary disease; EVs, extracellular vesicles; MVBs, multivesicular bodies; lncRNA, long non-coding RNA; rRNA, ribosomal RNA; mRNA, messenger RNA; AT2, alveolar type II epithelial cells; GMP, Good Manufacturing Practice; ARDS, acute respiratory distress syndrome; BALF, bronchoalveolar lavage fluid; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition; TME, tumor microenvironment; WHO, world health organization; TIMP2/3, tissue inhibitor of metalloproteinases 2/3; ALI, acute lung injury; SALI, sepsis-induced acute lung injury; 3D, three-dimensional; UC, ultracentrifugation; UF, ultrafiltration; PEG, polyethylene glycol; IEX, ion exchange chromatography; SAP, superabsorbent polymer; SEC, size exclusion chromatography; EXODUS, exosome detection via the ultrafast-isolation system; TLR4, toll-like receptor 4; SIRT1, silent information regulator 1; hUC-MSCs, human umbilical cord mesenchymal stem cells; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; IL-1β, interleukin-1 beta; NPs, nanoparticles; RGD, arginine-glycine-aspartic acid peptide; RVG, rabies virus glycoprotein; TAT, trans-activator of transcription protein; GPI, glycosylphosphatidylinositol;

EGFR, epidermal growth factor receptor; S-RBD, SARS-CoV-2 spike receptor binding domain; ACE2, angiotensin-converting enzyme 2; LIRI, lung ischemia-reperfusion injury; EVLP, ex vivo lung perfusion; PAH, pulmonary arterial hypertension; BMPR2, bone morphogenetic protein receptor type 2; ADSC, adipose-derived stem cells; BMSC, bone marrow mesenchymal stem cells; PMSC, placental mesenchymal stem cells; UC-MSC, umbilical cord mesenchymal stem cells; scRNA-seq, single-cell RNA sequencing; NGS, next-generation sequencing; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors declare that they have no conflict of interest in this review.

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