



tRNA-derived small RNAs in pulmonary diseases: Regulatory functions and clinical prospects

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ABSTRACT

Transfer RNA-derived small RNAs (tsRNAs) are evolutionarily conserved regulatory molecules produced by stress-responsive endonucleases that cleave mature tRNAs or their precursors. Emerging evidence suggests that aberrant tsRNA expression contributes to the pathogenesis and progression of pulmonary diseases. This review consolidates the current understanding of tsRNA biogenesis, molecular functions, and potential clinical applications in both malignant and non-malignant lung disorders. Clinically, tsRNAs show great promise as diagnostic biomarkers and therapeutic targets for lung diseases, although technical challenges in detection and functional validation necessitate further research.

1. Introduction

Pulmonary diseases, including lung cancer, chronic obstructive pulmonary disease (COPD), idiopathic pulmonary fibrosis (IPF), and pulmonary infectious diseases, continue to pose a major global public health challenge, contributing substantially to morbidity and mortality worldwide [1–3]. Lung cancer alone accounts for 1.8 million deaths annually, representing the leading cause of cancer-related deaths globally [4–6]. Non-neoplastic conditions, such as COPD and IPF, similarly impose significant morbidity, primarily due to chronic inflammation, oxidative stress, and epigenetic dysregulation [7]. Given the substantial impact of these pulmonary diseases on global healthcare systems, the advancement of precision diagnostics and treatments is imperative.

Transfer RNA-derived small RNAs (tsRNAs), also referred to as tRNA-derived fragments (tRFs), tRNA-derived stress-induced RNAs (tiRNAs), or tRNA halves, are expressed in a wide range of tissues and organs [8]. These molecules are generated through the cleavage of mature tRNAs or tRNA precursors by endonucleases (e.g., angiogenin (ANG), Dicer, RNase Z, and RNase P) [9,10]. Emerging evidence suggests that aberrant expression of tsRNAs is strongly associated with malignant and non-malignant lung pathogenesis [11]. The increasing interest in

tsRNAs underscores their potential as clinical biomarkers for diagnosis and as targets for therapeutic interventions [12]. This review aims to synthesize current research findings regarding the diagnostic, mechanistic, and therapeutic implications of tsRNAs in pulmonary diseases.

2. Classification, biogenesis and structure of tsRNAs

2.1. Classification of tsRNAs

Transfer RNA (tRNA), a non-coding RNA, plays a crucial role in protein synthesis by recognizing and transporting specific amino acids [13]. It has a cloverleaf-like secondary structure consisting of four arms (D arm, anticodon arm, T ψ C arm, and acceptor stem) and four loops (D loop, anticodon loop, T ψ C loop, and variable loop) [14]. In eukaryotic cells, tRNA genes are transcribed into precursor tRNAs (pre-tRNAs) by RNA polymerase III within the nucleus. Subsequently, these pre-tRNAs undergo post-transcriptional processing and modifications to yield mature tRNAs [15]. Ultimately, tsRNAs are generated following cleavage at specific sites by distinct endonucleases, such as ANG, RNase Z, Dicer, etc. [16,17]. According to the distinct tRNA cleavage patterns, tsRNAs are categorized into two primary types: tRFs and tRNA halves

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[18].

tRFs, which range from 14 to 30 nucleotides (nt) in length, are predominantly derived from the cleavage of mature tRNAs. Depending on the specific cleavage sites on pre-tRNAs or mature tRNAs, tRFs are further subdivided into six subtypes: tRF-5, tRF-3, tRF-1, tRF-2, internal tRF (i-tRF) and other tRFs [17,19] (Fig. 1A-E). tRNA halves are fragments measuring 31–40 nt in length, which are generated from cleaving the anticodon loop of the mature tRNAs during physiological and stress responses. tRNA halves can be classified into 5' tRNA halves and 3' tRNA halves, based on whether the fragment contains 5' or 3' sequence of the anticodon loop cleavage site [20,21] (Fig. 1F).

2.2. Biogenesis of tsRNAs

tRF-1s (14–30 nt) are generated through RNase Z/ELAC2-mediated cleavage at the 3' end of pre-tRNAs [19] (Fig. 1A). These fragments are characterized by a distinctive polyuridine tail and are alternatively referred to as 3'U tRF. The first identified tRF-1 (designated tRF-1001) initiates at the 3' terminus of mature tRNA, directly preceding the CCA addition site. A defining feature of tRF-1 fragments is the presence of five

or six consecutive thymine bases at their 3' end, indicative of a canonical RNA polymerase III termination motif. These attributes confirm that tRF-1s originate from the 3' trailer region of pre-tRNAs.

tRF-3s are produced by Dicer and ANG through cleavage of the T Ψ C loop of mature tRNAs [22] (Fig. 1B). Depending on the precise cleavage site (U/A or U/U), tRF-3s are categorized into two subtypes: tRF-3a (18 nt) and tRF-3b (22 nt). The tRF-3a subtype is generated by cleavage between nucleotides 58 and 59 within the T Ψ C loop, whereas the tRF-3b subtype results from cleavage between positions 54 and 55. Notably, these fragments retain the CCA sequence that is appended during tRNA maturation.

tRF-5s are produced by Dicer or ANG cleavage, originating from the 5' end of mature tRNA and preserving the intact 5' structural region, with termination occurring upstream of the anticodon loop [23] (Fig. 1C). Based on their cleavage termination sites, tRF-5s are classified into three primary isoforms: tRF-5a (14–16 nt), tRF-5b (22–24 nt), and tRF-5c (28–30 nt).

tRF-2s/i-tRFs (also known as internal tsRNAs) are a class of atypical tsRNAs typically ranging from 18 to 36 nucleotides in length [24,25] (Fig. 1D-E). These fragments are generated through internal cleavage of

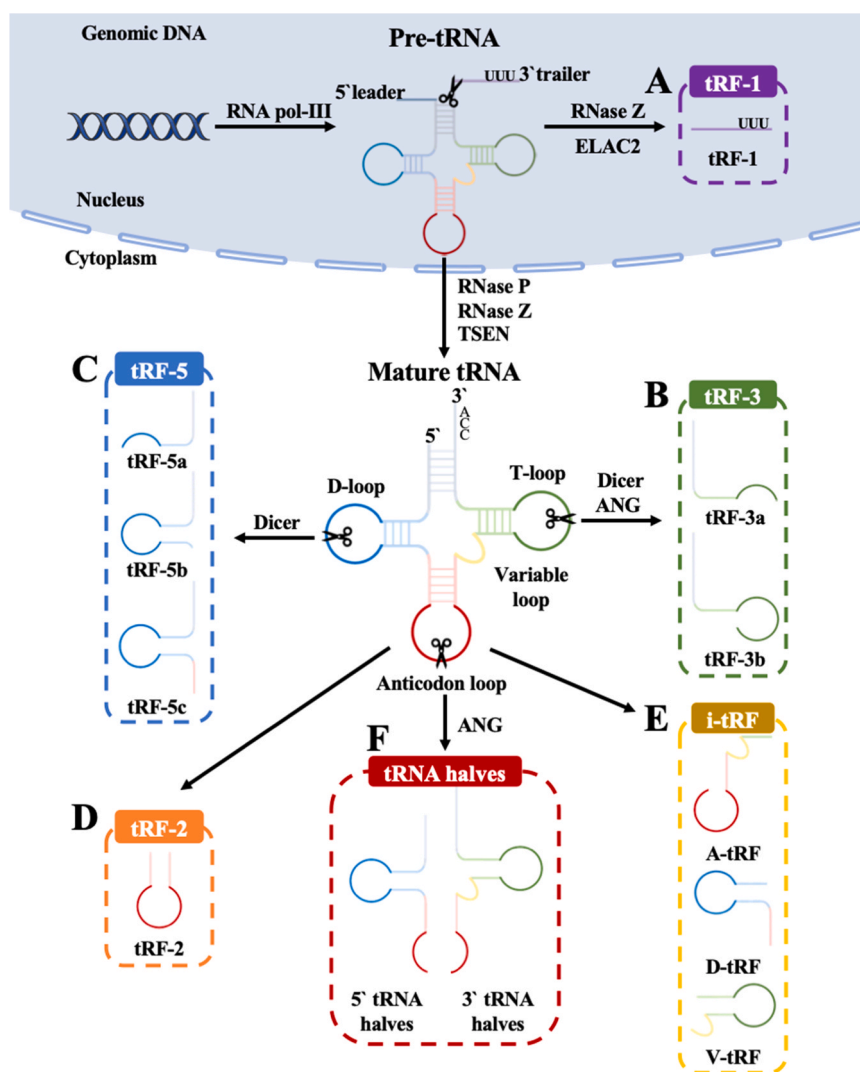


Fig., 1. Classification and biogenesis of tsRNAs. **A.** tRF-1 is generated from the 3' trailer region of the precursor tRNA by the cleavage of RNase Z/ELAC2. **B.** tRF-3 is generated through the cleavage of the T Ψ C loop by either Dicer or ANG within mature tRNA and is subdivided into tRF-3a and tRF-3b based on the location of the cleavage. **C.** tRF-5 is produced via Dicer-mediated cleavage of the D-loop, D stem, or anticodon stem from the 5' end of mature tRNA and can be categorized into three subtypes depending on the length. **D.** tRF-2 is produced through the cleavage of mature tRNA within the anticodon loop. **E.** i-tRF is mainly generated from the internal regions of mature tRNAs and is classified as A-tRF, D-tRF, and V-tRF. **F.** tRNA halves are produced through the cytoplasmic cleavage of mature tRNAs, encompassing 5' tRNA halves and 3' tRNA halves.

mature tRNA molecules and lack prototypical 5' and 3' terminal structures. Specifically, tRF-2 denotes subspecies originating from the anticodon stem-loop region of distinct tRNAs, including tRNA^{Asp}, tRNA^{Glu}, tRNA^{Tyr}, and tRNA^{Gly} (Fig. 1D). The classification of i-tRFs is defined by the 5'-end cleavage position within the mature tRNA. It includes A-tRF (derived from anticodon loop cleavage), V-tRF (originating from the variable loop region), and D-tRF (generated by D-loop cleavage) [17]

(Fig. 1E).

The 5' and 3' tRNA halves arise from cleavage at mature tRNAs' anticodon loops during physiological and stress responses. Based on the cleavage position, the tRNA halves are classified into two subtypes: 5' tRNA halves and 3' tRNA halves. 5' tRNA halves retain the 5' portion along with the anticodon loop, and the latter contains the 3' region and the anticodon loop (Fig. 1 F). A specific subset named tiRNAs are

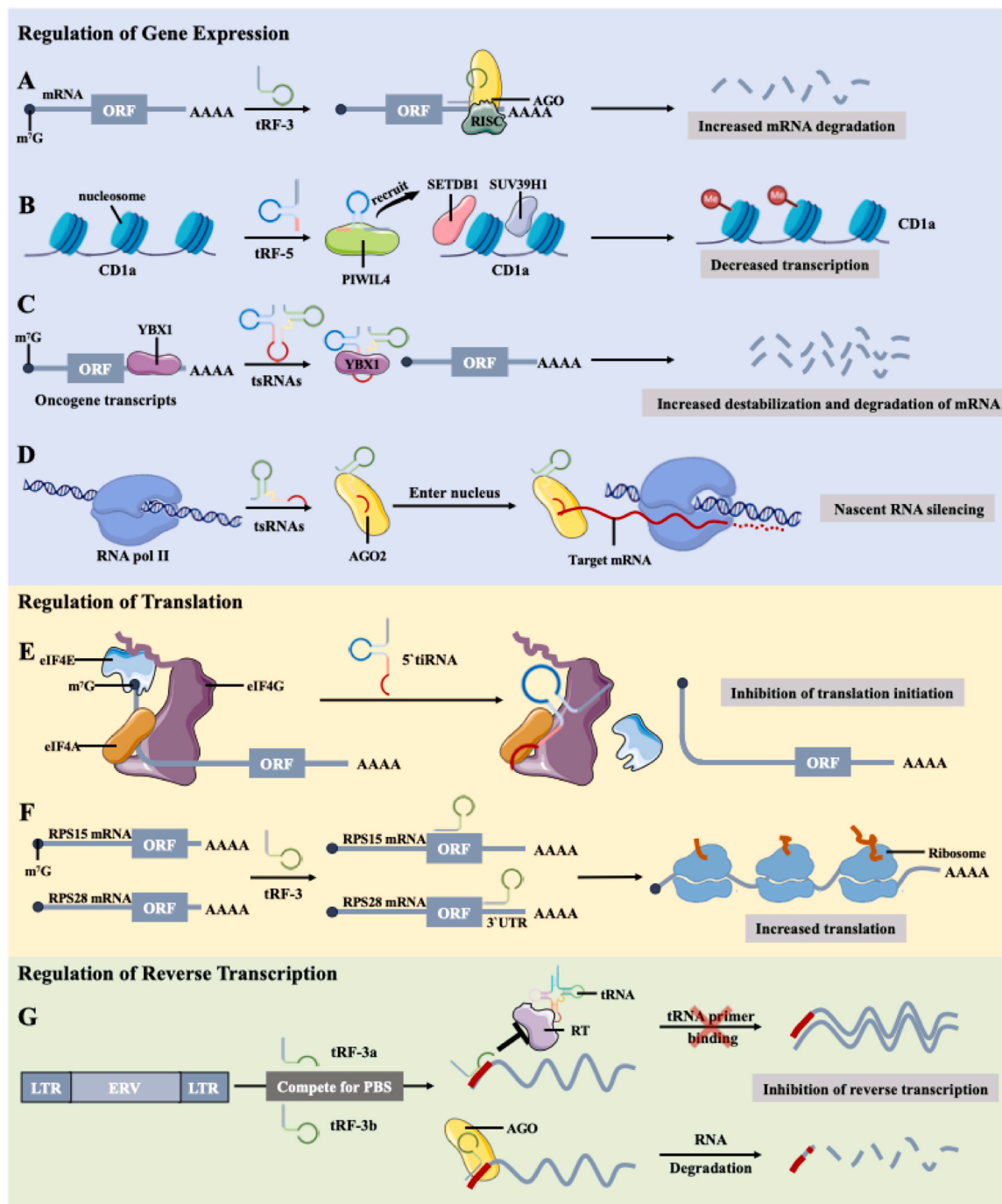


Fig. 2. Molecular functions of tsRNAs, Regulation of gene expression. **A.** tsRNAs can interact with AGO proteins to induce the formation of RISC and trigger mRNA degradation. **B.** tsRNAs can bind to PIWIL4 and recruit histone methyltransferases to the CD1a gene promoter, thereby enhancing histone methylation and suppressing CD1a expression. **C.** tsRNAs can bind to YBX1 and disrupt its interaction with various oncogenic transcripts, leading to the destabilization and degradation of mRNA. **D.** Nascent RNA silencing: tsRNAs can bind to the AGO2 protein and introns of nascent mRNAs simultaneously in the nucleus, resulting in RNA degradation through AGO2's slicer activity. **Regulation of translation.** **E.** tsRNAs can inhibit translation initiation by dissociating eIF4G and eIF4A from the eIF4F cap-binding complex. **F.** tsRNA can bind to ribosomes, thereby enhancing translation and promoting ribosome biogenesis. **Regulation of reverse transcription.** **G.** tsRNAs compete with tRNAs for binding to the PBS in the LTR of ERVs, thus suppressing tRNA-mediated priming of retroelement reverse transcriptase activity. Additionally, tsRNAs can bind to AGO2 and form the RISCs, thereby inducing silencing of coding-competent ERVs.

produced by ANG cutting the anticodon loop of the mature tRNAs under various stress conditions, such as hypoxia, oxidation, amino acid deficiency, etc. [26]. Initially, the processing of tRNAs under stress conditions was attributed to the enzymatic activity of ANG or Rny1p in mammalian and yeast cells [27]. Of particular interest is a specialized subset of tRNAs, known as sex hormone-dependent tRNA-derived RNAs, which are exclusively induced in response to sex hormone signaling [28].

Accumulating evidence highlights the essential regulatory role of tRNA modifications in the production and function of tsRNAs. These modifications enhance structural stability, influence tsRNA biogenesis, and improve translational accuracy [29]. Both tsRNAs and their precursor tRNAs undergo extensive modifications, such as 5-methylcytosine (m^5C), pseudouridine (Ψ), and N7-methylguanosine (m^7G). Enhancing the stability of tRNA structure is one of the most important functions performed by tRNA modifications. For example, m^5C is a common RNA modification that maintains tRNA stability and serves as a stress sensor [30]. Two methyltransferases, DNA methyltransferase (DNMT2) and RNA cytosine C5-methyltransferase (NSUN2), are primarily responsible for catalyzing m^5C formation [31,32]. Experimental evidence indicates that a deficiency in tRNA m^5C in Dnmt2 and NSun2 double-mutant mice leads to embryonic developmental defects due to increased tRNA degradation and disrupted protein synthesis rates [33]. Similarly, methyltransferase-like 1 (METTL1)-catalyzed m^7G tRNA modification protects tRNAs from stress-induced cleavage and the generation of tRF-5 [34]. Pseudouridine synthases 7 (PUS7)-mediated Ψ promotes tRNA ribonuclease cleavage, thereby enhancing tsRNA biogenesis and facilitating translation regulation [35]. Furthermore, Li et al. [36] have provided new insights into the regulation of tsRNA stability, demonstrating that ANG and RNASE1 play critical roles in stability control in response to cellular stress.

3. Molecular functions of tsRNAs

3.1. Regulation of gene expression

tsRNAs play a crucial role in regulating gene expression through various mechanisms, including RNA interference, transcriptional activation, and nascent RNA silencing [37,38]. Central to these regulatory processes is the incorporation of tsRNAs into Argonaute (AGO) protein complexes, leading to the formation of RNA-induced silencing complexes (RISCs) that mediate sequence-specific transcriptional and post-transcriptional repression.

A global meta-analysis has demonstrated that short tsRNAs can associate with AGO proteins, exhibiting microRNA-like functions to modulate target RNA expression and function. Specifically, tRF-3s utilize a 5' seed region of 7–8 nucleotides to bind target mRNAs, thereby facilitating the formation of tsRNA-mRNA-AGO complexes, analogous to the assembly of miRNA-mediated RISCs [39] (Fig. 2 A). tsRNAs exhibit preferential interactions with specific AGO proteins: tRF-1s predominantly associate with AGO3 and AGO4, whereas tRF-5s and tRF-3s engage with AGO1, AGO3, and AGO4. For example, tRF-29-79MP9P9NH525, a tRF5c derived from tRNA^{Val-ACC}, targets the 3' untranslated region (3'UTR) of Kinesin family member 14 (KIF14) mRNA by forming RISCs with AGO2, thereby silencing KIF14 expression and regulating gastric cancer development [40]. In chondrocytes, a specific tRF3a namely tRF-3003a (tRF-17-8871K92) can post-transcriptionally regulate JAK3 expression via AGO/RISC formation [41]. Additionally, a tRF5b namely tsRNA-5008a (tRF-23-QNR8VP949) interacts with SLC7A11 mRNA via AGO2, resulting in its silencing and contributing to the pathogenesis of atrial fibrillation and ferroptosis [42].

Emerging evidence suggests that specific tsRNAs can mediate gene silencing by interacting with PIWI proteins, a subfamily of AGO proteins. Specifically, tRF-1s such as ts-4521 (from tRNA^{Ser-GCT}) and ts-3676 (from tRNA^{Thr-AGT/CGT}) can bind to overexpressed PIWIL2 in HEK293

cells and exhibit piRNA-like silencing activity, although mechanistic validation remains limited [43,44]. Additionally, a tRF-5c from tRNA^{Glu} associates with PIWIL4 to repress transcription by recruiting histone methyltransferases to the CD1A promoter in monocytes [45] (Fig. 2B). This repression may depend on sequence complementarity between the tsRNA and target promoter.

Moreover, tsRNAs participate in regulating transcriptional processes and influencing gene expression. For example, 5' half-Val-CAC-2 (tRF-34-Q99P9P9NH57S15) facilitates the interaction between FUBP1 and the transcriptional regulatory element of the c-MYC gene, thereby promoting its transcription and upregulating c-MYC expression in pancreatic cancer [46]. Similarly, tsRNAs originating from tRNA^{Glu}, tRNA^{Asp}, tRNA^{Gly}, and tRNA^{Tyr} can displace the 3'UTR of the YBX1 transcript, leading to the destabilization of oncogenic mRNAs and the suppression of tumor progression [24] (Fig. 2 C). These findings illustrate the multifaceted regulatory roles of tsRNAs in governing gene expression, encompassing both transcriptional and post-transcriptional mechanisms.

A recent study has identified a novel gene silencing mechanism, nascent RNA silencing (NRS), which utilizes Dicer-dependent nuclear tRF-3s and tRF-1s to repress cancer-associated genes by targeting early introns of nascent transcripts [38] (Fig. 2D). This mechanism necessitates AGO2 endonuclease activity and reduces target expression at both the mRNA and protein levels. In zebrafish, 5'-tiRNAs derived from tRNA^{Glu-CTC} and tRNA^{Gly-GCC} are critical regulators during early embryonic development. These fragments interact with the genomic DNA templates of their corresponding tRNA genes to form R-loop structures. This interaction prevents the formation of transcription-inhibitory tRNA-DNA hybrids, thereby facilitating the transcription of their respective tRNA genes [47]. A recent significant study has demonstrated that the hypoxia-responsive tRNA-Asp-GTC-3'tDR binds and sequesters PUS7, and subsequently drives RNA autophagy by preventing the pseudouridylation and stabilization of PUS7-targeted histone mRNAs, to maintain cellular homeostasis in kidney cells [48].

3.2. Regulation of translation

Protein synthesis in eukaryotes is a fundamental process that requires coordination between the nucleus and cytoplasm, encompassing mRNA transcription, processing, nuclear export, and ribosomal translation. Dysregulation of translational control is implicated in tumor progression by accelerating the production of proteins and other biomolecules. Accumulating evidence underscores that tsRNAs can mediate translational control as their most prominently regulated molecular process through various biological mechanisms, primarily by suppressing protein biosynthesis, while occasionally enhancing translation [48–50]. Recent findings indicate that tsRNAs regulate protein synthesis via two principal mechanisms: modulating ribosome biogenesis (e.g., by controlling the synthesis of ribosomal proteins) and directly targeting components of the translational machinery [51,52].

Under stress conditions, cells inhibit global protein synthesis through two distinct pathways to conserve energy for damage repair [53]: (1) phosphorylation of eukaryotic translation initiation factor 2 α (eIF2 α) coupled with stress granule (SG) assembly, and (2) an eIF2 α phosphorylation-independent mechanism. Early studies demonstrated that the accumulation of ANG-dependent 5'-tiRNAs, particularly derived from tRNA^{Ala} and tRNA^{Cys} in humans, significantly suppresses protein synthesis under stress conditions [54,55] (Fig. 2E). Mechanistically, these fragments interfere with cap-dependent translation initiation by displacing eIF4G and eIF4A from the eIF4F translation initiation complex. Meanwhile, this inhibition is mediated by RNA G-quadruplex (RG4) structures formed by terminal oligoguanine (TOG) motifs located at their 5' ends. RG4 structures in mRNA impede the progression of scanning pre-initiation and ribosomal complexes, thereby inhibiting translation. Alternatively, RG4 may recruit factors that also inhibit translation. Furthermore, these 5'-tiRNAs can also promote the assembly

of SGs in an eIF2 α -independent manner, which are cytoplasmic condensates containing YBX1 and translationally stalled ribonucleoprotein complexes [56,57]. Notably, shorter 5'-tRNA isoforms derived from TOG-bearing tRNAs, known as mini-TOGs (mTOGs), inhibit translation by binding to PABPC1, a core component of the eIF4F complex [35]. Remarkably, their activity is regulated by PUS7-mediated pseudouridylation at U8, which modulate mTOG–PABPC1 binding and PAIP1 recruitment, thereby stabilizing PABPC1–eIF4F interactions. Other mechanisms include 5'-tRNAs derived from tRNA^{Pro} binding to 18S rRNA, which inhibits 80S ribosomes [58]. Additionally, a specific tRF-1 derived from tRNA^{Gln} has been reported to potentially suppress global protein synthesis by interfering with TSR1 binding to pre-40S ribosomes in mice [59].

tsRNAs play a crucial role in enhancing protein translation by facilitating the synthesis of ribosomal proteins and RNA-binding proteins. A seminal study demonstrated that a 22-nt tRF-3 derived from tRNA^{Leu-CAG} promotes ribosome biogenesis by selectively binding to the mRNAs of RPS28 and RPS15, thereby enhancing their translation [51] (Fig. 2 F). This tsRNA promotes downstream translation by binding to and destabilizing RNA secondary structures within the 3' UTR or coding sequences of target mRNAs. In another separate investigation, two 5'-tRNAs, derived from tRNA^{Pro-CGG} and tRNA^{Cys-GCA}, respectively, were found to stimulate cap-dependent translation in granulocyte-monocyte progenitors, although the precise mechanisms remain unclear [60].

3.3. Regulation of reverse transcription

Emerging evidence suggests that tsRNAs play diverse roles in modulating viral infections, particularly influencing the reverse transcription process of RNA viruses [61]. For example, tRF-3019 (tRF-18-HR6HFRD2), a tRF3a derived from tRNA^{Pro}, was identified as the most abundant tsRNA in human T-cell leukemia virus (HTLV-1)-infected CD4⁺ T cells compared to normal CD4⁺ T cells [62]. Ruggero et al. demonstrated that tRF-3019 (tRF-18-HR6HFRD2) exhibits perfect complementarity to the primer binding site (PBS) in HTLV-1 retroviral RNA, enabling it to prime reverse transcription initiation and enhance viral replication. Conversely, 18-nt tRF-3s inhibit reverse transcription of endogenous retroviruses (ERVs) by competing with tRNAs for binding to the PBS in mouse stem cells [63] (Fig. 2 G). Collectively, these findings highlight the dual role of tsRNAs in functioning either as primers or suppressors of viral reverse transcription [64].

4. Clinical application value of tsRNAs in lung diseases

4.1. tsRNAs can serve as novel biomarkers of lung diseases

Traditionally, the diagnosis and monitoring of pulmonary diseases mainly rely on clinical symptoms, imaging techniques, and histopathology of biopsies. However, the invasive nature of these procedures poses challenges for early screening and efficacy prediction of pulmonary diseases. Emerging evidence highlights the dual roles of tsRNA in the pathogenesis of pulmonary diseases, indicating their potential utility in early detection, clinical diagnosis, and therapeutic interventions, particularly with advancements in high-throughput technologies [65–67]. Various tsRNAs have been identified in tumor tissues and biological fluids (e.g., plasma, serum, urine, cerebrospinal fluid, and saliva) of cancer patients [23]. Additionally, tsRNAs can be encapsulated within extracellular vesicles, such as exosomes, facilitating intercellular communication [68]. This section will explore the clinical applicability of tsRNAs in human lung diseases.

4.1.1. tsRNAs can serve as novel biomarkers of lung cancers

Gao et al. [66] identified 52 differentially expressed circulating tsRNAs from 1550 patient samples of non-small cell lung cancer (NSCLC), with six tsRNAs being selected as valuable diagnostic

biomarkers, achieving an area under the curve (AUC) of up to 0.90 in independent validation studies. Notably, tRF5a-Ile-AAT/GAT can serve as a prognostic biomarker for the early stage of NSCLC. In addition, the expression of 5'-half-Leu-CAG (tRF-34-SP5830MMUKLYHE) is notably upregulated in patients with advanced NSCLC, where it plays a role in cell cycle progression by targeting aurka [69]. Another investigation [70] demonstrated a significant downregulation of exosomal tsRNAs, including tRF5a-Leu-TAA-005 (tRF-16-0P5830E), tRF2-Asn-GTT-010, tRF5a-Ala-AGC-036, tRF5a-Lys-CTT-049, and tRF5a-Trp-CCA-057 in NSCLC patients compared to healthy individuals. Furthermore, research [65] has indicated that exosomal tRF5b-Tyr-GTA (tRF-24-395P4PZ3ES) and tRF5b-Val-TAC can serve as potential biomarkers for NSCLC, with tRF5b-Tyr-GTA (tRF-24-395P4PZ3ES) being correlated with tumor stage and lymph node metastasis (Fig. 3 A).

tsRNAs also exhibit aberrant expression patterns in lung adenocarcinoma (LUAD). Zhang et al. [71] reported a significant upregulation of certain tsRNAs, such as 5'-half-Lys-CTT-002 (tRF-34-PSQP4PW3FJIOE5), tRF5c-Val-CAC-010 (tRF-31-Q99P9P9NH57SD), and tRF5c-Val-CAC-011 (tRF-32-Q99P9P9NH57SJ), alongside the downregulation of tRF1-Ser-TGA-005 in LUAD tissues. You et al. [72] analyzed differential expression profiles of plasma tsRNAs in LUAD patients, encompassing both early and advanced stages, as well as in healthy controls. They found that the AUCs values for tRF5c-1:29-Pro-AGG-1-M6 (tRF-29-6978 WPRLXNHK) and tRF3b-55:76-Tyr-GTA-1-M2 (tRF-22-WB884U1D2) were as high as 0.882 and 0.896, respectively. Moreover, the expression levels of these tsRNAs exhibited significant correlations with various clinicopathological features, including tumor-node-metastasis stage, node stage, and carcinoembryonic antigen expression levels, indicating their substantial diagnostic potential for LUAD. A recent study [73] examined the mediating roles of tsRNAs in benzo[a]pyrene (BaP)-associated lung cancer. It identified four differentially expressed tsRNAs that were potentially involved in the development of BaP-associated lung cancer (Fig. 3B).

A primary challenge in diagnosing lung cancer lies in accurately distinguishing it from other pulmonary conditions, such as benign pulmonary nodules and pulmonary tuberculosis, which share similar clinical and radiological characteristics. Zhou et al. [74] developed a plasma exosomal tsRNA signature (3'-half-43-Gly-GCC-4 (tRF-43-X6LP5JKKZUY76RIFD2), tRF3a-17-Gly-TCC (tRF-17-8SPOL52), 5'-Leader-Val-AAC-1-2) to facilitate the differential diagnosis of benign pulmonary nodules and early-stage lung cancer. The predictive model that integrates these tsRNAs with clinical parameters demonstrated outstanding performance, achieving an AUC of 0.9559, with sensitivity and specificity rates of 91.06% and 91.53%, respectively. Gu et al. [75] identified a peripheral blood mononuclear cell based non-coding RNA signature, which includes tsRNAs, rRNA-derived small RNAs and YRNA-derived small RNAs, capable of accurately differentiating between lung cancer patients and healthy controls, as well as distinguishing lung cancer patients from those with pulmonary tuberculosis. This RNA signature exhibited superior diagnostic performance, with an AUC of 0.930, compared to miRNA-based biomarkers in validation cohorts, highlighting its potential as a non-invasive tool for lung cancer screening.

Beyond their aberrant expression in cancer cells and tissues, certain functional tsRNAs are known to regulate key signaling pathways, thereby influencing multiple aspects of tumor progression, including malignant proliferation, invasion and metastasis, angiogenesis, immune response, therapeutic resistance, and tumor metabolism. Consequently, tsRNAs hold promise as biomarkers for cancer diagnosis and prognosis.

4.1.2. tsRNAs can serve as novel biomarkers of COPD

COPD is a chronic airway disorder primarily characterized by persistent inflammation and airway remodeling, with features including goblet cell metaplasia, mucus hypersecretion, and airway wall thickening. Recent investigations have revealed a marked accumulation of the 5' half derived from tRNA^{Val-CAC} (tRF-34-79MP9P9NH57S15) in the plasma of COPD patients, in contrast to healthy individuals, where the 5'

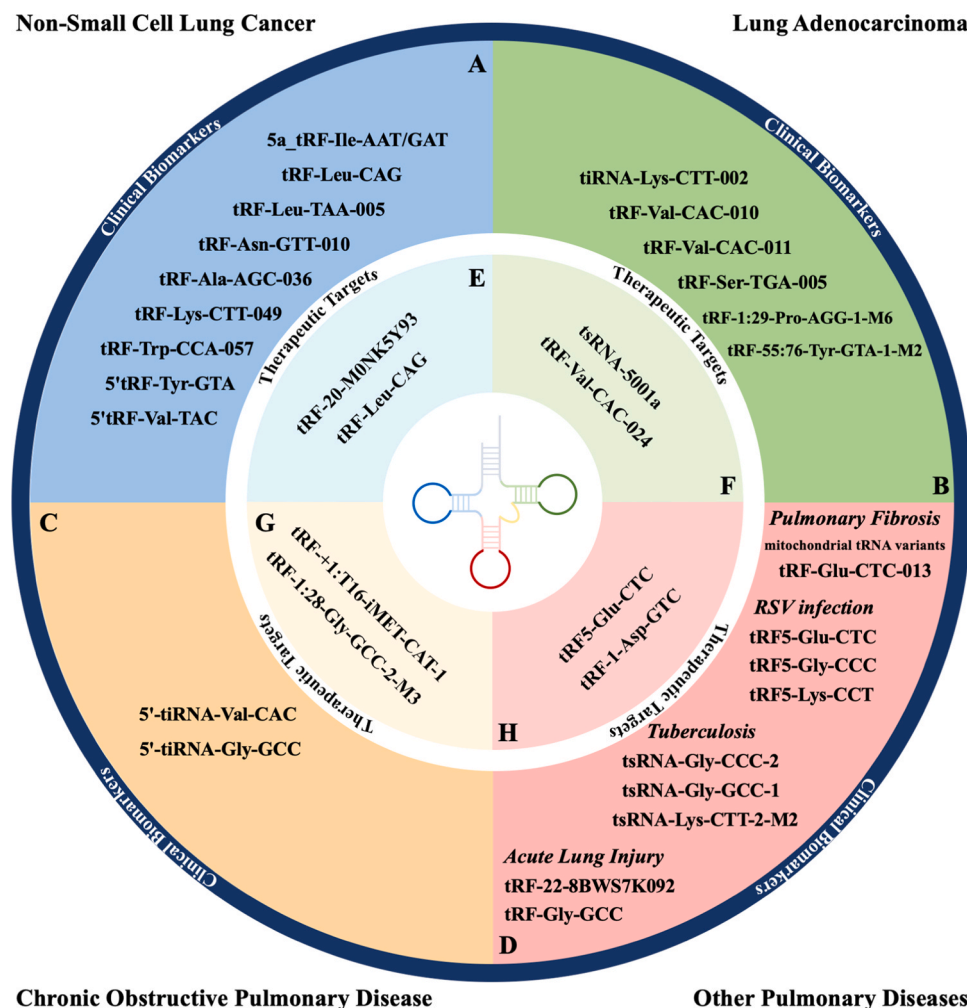


Fig. 3. Clinical application value of tsRNAs in lung diseases. **A.** tsRNAs can serve as novel biomarkers of non-small cell lung cancer (NSCLC). **B.** tsRNAs can serve as novel biomarkers of lung adenocarcinoma (LUAD). **C.** tsRNAs can serve as novel biomarkers of chronic obstructive pulmonary disease (COPD). **D.** tsRNAs can serve as novel biomarkers of other pulmonary diseases (pulmonary fibrosis, respiratory syncytial virus infection, tuberculosis and acute lung injury). **E.** tsRNAs can serve as therapeutic targets of NSCLC. **F.** tsRNAs can serve as therapeutic targets of LUAD. **G.** tsRNAs can serve as therapeutic targets of COPD. **H.** tsRNAs can serve as therapeutic targets of other pulmonary diseases.

half derived from tRNA^{Gly-GCC} (tRF-34-PNR8YP9LON4VHM) is more prevalent. Moreover, tRF-34-79MP9P9NH57S15 could activate human macrophages through Toll-like receptor 7, subsequently inducing cytokine production [76]. These findings suggest that tsRNAs may play critical roles in the host immune response and could serve as novel molecular targets for COPD treatment (Fig. 3 C).

4.1.3. tsRNAs can serve as novel biomarkers of pulmonary fibrosis

To date, the role of tsRNAs in the progression of IPF remains underexplored. Mitochondrial dysfunction is a central driver of IPF. A recent investigation revealed that increased leukocyte mitochondrial tRNA variants (mainly in the right half of the clover-leaf structure) predict poor prognosis of IPF [77]. Additionally, there have been limited reports on fibrosis induced in organs other than the lungs, such as the liver, heart, and kidneys. For example, Shen et al. [78] identified tRF5c-Glu-CTC-013 (tRF-30-87R8WP9N1EWJ) as having a protective role in ameliorating angiotensin II-induced hypertrophic remodeling by inhibiting fibrosis and inflammation (Fig. 3D).

4.1.4. tsRNAs can serve as novel biomarkers of other pulmonary diseases

tsRNAs are generated under a variety of stress conditions, including oxidative stress and pathogen infections. Prolonged exposure to stress can result in chronic inflammation, thereby contributing to disease

pathogenesis through the induction of these fragments. Respiratory syncytial virus (RSV) infection in host cells is facilitated by ANG-mediated tRNA cleavage, resulting in increased tsRNA production [79]. Deng et al. [80] demonstrated that RSV infection specifically induces the tRF5c-Glu-CTC (tRF-31-87R8WP9N1EWJ), a specific tRNA fragment upregulated during viral challenge. In addition, two novel RSV-induced tsRNAs were identified as tRF5c-Gly-CCC (tRF-29-Q1Q89P9L84FS) and tRF5c-Lys-CCT (tRF-30-PSQP4PW3FJIO), which play crucial roles in enhancing viral replication and modulating cytokine signaling [81]. Stress responses can also be triggered by other pathogens. Huang et al. [82] identified a serum tsRNA signature, comprising tRF5c-Gly-CCC-2 (tRF-30-Q1Q89P9L8422), tRF5c-Gly-GCC-1 (tRF-32-P4R8YP9LON4V3), and tRF5c-Lys-CTT-2-M2 (tRF-31-PSQP4PW3FJIOB), which is useful for the diagnosis and severity assessment of multiclass tuberculosis, including lung injury severity and acid-fast bacilli grades (Fig. 3D).

Furthermore, in other models of acute lung injury (ALI), Deng et al. [83] found that tRF5c-Gly-GCC-1 (tRF-28-P4R8YP9LOND5), derived from pulmonary epithelial cells, is associated with oxidative stress in the context of radiation-induced lung injury. In a murine model of ALI, exosomes derived from alveolar macrophages deliver tRF-22-8BWS7K092, which activates the Hippo signaling pathway to induce ferroptosis, thereby contributing to the pathogenesis of ALI [84] (Fig. 3D). The pathogenesis of pulmonary arterial hypertension (PAH) pathogenesis is critically driven by

hypoxia-induced damage and systemic oxidative stress. Chen et al. [85] identified dysregulated tsRNA profiles in idiopathic PAH, proposing these dysregulated tsRNAs as dual-function biomarkers and therapeutic targets.

4.2. tsRNAs can serve as potential targets of lung diseases therapy

Substantial evidence indicates that tsRNAs are not only highly promising novel biomarkers for the early detection and molecular subtyping of tumors but also prime candidates for therapeutic intervention. By directly targeting tsRNAs or modulating their biogenesis, novel therapeutics have the potential to disrupt tumorigenesis and progression, offering promising new avenues for precision oncology. Critically, tsRNAs are key modulators of tumor progression, orchestrating multiple signaling pathways that govern critical hallmarks of cancer, including uncontrolled proliferation, enhanced invasion/metastasis, aberrant angiogenesis, dysregulated immune responses, and altered cellular metabolism. Collectively, this pervasive involvement in oncogenic signaling underscores the compelling rationale for developing tsRNA-directed therapeutic strategies in oncology.

4.2.1. tsRNAs can serve as potential targets of lung cancer therapy

In LUAD, a tRF5a named tsRNA-5001a is upregulated and contributes to tumor cell proliferation, associating with poor prognosis and increased recurrence risk [86]. Mechanistically, tsRNA-5001a downregulates the tumor suppressor gene GADD45G. In studies on NSCLC, Shao et al. [69] found that 5' half-Leu-CAG (tRF-34-SP5830MMUKLYHE) expression was significantly elevated in NSCLC tissues, influencing cell proliferation and cell cycle progression. Additionally, tRF-Leu-CAG (tRF-34-SP5830MMUKLYHE) directly targets the gene of transcription elongation factor A3 (TCEA3), which can inhibit the proliferation and migration of NSCLC cell. However, tRF-Leu-CAG (tRF-34-SP5830MMUKLYHE) facilitates NSCLC proliferation and migration by mediating TCEA3 silencing and promotes paclitaxel resistance through autophagy activation [87]. Additionally, the overexpression of an i-tRF named tRF-20-MONK5Y93 was observed to inhibit NSCLC cell proliferation and migration, accelerate apoptosis *in vitro*, and suppress tumor growth by targeting PLOD1 [88] (Fig. 3E). The overexpression of ts-46 and ts-47 in lung cancer cells suppressed colony formation, confirming their tumor-suppressive roles and potential as therapeutic targets [43]. Two novel tumor-suppressive tiRNAs, ts-3676 and ts-4521, have been identified as down-regulated and mutated in lung cancer and chronic lymphocytic leukemia. This discovery underscores the dual oncogenic and tumor-suppressive functions of tiRNAs across hematopoietic malignancies and solid tumors [44]. Wang et al. [89] characterized tRF3a-Val-CAC-024 (tRF-17-08P2F52) as an oncogene that facilitates the progression of LUAD by interacting with ALDOA at residues Q125 and E224, promoting its oligomerization, thereby enhancing enzymatic activity and driving aerobic glycolysis. The study also demonstrated the promising therapeutic potential by lipid nanoparticle-delivered tRF3a-Val-CAC-024 (tRF-17-08P2F52) antagonists suppressed tumor growth in preclinical models (Fig. 3F).

4.2.2. tsRNAs can serve as potential targets of other lung disease therapies

tsRNAs represent promising therapeutic targets for lung diseases, as their distinct signatures correlate with inflammation or apoptosis biomarkers, highlighting their clinical applicability. tsRNAs exhibit COPD-specific signatures linked to disease severity. Notable species, such as tRF1-1:T16-iMET-CAT-1 and tRF5c-1:28-Gly-GCC-2-M3 (tRF-28-PNR8YP9LOND5), correlate with markers of inflammation and apoptosis, and respond dynamically to stem cell therapy [90] (Fig. 3G). Additionally, tRF5c-Glu-CTC (tRF-31-87R8WP9N1EWJ0) targets the apolipoprotein E receptor 2 by binding to its 3'UTR, thereby suppressing the antiviral host factor and enhancing RSV replication [80]. The biogenesis of this tsRNA is further regulated by parental tRNA methylation and involves AGO1/4 proteins [91,92]. A recent study initially observed that tRF5c-Glu-CTC (tRF-31-87R8WP9N1EWJ0) may

modulate viral infection through its interaction with the RSV matrix protein PABPC1 [93]. Importantly, inhibition of these pathogenic tsRNAs may reverse cellular phenotypes, achieving their potential as druggable targets for precision interventions in lung diseases. Zhang et al. [94] demonstrated that inhibition of the oxidatively modified tsRNA, tRF1-Asp-GTC (8-oxoguanine-modified tRF), reverses hypoxia-induced hyperproliferation and resistance to apoptosis in pulmonary artery smooth muscle cells, thereby establishing this tsRNA as a potential therapeutic target for pulmonary hypertension (Fig. 3H).

5. Conclusions and future perspectives

tsRNAs are generated by site-specific cleavage of precursor or mature tRNAs, which function as stable and specific regulators detectable in body fluids. These molecules hold significant promise as biomarkers for early diagnosis, prognostic stratification, and therapeutic monitoring in pulmonary diseases. This review synthesizes their dual roles in regulating gene expression, reverse transcription, and translation. Importantly, the dysregulation of tsRNAs is implicated in fundamental pathological processes, such as homeostatic disruption, uncontrolled cellular proliferation, metastasis, immune inflammation, and oxidative stress, in conditions including lung cancers, COPD, pulmonary hypertension, and pulmonary infections. Although research on tsRNAs has attracted increasing attention, our current understanding remains at an incipient stage. Several challenges persist, including the limited comprehension of the biological functions of tsRNAs, the absence of standardized nomenclature systems, inadequate technological advancements, and the fact that potential therapeutic strategies involving tsRNAs for lung diseases have yet to be developed.

Currently, although the diverse biological functions of tsRNAs have been extensively documented, there remain potential novel mechanisms that are yet to be fully explored. For example, emerging evidence demonstrates that ANG and RNase1 not only regulate tsRNA biogenesis but also play a role in the stability regulation of a subset of cellular tsRNAs, underscoring essential biological functions that warrant further investigation [36]. Additionally, the potential functional crosstalk between tsRNAs and their parental tRNAs remains undefined. Furthermore, the cooperative regulatory interplay between RNA modifications and tsRNA biogenesis and biological activity is still not fully elucidated. Precision modification of individual tsRNAs enhances the tertiary-structure stability of tRNA fragment analogs, thereby augmenting their biological function [95].

Of note, tsRNAs are not merely random degradation byproducts of tRNA; rather, they represent a rich and novel class of short RNAs characterized by precise sequence structures, specific expression patterns, and distinct biological functions. Comprehensive annotation of the global tsRNA landscape is hindered by technical challenges, including incomplete purification of modified tsRNAs and sequencing distortions arising from nucleotide modifications [96]. Traditional tsRNA profiling methods, which rely on hybridization-based techniques and conventional RNA sequencing, encounter significant limitations in capturing the full complexity of tsRNAs. The presence of modified nucleotides in tsRNAs obstructs reverse transcription during library preparation, leading to premature termination and misincorporation errors, thereby compromising both tsRNAs quantification and base-calling accuracy. To address these challenges, a methodology known as AlkB-facilitated RNA methylation sequencing (ARM-Seq) has been developed [97]. This approach enzymatically removes key methyl groups prior to adapter ligation and cDNA synthesis [97]. Utilizing ARM-seq, a recent study has systematically established the first comprehensive atlas of stress-induced tRNA signatures, revealing dynamic extracellular tsRNA remodeling in response to stress [36]. This work demonstrates a substantial improvement in the detection of key tsRNAs under cellular stress, surpassing traditional methods. We anticipate the development of a more precise and comprehensive atlas of tsRNAs signatures in the future, facilitated by advanced molecular technologies.

Several specialized tsRNA databases have been established, such as tRFdb [98] and MINTbase v2.0 [99], each employing distinct classification systems. This heterogeneity in nomenclature leads to non-overlapping terminologies, posing a significant challenge to the reproducibility of cross-study identification of tsRNA signatures. The field has reached a consensus on adopting a standardized nomenclature using the tDR naming system called tDRnamer [100]. This tsRNA nomenclature consists of three mandatory components and two optional annotations: (1) the prefix "tDR" (a functionally neutral designation); (2) start and end positions relative to the source tRNA in Sprinzl numbering, ensuring cross-tRNA positional consistency across tRNAs; (3) Source tRNA identifier from GtRNAdb [101]. Optionally included are: (4) "M" with the number of matching source transcripts; and (5) nucleotide variation annotation. Future research should prioritize the development of comprehensive databases that integrate information on tsRNA biogenesis and modifications.

tsRNAs demonstrate significant regulatory roles under various stress stimuli, such as nutritional deprivation, hypoxia, and oxidative stress [36], suggesting their high relevance to the immune microenvironment associated with pulmonary tumors and inflammation. Although tsRNAs are well-established diagnostic biomarkers for lung cancer, their roles in non-malignant lung diseases remain substantiated, primarily due to constraints related to human sample sources. Moreover, the respiratory and cardiovascular systems are complex, dynamic and intricately connected, necessitating further investigation into the communicative functions of tsRNAs. Additionally, tsRNAs exhibit diverse expression patterns across different bodily tissues and fluids. For tsRNAs to serve as clinically viable biomarkers for disease diagnosis and prognosis, they must exhibit both high abundance and stability in bodily fluids. Further investigation is warranted to evaluate whether the expression profiles and functional efficacy of tsRNAs differ across various biological sources. Currently, most tsRNA therapeutic studies are in the preclinical stage, focusing on tsRNA-targeted interventions in *in vivo* models. Moving forward, the strategic development of chemical modifications and advanced delivery platforms, such as lipids, exosomes, and inorganic nanoparticles, may offer viable pathways toward the development of clinically relevant tsRNA therapeutics.

In summary, the specific functions and mechanisms of tsRNAs in pulmonary diseases remain inadequately understood. More comprehensive understandings of their functions are essential for leveraging their potential as diagnostic markers and therapeutic targets, thereby necessitating ongoing scientific exploration and innovation.

Ethical approval

Not applicable.

Contributions

Y.W., X.S. and G.M. searched and reviewed the literatures. Y.W., X.L. and A.C. prepared the first draft of the manuscript. X.H. and M.T. critically edited the manuscript. C.G. draw the figure. A.C. supervised, and Y.W. published the manuscript. All authors read and approved the final manuscript.

Ethics declarations

None

Consent to participate

Not applicable.

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CRediT authorship contribution statement

Xiaoyu Song: Data curation, Conceptualization. **Ye Wang:** Writing – original draft, Investigation. **Xu Han:** Writing – review & editing, Supervision. **Man Tian:** Writing – review & editing. **Xing Liu:** Writing – original draft. **Ge Ma:** Data curation. **Apeng Chen:** Writing – review & editing, Writing – original draft. **Cong Gan:** Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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