Molecular Therapy
Nucleic Acids
Review

Aberrant MicroRNAomics in Pulmonary Complications: Implications in Lung Health and Diseases

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Over the last few decades, evolutionarily conserved molecular networks have emerged as important regulators in the expression and function of eukaryotic genomes. Recently, miRNAs (miRNAs), a large family of small, non-coding regulatory RNAs were identified in these networks as regulators of endogenous genes by exerting post-transcriptional gene regulation activity in a broad range of eukaryotic species. Dysregulation of miRNA expression correlates with aberrant gene expression and can play an essential role in human health and disease. In the context of the lung, miRNAs have been implicated in organogenesis programming, such as proliferation, differentiation, and morphogenesis. Gain- or loss-of-function studies revealed their pivotal roles as regulators of disease development, potential therapeutic candidates/targets, and clinical biomarkers. An altered microRNAome has been attributed to several pulmonary diseases, such as asthma, chronic pulmonary obstructive disease, cystic fibrosis, lung cancer, and idiopathic pulmonary fibrosis. Considering the relevant roles and functions of miRNAs under physiological and pathological conditions, they may lead to the invention of new diagnostic and therapeutic tools. This review will focus on recent advances in understanding the role of miRNAs in lung development, lung health, and diseases, while also exploring the progress and prospects of their application as therapeutic leads or as biomarkers.

MicroRNAs (miRNAs) have provided a new and outstanding molecular biology paradigm to understand the molecular mechanisms underlying pulmonary diseases. In the last few decades, non-coding RNAs (ncRNAs) such as miRNA have been identified as emerging mediators in human lung disease. miRNA have been shown to play crucial roles in fundamental biological mechanisms by post-transcriptional regulation of their cognate mRNA, impacting cellular events such as metabolism, growth, cell differentiation, and development (thereby regulating organogenesis), apoptosis, inflammation, and cell signaling.[1] Dysregulation of miRNA in diseased states often produces signature microRNA profiles that can be used as biomarkers for specific diseases.[2] The role of an aberrant microRNAome in the pathophysiology of diseases has identified miRNA as biomarkers, therapeutic targets, or indicators of prognosis. The first miRNA identified was Lin-4 in *Caenorhabditis elegans*, followed by the discovery of the let-7 family of miRNA, which are crucial regulators in the development of *C. elegans*.[3,4] Since then, around 2,000 validated miRNA have been identified in the human genome.[5,6] About 60% of human protein-coding genes are now known to be subject to miRNA-mediated post-transcriptional regulation.[7] The relation between an aberrant lung microRNAome and lung diseases is becoming increasingly evident, introducing novel paradigms in the molecular mechanisms underlying lung diseases, such as lung cancer, pulmonary fibrosis, chronic obstructive pulmonary disease (COPD), asthma, and pulmonary hypertension.[8–12] As potential regulators of an immune response, miRNA play a vital role in lung immunity. Hence they can also determine the magnitude of inflammation and tissue damage in lung diseases.[13,14] This review focuses on the recent discoveries regarding the influence and contribution of miRNA in inflammatory airway diseases and lung cancer with specific emphasis on therapeutic development.

Biogenesis of MicroRNA

The discovery of miRNA-mediated post-transcriptional gene regulation was one of the watershed events in the field of gene regulation. miRNA are short, single-stranded, non-coding RNA molecules about 22 nt in length. In early 1960, Britten and Davidson[15] first proposed that the regulatory mechanisms in higher organisms are controlled by activator RNAs. These activator RNAs are found in the nucleus and transcription product of the redundant genome. They also suggested that redundant genome sequences were found among the integrator genes and that the activator RNAs move from their synthesis site to active transcription of the producer genes (presently called exon). Since the first report of the discovery of lin-4 miRNA by Lee et al., 2,000 different miRNA have been identified and validated to play essential roles in different developmental stages and pathophysiological processes.[4] miRNA are commonly encoded either within protein-coding genes called introns or as independent genes in intronic

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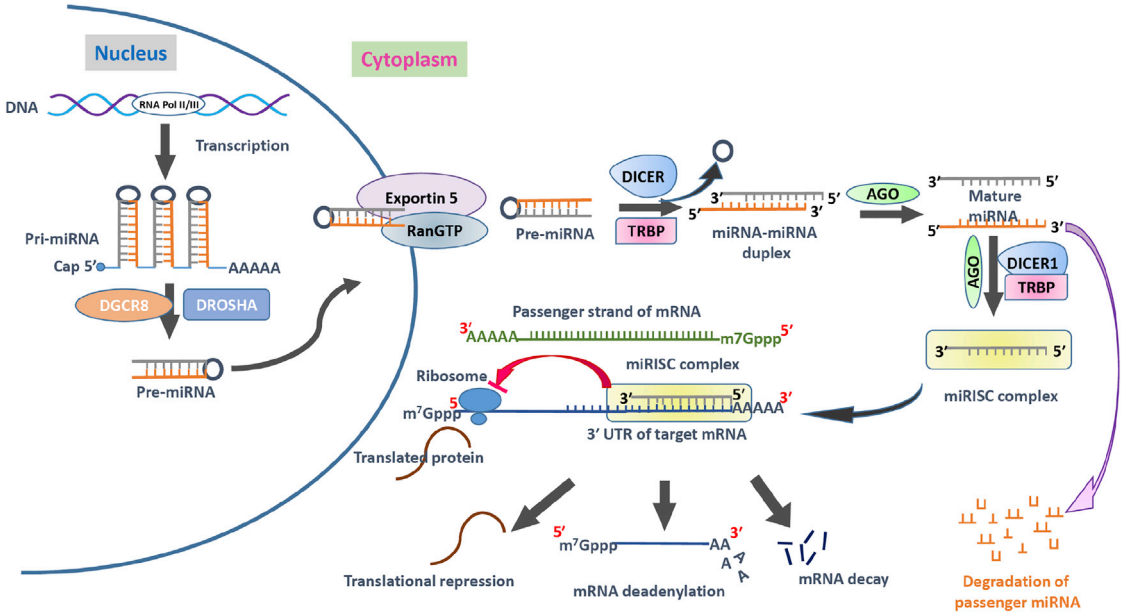

Figure 1. Mechanism of MicroRNA Processing and Their Inhibitory Mechanism

The microRNA (miRNA) processing pathway begins with transcription of their genes with the help of RNA polymerase II (Pol II) or polymerase III (Pol III) to produce pri-miRNAs in the nucleus. Then a microprocessor complex, composed of RNA-binding protein DGCR8 and type III RNase Drosha, cleaves pri-miRNA into a ~85-nt stem-loop structure called pre-miRNA. The exportin 5-RAN/GTP complex mediates the transport of pre-miRNA from the nucleus into the cytoplasm. The RNase DICER in complex with double-stranded RNA-binding protein TRBP cleaves the pre-miRNA hairpin to a ~20- to 22-nt miRNA/miRNA duplex. After the duplex is unwound, the functional strand of the mature miRNA (the guide strand) is loaded into the miRISC-containing DICER1, TRBP, and Argonaute (AGO) proteins. This miRISC silences/inhibits the target mRNAs expression/function through mRNA cleavage, translational repression, or deadenylation. The passenger strand of the miRNA is degraded. AGO, Argonaute proteins; DGCR8, DiGeorge syndrome critical region gene 8; m7G cap, 7-methylguanosine; miRISC, miRNA-induced silencing complex; miRNA, microRNA; pre-miRNA, miRNA precursor; pri-miRNA, primary miRNA; RAN-GTP, Ras-related nuclear protein coupled with guanosine-5′-triphosphate; TRBP, transactivating response RNA-binding protein.

regions.[16] Some other studies demonstrated that they are also derived from transposon elements or across two tandem, inverted repetitive elements.[17] miRNA are first transcribed as much longer RNAs called primary miRNA, or pri-miRNA.[18] Biogenesis of miRNA involves two distinct steps involving both nuclear and cytoplasmic events (Figure 1). In the nucleus, the long pri-miRNA transcript is initially altered with a 5′ 7-methylguanosine cap and a 3′ poly(A) tail and transcribed by RNA polymerase II (Pol II) or III.[19–21] This is subsequently processed by DGCR8 and Drosha to form short hairpin structures of 70–90 nt called a precursor of mature microRNA (pre-miRNA). Exportin-5 exports pre-miRNA to the cytoplasm, where it is further processed by RNase III enzyme Dicer and *trans*-activating response RNA binding protein (TRBP) to form mature miRNA.[22,23] Subsequently, Argonaute proteins (Ago1 and Ago2) recruit miRNA in the miRNA-induced silencing complex (miRISC) (Figure 1). The loaded RISC complex binds selectively to the miRNA recognition elements (MREs) within the 3′ UTR of target mRNA transcripts.[24,25] To bind selectively to the target sequence within mRNA, the miRNA uses a 2- to 8-nt long "seed sequence," which is present at the 5′ region of the miRNA.[26] Target genes with longer 3′ UTR generally have the maximum density for miRNA binding sites rather than the shorter 3′ UTR. Most of the miRNA-mRNA interaction occurs at the 3′ UTR region, although targeting and silencing of mRNA have also been reported to occur at the 5′ UTR with similar efficiency.[14,27] miRNA:mRNA interaction inhibits protein translation by suppressing translation or degrading the target mRNA, and subsequently plays a critical role in cellular growth and differentiation.

Function and Mechanism of miRNA

miRNA can regulate multiple genes and vice versa, i.e., each mRNA may be targeted by multiple miRNA. miRNA mediate their function as part of an effector unit containing an Argonaute protein and is known as miRNP, miRgonaute or miRISC.[28] Several factors affect the biological outcome of a miRNA:mRNA interaction, such as the binding of the miRNA seed region with the target site via base pairing, the number and relative positions of multiple target sites on the same miRNA, target site accessibility as a function of the secondary structure, and flanking target sequences of other miRNA.[29–31] miRNA can mediate transcriptional or post-transcriptional gene silencing.[7] Transcriptional gene silencing involves a unique cellular complex called RNA-induced transcriptional silencing (RITS) that contains argonaute molecules responsible for chromatin remodeling.[32] Post-transcriptional gene silencing involves suppression of translation and degradation of target mRNA transcripts. Target mRNA degradation by miRNA involves 5′ end decapping and 3′ deadenylation followed by degradation by several endo- and exo-nucleolytic nucleases such as

polysomal ribonuclease 1 (PMR1) and Xrn1p, respectively.[32–34] Wakiyama et al.[35] have shown that the closed-loop structure of mRNA enhances translation, but by deadenylating the poly(A) tail, miRNA prevent binding of cytoplasmic poly(A)-binding protein (PABPC1) and consequently repress translation. Argonaute protein can also directly repress translation of the target mRNAs by competing with the eukaryotic transcription factor, elF4E, that directs the ribosomes to the target mRNAs.[36] Chendrimada et al.[37] demonstrated that Ago2 binds to eIF6, preventing the binding of the large ribosomal subunit to the small ribosomal subunit and inhibiting translation. On the other hand, the RNA-binding protein HuR can relieve miRNA-mediated repression. Under normal conditions, HuR protein is primarily localized in the nucleus, but upon several stress conditions, it relocates to the cytoplasm and plays a potential regulatory role by relieving the miRNA-mediated repression.[28] Bhattacharyya et al.[38] have reported that the miR-122-mediated translational suppression of cationic amino acid transporter 1 (CAT-1) is relieved by binding of HuR protein to the 3′ UTR of CAT-1 mRNA.

Regulation of MicroRNA Expression

Most of the miRNA genes are found in intergenic locations or antisense orientation to the annotated gene, implying that they have their transcription machinery.[39,40] Lee et al.[20] first demonstrated that miRNA are transcribed by Pol II, although recently other studies have found that miRNA transcription is also mediated by Pol III.[21] Saito et al.[41] first showed that chromatin remodeling and epigenetic alterations by DNA methylation and histone tail modifications could regulate the expression of several miRNA with consequent effects on cellular functions. To examine miRNA expression in human cancer cells, they treated the cells with a DNA-demethylating drug named 5-Aza-CdR and histone deacetylase inhibitor named 4-phenyl butyric acid, and found that miR-127 was upregulated significantly among other miRNA targeting the proto-oncogene BCL-6 that is upregulated in cancer cells.[42,43] This study was supported by another study that showed that using the histone deacetylase (HDAC) inhibitor named LAQ824 in breast cancer cell line SKBr3 caused a significant change in 40% of the different miRNA species.[44] Most of the miRNA are intragenic or found in the introns of the protein-coding genes and are reasonable to demonstrate that transcription of miRNA is cooperatively regulated with the host genome. Because miRNA have their promoter, it is believed that CpG islands of host promoter also found within the same intron and transcription of both protein-coding gene and miRNA are regulated by DNA methylation.[45,46]

Apart from the epigenetic modulation, several other nuclear proteins or factors are responsible for regulating the miRNA expression. Fukuda et al.[47] demonstrated that DEAD-box RNA helicases p68 and p72, components of a large Drosha-mediated processing complex, could interact with several transcription factors such as Smads, p53, and estrogen receptor to correctly recognize and bind to a subset of pri-mRNAs, and initiate the cleavage to form pre-miRNA. Guil and Cáceres[48] reported that heterogeneous nuclear ribonucleoproteins (hnRNP proteins), RNA-binding proteins, play a role in the processing of endogenous pri-miR-18a, which is context dependent and regulating the activity of miR-18a. Importantly, they found that depletion of hnRNP A1 affects able processing of miR-18a, which increases cell proliferation and promotes the anchorage-independent growth of cancer cells.[49] KH-type splicing regulatory protein (KSRP), a multifunctional single-strand RNA-binding protein, binds to the conserved G-rich elements in the terminal loop (TL) of a cohort of miRNA precursors and interacts with both Drosha and Dicer to promote miRNA maturation.[50] KSRP interacts with heterogeneous nuclear RNA-binding proteins (hnRNPs), which are involved in mRNA maturation, as well as acts as an auxiliary factor for the Drosha-mediated processing of a microRNA precursor by binding to the TL of a group of pri-miRNA.[48,51] High mobility group A (HMGA) proteins are extensively synthesized during the early embryonic stage, as well as growth and development, and also are involved in regulating miRNA expression by regulating chromatin structure and gene expression.[52,53]

Role of miRNA in Lung Development

Lung development and maturation is a complex and vital morphogenetic process that is temporally and spatially regulated by a defined set of genes.[54] In the fetus, lung development goes through six defined stages: embryonic, glandular, canalicular, saccular, alveolar, and vascular expansion.[55] Several cytokines and their signaling pathways such as transforming growth factor-beta (TGF-β), fibroblast growth factors (FGFs), sonic hedgehog (Shh), and wingless-type MMTV integration site family (WNT)/β-CATENIN are involved in lung development.[56–58] Stage-specific and tissue-specific miRNA expression are crucial for lung development and in maintaining lung homeostasis.[59]

For instance, members of the miR-17-92 cluster (miR-17, -18a, -19a, -20a, -19b-1, and -92-1) are highly expressed in embryonic lungs.[60] Expression of these same miRNA decreases during lung maturation. Conversely, the let-7 family miRNA are elevated in the adult lung compared with early embryonic stages.[61] Hayashi et al.[62] reported that the expression of miR-21 is required and has a crucial role in branching morphogenesis, a primary developmental process in the lung. Furthermore, expression of miR-142-3p and miR-326 regulates the proper differentiation and proliferation of mesenchymal cells by WNT signaling and Shh signaling pathways, respectively.[63,64] In the development of vascular smooth muscle cells (vSMCs), the role of miRNA and proteins involved in the miRNA pathway was studied extensively. The cooperative role of both miRNA (miR-145 and miR-143) was reported in maintaining proper SMC phenotype, whereas miR-133 and miR-206 play crucial roles in the proliferation, migration, and development of vSMCs by targeting transacting transcription factor-1 and Notch3, respectively.[65] Inhibition of miRNA processing by conditional inactivation of DICER, during the embryonic stage, resulted in deformed lung development, and excessive epithelial cell death was reported.[66] At the embryonic stage, reduced expression of Ago1 and Ago2 in the distal epithelium and mesenchyme, respectively, suggested that miRNA-regulated gene expression is involved in the lung developmental processes.[67,68]

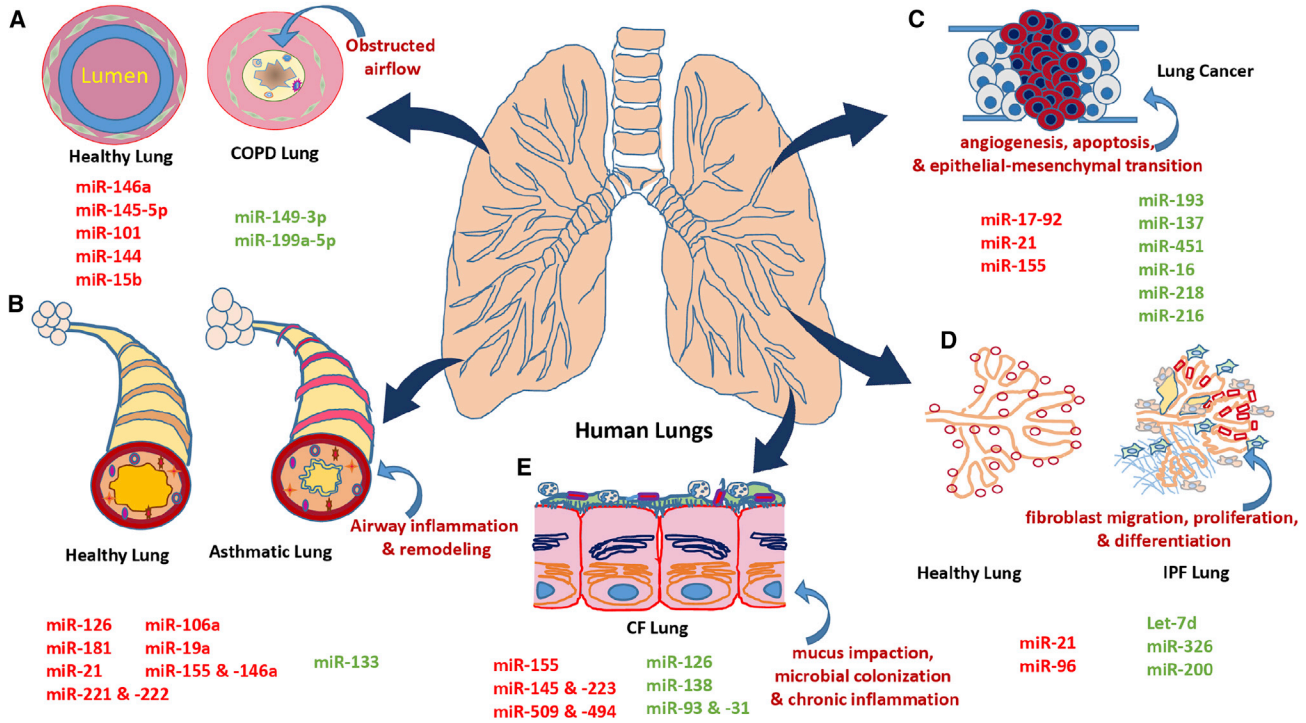

Figure 2. Schematic of MicroRNAs Implicated in Lung Pathophysiology in Different Lung Diseases

(A) Chronic obstructive pulmonary disease (COPD) is a chronic inflammatory lung disease that causes obstructed airflow from the lungs and attenuates mucociliary clearance (MCC), leading to mucous obstruction, and provides a nutrient-rich environment for bacterial reproduction, leading to pulmonary infections and chronic inflammation. (B) Asthma, characterized by the hallmarks of airway inflammation, airway remodeling, airway hyperresponsiveness, and reversible airway obstruction. (C) Lung cancer is associated with excessive pulmonary cell proliferation, apoptosis, angiogenesis, and epithelial-mesenchymal transition. (D) In idiopathic pulmonary fibrosis, the normal lung tissue is replaced by more heavily scarred lung tissue, which makes it difficult for the patient to breathe and deliver needed oxygen to the body. This causes the aberrantly activated lung epithelium to produce mediators of fibroblast migration, proliferation, and differentiation into active myofibroblasts. (E) In cystic fibrosis, aberrant or nonexistent CFTR function compromises the airway surface liquid, leading to mucous impaction and sub-optimal ciliary beating. This results in microbial colonization and chronic inflammation, which further compromise mucociliary clearance. microRNAs in red and green indicate whether the miRNA is elevated or reduced in lung-associated diseases, respectively. CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis.

Role of miRNA in Lung Health and Disease

miRNA play an important role in lung health and diseases. Dysregulation of miRNA plays an important role in pathological hallmarks of several lung diseases (Figure 2). Several studies identified altered miRNA expression profiles, which may be associated with pathological processes within the lung and lead to the development of several respiratory diseases, ranging from inflammatory diseases (chronic airway diseases such as COPD, asthma, and cystic fibrosis [CF]) to lung cancers.[69,70] A group of miRNA has been identified to play a role in inflammatory responses in chronic airway diseases, such as COPD, asthma, and CF. Likewise, other groups of both pro-fibrotic and anti-fibrotic miRNA have been identified to play a role in interstitial pulmonary fibrosis. Lung diseases are the leading cause of morbidity and mortality worldwide.[71] According to the World Health Organization (WHO), COPD is the fourth leading cause of death worldwide and predicted to become the third leading cause by 2030.[72] On the other hand, asthma, a complex, heritable disease, affects more than 300 million people globally, and idiopathic pulmonary fibrosis (IPF), a chronic fibrotic lung disease, affects approximately 3 million people worldwide, with the incidence increasing with age.[73,74] The GLOBOCAN 2018 database reports 2.09 million new cases and 1.76 million deaths from lung cancers.[75] Hence identifying the molecular mechanisms involved in the development and progression of these diseases is important to public health. Many reports are now investigating microRNA-mediated post-transcriptional gene silencing in lung diseases. Much attention and research remain to be conducted to explore the function and pathological role of miRNA in respiratory diseases. In the following sections, we will be focusing on aberrant miRNA expression, their target sites, and findings in the five most common lung diseases (Table 1).

miRNA in COPD

COPD is a common airway complication that comprises chronic obstructive bronchitis and lung emphysema.[76] COPD is a multifactorial disease that represents the leading cause of higher morbidity and mortality globally, and it is also expected that COPD will become the third leading cause of death worldwide by 2030 because of increased prevalence with older age, environmental risk factors, excessive

Table 1. List of miRNAs that Are Differentially Expressed in Lung Diseases (COPD, asthma, lung cancer, IPF, and CF), with Their Respective Target Sites and Findings Listed Above

Lung Diseases	Specific miRNA	Expression Level	Target Site/Host Gene	Findings	References
COPD	miR-146a	high	COX-2	targets 3′ UTR of the Cox2 mRNA and suppresses the expression	1
	miR-149-3p	low	TLR-4, MyD88	reduced expression causes overexpression of TLR-4 and MyD88	2
	miR-145-5p	high	SMAD3, CFTR, SLC26A9	involved in Th2 response activation, blocks chloride ion channel	3,4
	miR-199a-5p	low	Unfolded protein responses	intensification of the UPR	5
	miR-101 and miR-144	high	MKP-1, TGF-β signaling	induce inflammatory responses	6
	miR-15b	high	SMAD7	induces TGF-β signaling	7
Asthma	miR-126	high	TLRs	activation of inflammatory pathways	8
	miR-21	high	IL-12p35	modulates IL-12 expression and polarizes Th cells toward Th2 response	9
	miR-155 and miR-146a	high	transcription factor PU.1 and IL-4	contributes to immediate inflammation and allergic reactions	10
	miR-133a	low	RhoA	excessive bronchial smooth muscle (BSM) contraction	11
	miR-221 and miR-222	high	$p21^{WAF1}$ and $p27^{kip1}$	involved in mast cell activation and release several growth factors	12
	miR-106a	high	IL-10	increases pro-inflammatory cytokines release	13,14
	miR-181	high	NF-κB	induces increased TCR sensitivity	15
	miR-19a	high	PI3K, JAK-STAT, NF-κB signaling	promotes allergic inflammatory phenotype	16,17
Lung cancer	miR-193	low	KRAS	promotes cellular proliferation, differentiation, and migration	18
	miR-17-92	high	myc	promotes hyper-proliferation of lung epithelial cells	19
	miR-21	high	PTEN, PDCD4	promotes growth and invasion in NSCLC	20
	miR-137	low	SLC22A18	promotes aggressive tumor progression	21
	miR-451	low	RAB14	induces tumor differentiation and shorter survival	22
	miR-16	low	p27, Bcl-2, Bax, and caspase-3	induces cell proliferation and apoptosis	23
	miR-218	low	HMGB1	leads to aggressive cell proliferation, migration, and invasion	24
	miR-155	high	Apaf-1	resistance to therapy and associated with shorter survival	25
	miR-216	low	eIF4B, ZEB1	tumor growth, proliferation, metastasis, and chemoresistance	26
IPF	Let-7d	low	HMGA2	increases mesenchymal markers (ACTA2, VIM) and decreases epithelial markers (cytokeratin and TJP1)	27
	miR-21	high	SMAD7	promotes excessive extracellular matrix (ECM) gene transcription	28
	miR-96	high	FoxO3a	increases PI3K-Akt activity, thereby promoting IPF fibroblasts	29
	miR-326	low	3′ UTR of TGF-β	upregulates profibrotic genes	30
	miR-200	low	TGF-β signaling	induces epithelial-mesenchymal transition and tumor metastasis	31

(*Continued on next page*)

Table 1. *Continued*

Lung Diseases	Specific miRNA	Expression Level	Target Site/Host Gene	Findings	References
CF	miR-126	low	TOM1	causes excessive inflammatory response and airway obstruction	32,33
	miR-138	low	SIN3A	resuscitates the CFTR expression	
	miR-155	high	MAPK and PI3K/Akt signaling	activates proinflammatory cytokine IL-8 to attract neutrophils	34
	miR-145 and miR-223	high	3′ UTR of CFTR	decrease CFTR expression and cause inflammation	35
	miR-509 and miR-494	high	NF-κB signaling	repress CFTR expression and induce pro-inflammatory cytokines	36
	miR-93 and miR-31	low	3′ UTR of IL-8, IRF-1	promote increased production of cathepsin S	37

High and low indicate whether the miRNA is elevated or reduced in lung-associated diseases, respectively. CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; IPF, idiopathic pulmonary fibrosis.

cigarette smoking, and noxious gases.[77,78] The hallmarks of COPD are characterized by chronic inflammation in the lungs, a shorter interval between breathing, severe cough, and repetitive impediment across the tracheal wall during inhalation (Figure 2A).[79] Several miRNA have been implicated in the pathobiology of COPD.[80–84]

In COPD patients, increased secretion of prostaglandin E_2 (PGE_2) results in collagen overproduction, ultimately reducing lung capacity and accelerating COPD.[69] COPD patients demonstrate decreased miR-146a expression and increased expression of its target, Cox-2, with a consequent increase in PGE_2 levels.[80] Matrix metalloproteases (MMPs) play a major role in respiratory inflammation and structural remodeling in COPD patients. During the early stage of COPD, cigarette smoke induces macrophages, lymphocytes, and neutrophils to be deposited in the walls of bronchioles, alveolar ducts, and alveoli.[85,86] Macrophage-derived MMPs including MMP-2, MMP-9, and MMP-12 degrade and solubilize extracellular matrix proteins, collagen, and elastin.[87,88]. MMP-12 is overexpressed in the lungs of COPD patients.[89] Graff et al.[90] demonstrated that miR-452, an MMP-12-targeting microRNA, is significantly downregulated in COPD patients, resulting in overexpression of MMP-12.

Shen at al.[91] have shown that levels of miR-149-3p play a protective role in COPD by suppressing the Toll-like receptor 4/nuclear factor κB (TLR4/NF-κB) pathway by targeting two distinct signaling intermediates, namely, TLR4 and MyD88.[91–93] miR-149-3p levels are progressively suppressed in non-COPD smokers, followed by stable COPD smokers, with maximal suppression observed in smokers with acute exacerbation COPD.[91] Dysregulation of TLR4 expression has multiple downstream effects by increasing the expression of proinflammatory cytokines interleukin-1β (IL-1β), IL-6, IL-8, IL-10, tumor necrosis factor-alpha (TNF-α), and interferon-γ (IFN-γ).[94–96] Persistent activation of the TLR-4 signaling and MyD88 dysregulation also induces matrix metalloproteinase 1 (MMP-1) via MyD88 and IRAK1 pathways, which plays an important role in COPD.

Another microRNA, miR-145-5p, is significantly upregulated in patients with COPD and smokers, and can serve as a promising biomarker of COPD.[97] Tobacco smoking is the principal risk factor for COPD. Cigarette smokers and COPD patients demonstrate chronic induction of TGF-β signaling.[98–102] We have demonstrated that TGF-β upregulates miR-145-5p in bronchial epithelial cells.[100] miR-145-5p dysregulation can have multiple downstream effects, which can lead to a progressive decline in lung function. For instance, miR-145-5p is involved in Th2 response activation, macrophage differentiation, and recruitment of eosinophils.[103,104] Likewise, TGF-β-mediated miR-145-5p induction plays an important role in the regulation of airway smooth muscle (ASM) function in COPD patients by targeting SMAD3 that negatively regulates the release of pro-inflammatory cytokines.[105] Cystic fibrosis transmembrane conductance regulator (CFTR) functions as a chloride channel involved in maintaining airway fluid homeostasis, as well as regulating innate immune responses in the airway. Smokers and COPD patients demonstrate an acquired CFTR dysfunction even though they have regular copies of the CFTR gene.[106,107] Acquired CFTR dysfunction results in impaired mucociliary clearance and dysfunctional airway innate immune responses, which result in chronic microbial infection and lung inflammation.[108–115] In COPD patients, expression of CFTR-targeting miRNA miR-101 and miR-144 is upregulated with consequent CFTR suppression.[116] We have recently demonstrated that TGF-β signaling and cigarette smoke (via TGF-β signaling) upregulate miR-145-5p to suppress CFTR, as well as an important CFTR modifier SLC26A9, which also functions as a backup Cl^- channel.[100]

miR-144 and miR-15b are potential mediators of the TGF-β signaling cascade and genes that are functionally associated with the TGF-β superfamily involved in the development and progression of COPD, inflammatory response, and airway epithelial repair after injury.[99,117] The miR-15b expression is higher in COPD patients with a concomitant decrease in the inhibitory SMAD7.[118] Expression of another microRNA, miR-199a-5p, is diminished in COPD patients because of hypermethylation of CpG sites in the miR-199a-5p promoter.[119] Decreased expression of miR-199a-5p leads to an intensification of the unfolded protein responses (UPRs) and contributes to lung cell apoptosis and lung inflammation.[119]

miRNA in Asthma

Asthma is a chronic inflammatory disease of the airway system characterized by fatal airway obstruction, tissue remodeling, bronchial epithelial hyperresponsiveness, and chronic inflammation (Figure 2B). Asthmatic patients also experience intermittent periods of wheezing, heavy tightness in the chest, and shortness of periodic breathing.[120–122] According to the WHO, asthma caused 225,000 deaths all over the world in 2005, and with this current trend, the number will reach 430,000 by 2030.[123] Both genetic and environmental factors are considered important triggers to the pathogenesis of asthma. Chronic inflammation in asthmatic patients is associated with persistent deposition of mast cells and eosinophils that promotes increased cytokine production by Th2 cells, resulting in mucous hypersecretion, bronchial hyperactivity, elevated immunoglobulin E (IgE) levels, and eosinophils infiltration.[124,125]

Several miRNA play important roles in the pathology of asthma. These can be broadly categorized into pro-inflammatory miRNA and anti-inflammatory miRNA. MicroRNA-126 promotes inflammation by inducing the overexpression of Toll-like receptors (TLRs) present on T helper 2 cells (Th2).[121] miR-126 antagonism activates the PU.1 transcription factor, which modulates Th2 cell function via negative regulation of GATA3 expression.[126] Another study confirmed this role for miR-126 using antagomiRs to miR-126.[127] They showed that miR-126 antagonism suppresses Th2-driven bronchial inflammation, mucous hypersecretion, and airway hyperresponsiveness (AHR) in mice.

IL-13, a pleiotropic Th2 cell-derived effector cytokine, plays a central role in the pathogenesis of asthma.[128] Lu et al.[122] showed that IL-13 induces the overexpression of miR-21 and underexpression of miR-1 in transgenic mice compared with the control mice. They also demonstrated that IL-13-induced miR-21 overexpression is IL-13Rα1 dependent, whereas allergen-induced miR-21 overexpression is IL-13Rα1 and STAT6 independent, meaning that miR-21 induction is associated with leukocytes recruitment/activation in *IL-13Rα1*$^{-/-}$ mice. By using bioinformatics tools and target site validation approaches, Lu et al.[122] found that IL-12p35 is the putative target of miR-21. On the other hand, increased levels of miR-21 negatively modulate the expression of IL-12, which is a pro-inflammatory cytokine that induces the production of IFN-γ, involved in adaptive immune responses including Th1 cell polarization.[129] IL-13 also upregulates RhoA expression, which is responsible for bronchial smooth muscle (BSM) contraction that contributes to airway narrowing in people with asthma.[130] IL-13-induced RhoA upregulation was found to be a consequence of miR-133a suppression and STAT6 dependent.[131] IL-13 secretion itself is subject to miRNA-mediated regulation. miR-145 plays a vital role in the onset and pathogenesis of allergic airways disease by inducing Th2 cells to release IL-5 and IL-13.[103]

Altered expression of miR-155 and miR-146a affects the local immune response in allergic asthma.[132–135] Increased expression of miR-155 and miR-146a is associated with Th2-mediated increased cytokine IL-4 release that induces B cells to undergo class switching to secrete more IgE and contributes to immediate inflammation and allergic reactions.[136] Intranasal administration of miR-155 in mice activates the expression of chemokine eotaxin-1/CCL11 and eotaxin-2/CCL24, as well as an eotaxin-1/2/CCR3 pathway, which are essential for eosinophil recruitment.[134,137] miR-155 knockout mice demonstrated elevated levels of PU.1, transcription factor suggesting that PU.1 is a direct target of miR-155. PU.1 negatively regulates Th2 cytokines (IL-4, IL-5, IL-9, and IL-13), which play a vital role in the pathophysiology of asthma.[138] Antagonism with miR-155 suppresses the inflammation in asthmatic patients and can be considered a novel lead to asthma therapy.[139]

In severe asthmatic patients, TGF-β increases expression of miR-221 and miR-222 with consequent ASM hyper-proliferation and IL-6 secretion. IL-6 is involved in mast cell activation and release of several growth factors.[140] Mast cells are responsible for the early-phase reaction of allergic inflammation and involved in the secretion of preformed and lipid-derived mediators.[141] Perry et al.[142] discovered that increased secretion of IL-6 in ASMCs was associated with reduced expression of cyclin-dependent kinase inhibitor p21WAF1 and tumor suppressor p27kip1. They also found that miR-221 and miR-222 regulate the level of p21WAF1 and p27kip1 expression in mast cells and induce the abnormal inflammatory and proliferative responses with severe asthma.[143]

miR-181a and miR-19a are also considered inflammatory miRNA in asthma. miR-181a upregulation induces increased TCR sensitivity and lowers the T cell activation threshold.[144,145] Likewise, upregulation of miR-19a stimulates Th2 cytokine production by augmenting the phosphatidylinositol 3-kinase (PI3K), JAK-STAT, and NF-κB signaling pathways, and drives asthma pathogenesis.[146,147] Decreased secretion of anti-inflammatory cytokines such as IL-10 plays an important role in asthma.[148] miR-106a significantly decreases the synthesis of anti-inflammatory cytokine IL-10 with a concurrent augmentation of proinflammatory cytokines release.[149,150] miR-106a antagonism promotes IL-10 secretion and helps mitigate asthmatic conditions by increasing Th2 response in mouse models of asthma. Other miRNA are also reported to regulate IL-10 synthesis as well. Likewise, IL-10 induces expression of other miRNA such as miR-146a, miR-146b, miR-155, miR-132, miR-21, and miR-125a in asthmatic patients by regulating TLR and NF-κB-mediated signaling pathways.[151,152]

miRNA in Lung Cancer

Lung cancer is the leading cause of cancer morbidity and mortality worldwide.[153] Impairment in proper microRNA processing, frequent epigenetic changes in cellular regulatory elements, activation of oncogenes, suppression of tumor suppressor genes, impairment with Drosha, DGCR8, and Dicer activity, and potential effects of cigarette smoke or allergens have all been found as possible mechanisms for microRNA dysfunction in lung cancer.[1] Figure 2C summarises the

dysregulation of miRNA in lung cancer. Although most reports have demonstrated suppression of miRNA targeting oncogenes with a concomitant increase in their target gene expression, some miRNA such as miR-17-92 cluster and miR-155 are overexpressed in small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC), respectively. Hayashita et al.[154] showed that the overexpression of miR-17-92 promotes the hyper-proliferation of lung epithelial cells and leads to lung cancer. Upregulation of miR-17-92 cluster by Myc plays an important role in the oncogenesis of the more aggressive SCLC.[155] miR-155 upregulation in NSCLC has been associated with resistance to chemotherapy.[156] miR-155 targets the apoptotic protease-activating factor 1 (Apaf-1), which increases the sensitivity of lung cancer cells to cisplatin-induced DNA damage and apoptosis.[157] Roosbroeck et al.[156] have shown that overexpression of miR-155 also suppresses the expression of tumor protein (TP53) in lung cancers. They reported that the miR-155/TP53-negative regulatory feedback loop is involved in resistance to therapy and decreased survival in lung cancer.

miR-21 is one of the most extensively identified and studied miRNA in different types of cancer, including NSCLC.[158] miR-21 functions as an anti-apoptotic and pro-survival factor, and overexpression of miR-21 is a prognostic and diagnostic biomarker for lung cancer.[159] Shen et al.[160] have reported that miR-21 post-transcriptionally silences the expression of the tumor suppressor PTEN and promotes growth and invasion in NSCLC.

miR-193a (miR-193a-3p and miR-193a-5p) functions as a tumor suppressor in lung cancer.[161] Fan et al.[162] first reported the detailed association between miR-193a-3p expression and lung cancer, and also identified the unique target genes for miR-193a-3p by using a xenograft mouse model. Their study indicated that overexpression of miR-193a-3p in NSCLC tissues suppressed cell viability, cellular migration, and proliferation by targeting the KRAS oncogene that regulates cellular proliferation, differentiation, and migration in lung cancer.[163]

SLC22A18, an organic cation transporter, plays an important role in lung cancer.[164] Using a large cohort of NSCLC patients, the study demonstrated that overexpression of miR-137 drastically suppresses the proliferation and migration in NSCLC patients. Conversely, decreased expression of miR-137 directly suppresses SLC22A18 expression and promotes aggressive tumor progression.[165] Zhang et al.[165] demonstrated that overexpression of SLC22A18 was associated with tumor progression and patients' prognosis. They also showed that miRNA-137 serves as a tumor suppressor by directly targeting SLC22A18 and inhibiting NSCLC cell proliferation, invasion, and migration. Moreover, in NSCLC, miR-137 also targets paxillin, Cdc42, and Cdk6, and inhibits the proliferation and migration of NSCLC cells.[166,167]

miR-218 is a putative tumor suppressor in NSCLC.[168] Expression of a mature miR-218 is depleted in NSCLC, and overexpression of miR-218 negatively regulates cell proliferation and invasiveness by reducing the expression of JAK3, IL-6R, and phosphorylated STAT3 in lung cancer. miR-218 also targets high mobility group box-1 (HMGB1) that binds to chromatin and facilitates access of transcriptional factors.[169,170] Overexpression of HMGB1 leads to aggressive cell tumorigenesis and tumor metastasis, and this can be diminished by miR-218.[171,172] Likewise, miR-451 and miR-216 are suppressed in NSCLC. Wang et al.[173] demonstrated that reduced expression of miR-451 in NSCLC tissues was significantly associated with tumor differentiation, pathological stage, and shorter overall survival in NSCLC patients. miR-451 targets the oncogene Ras-related protein (RAB14), a member of the RAS oncogene family, and its dysfunction has been reported in various types of lung cancer.[174]

mir-216 functions as a tumor suppressor by regulating eukaryotic initiation factor 4B (eIF4B) and zinc-finger E-box-binding homeobox 1 (ZEB1), and downregulation of miR-216a expression causes tumor growth, proliferation, metastasis, and chemoresistance contributing to NSCLC progression.[175] The mir-216 expression can also serve as a biomarker for NSCLC progression. Both eIF4B and ZEB1 act as oncogenes and induce several proto-oncogenic signaling pathways such as Ras-mitogen-activated protein kinase (MAPK) and PI3K/mammalian target of rapamycin (mTOR).[176,177] Zinc-finger E-box miR-145-5p also plays an overall important role in NSCLC. Recently, Hu et al.[178] demonstrated that expression of miR-203 and miR-145 is downregulated in NSCLC and suggested that both function as tumor suppressors. It has been shown that TGF-β signaling plays an important role in epithelial-mesenchymal transition (EMT).[179] EMT is considered an important step in tumor progression. miR-145 and miR-203 suppress the TGF-β-induced EMT and invasion by repressing SMAD3 in NSCLC cells where SMAD3 has an important role in the EMT and tumor metastasis.[178]

miR-34a is suppressed in human NSCLC tissues, and restoration of miR-34a expression inhibits cell growth and tumor formation.[180] miR-34 loci were frequently hypermethylated and downregulated in human NSCLCs.[181] miR-34a regulates epidermal growth factor receptor (EGFR) directly or EGFR signaling and function as a vital tumor suppressor in NSCLC with EGFR as a novel target.[182] Because EGFR is involved in oncogenesis such as excessive DNA synthesis, dysregulated cell cycle, uncontrolled cell proliferation, cell invasion, and metastasis, miR-34a-mediated EGFR downregulation inhibits cellular proliferation, promotes cellular apoptosis, and induces cell-cycle progression in NSCLC cell lines.[183]

miRNA in IPF

IPF is a chronic, progressive, and fibrotic lung disease.[184] The disease is characterized by fibroblast proliferation, extracellular matrix remodeling, epithelial scarring, and excessive accumulation of collagen in parenchymal tissue (Figure 2D).[185] Several studies have identified cigarette smoking, exposure to commonly prescribed drugs, environmental factors, and genetic predisposition as the potential causes for IPF.[186]

Pulmonary fibrosis in IPF is characterized by excessive synthesis and secretion of cytokines such as TGF-β, TNF-α, FGFs, interleukin-1 (IL-1), and monocyte chemoattractant protein-1 (MCP-1) from activated inflammatory cells, such as macrophages and eosinophils.[187] Other studies reported that several miRNA affect the networks of cytokines and exacerbate the disease.[188–190] TGF-β expression is tightly regulated at different stages such as transcription, post-transcriptional mRNA stability, and processing and posttranslational processing.[191] TGF-β promotes fibroblast differentiation into more fibrogenic myofibroblasts and acts as the primary regulator of fibrotic lung diseases.[192] TGF-β signaling can lead to transcription activating SMAD3, or the inhibitory SMAD7.[193] TGF-β induces SMAD3, which has been shown to suppress the expression of let-7d by binding to the upstream region of let-7.[194] TGF-β induces high mobility group A2 (HMGA2) by inhibiting let-7 expression. TGF-β-induced EMT is associated with smad-dependent overexpression of HMGA2 which results in transcription of multiple factors involved in EMT.[195] Downregulation of let-7 expression and consequent overexpression of HMGA2 increased expression of mesenchymal markers ACTA2 and VIM and decreased expression of epithelial markers cytokeratin and TJP1. Liu et al.[184] demonstrated that TGF-β signaling leads to significant overexpression of miR-21 in the bleomycin-induced lungs of mice, and this functions as an amplifying circuit to increase the fibrogenic activity of TGF-β, thereby promoting lung fibrosis. miR-21 overexpression suppresses the inhibitory SMAD7, as well as leading to enhanced phosphorylation of SMAD2 with consequent fibrogenic effects.

Das et al.[196] showed that miR-326 plays a protective role in lung fibrosis by downregulating TGF-β expression and attenuating fibrotic response. Downregulation of miR-326 expression is a crucial mediator of IPF by acting on different components of TGF-β signaling pathways. In bleomycin-induced lung fibrosis, the miR-326 expression is suppressed. miR-326 mimics decreased TGF-β expression and consequently attenuated the bleomycin-induced fibrotic response. miR-326 was also implicated in the downregulation of other profibrotic genes, such as Ets1, Smad3, and MMP-9, and upregulation of antifibrotic genes, such as Smad7, involved in the TGF-β signaling pathway. Nho et al.[197] demonstrated that increased expression of miR-96 correlates with decreased expression of the FoxO3a transcription factor in most IPF fibroblasts. FoxO3a is ubiquitously expressed in cells and regulates cell proliferation and survival, and coordinates responses to DNA damages.[198] FoxO3a regulates functions as a checkpoint in the cell cycle, triggers the repair of DNA damage, and protects cells from oxidative stress.[199] By suppressing FoxO3a, miR-96 suppresses p27, p21, and Bim-1 expression, which leads to increased cell proliferation.[200]

Yang et al.[201] showed that miR-200 family members (200a, 200b, 200c) suppress EMT and reverse the fibrogenetic function of pulmonary fibroblasts. miR-200 family members target GATA3, ZEB1, and ZEB2 genes implicated in EMT and tumor metastasis.[202,203] miR-200 mimics suppressed the overexpression of SMA-α and Fn, the marker of the myofibroblasts in lung fibroblasts of mice with pulmonary fibrosis.[204] Yang et al.[201] also showed that members of the miR-200 family act as negative regulators of TGF-β-mediated lung fibrosis and attenuate the TGF-β-mediated expression of mesenchymal markers, and could serve as candidate therapeutics to treat lung fibrosis.

miRNA in CF

CF is one of the monogenic, lethal genetic (autosomal recessive) lung disorders common in Caucasian populations. CF is also reported in African and Asian populations with a lower incidence.[79,205] Several studies demonstrated that the underlying reason for CF is a dysfunctional CFTR as a consequence of mutation in the CFTR gene. CFTR localizes to the mucosal side of the airway epithelium and is involved in Cl^- efflux and Na^+ absorption. The net effect of CFTR action is a mild osmotic gradient that drives paracellular water flow, maintaining the airway surface liquid, which is critical for ciliary beating and mucous clearance.[111] CF, as a consequence of CFTR dysfunction, is best characterized by altered chloride ion transport, depletion of airway surface liquid, airway obstruction, and an excessive inflammatory response (Figure 2E).[206] Mucociliary dysfunction facilitates both chronic and acute bacterial infection by several opportunistic microorganisms named *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and causes excessive inflammation.[207,208]

miRNA in CF pathophysiology can be broadly categorized into two types, those directly regulating CFTR and those that modulate the consequent inflammation and remodeling because of CFTR dysfunction. miR-138 belongs to the first category in that it regulates SIN3A expression.[209] SIN13A is a transcriptional repressor that is mobilized to the promoter region of CFTR repressing its expression.[210] ΔF508 is the most predominant mutation in the CFTR mutation in CF and promotes the misfolding and degradation of CFTR.[211,212] However, a small proportion of ΔF508 does make it to the surface, and once on the surface is active; hence suppression of CFTR expression will further exacerbate CF. miR-138-mediated SIN13A suppression resuscitates CFTR expression and, consequently, activity.

miR-509-3p and miR-494 are upregulated in CF lungs compared with non-CF healthy controls.[213] The NF-κB signaling pathway regulates the expression of both miRNA. Both these miRNA are known to directly regulate CFTR, suggesting that under inflammatory stimuli predisposing to NF- κB signaling, miR-509-3p and miR-494 repress CFTR expression and consequently its function cooperatively by binding to its 3′ UTR. Likewise, increased expression of miR-145, miR-223, and miR-494 in CF individuals who are carrying homozygous or heterozygous ΔF508 CFTR mutation leads to decreased CFTR expression.[214] Oglesby et al.[214] have shown that microbial colonization in CF alters miRNA expression, which can directly modulate CFTR expression or indirectly affect ΔF508 CFTR by promoting inflammation. They showed that of the 255 miRNA with potential seed target sites in CFTR mRNA, miRNA miR-145, -223, and -494, with targeting a highly conserved region of 3′ UTR of CFTR, are upregulated. Interestingly, they showed that *Pseudomonas*-conditioned media, including lipopeptides, lipopolysaccharide (LPS), and CpG

DNA, induce the overexpression of these miRNA. This study supports the previous reports by Gillen et al.[215] and Megiorni et al.[216] demonstrating the role of these miRNA in CFTR mRNA suppression.

miRNA that promote or suppress inflammation can indirectly alter CFTR expression. As discussed above, inflammatory stimuli can lead to the expression of miR-509-3p and miR-494 repressing CFTR mRNA. Oglesby et al.[217] reported that the expression of miR-126 is significantly suppressed in CF lungs with a concomitant increase in its target TOM1. TOM1 has also been considered a negative regulator of TLR2, TLR4, and IL-1β and the TNF-α-induced signaling pathway, and inhibits the activity of transcription factors NF-κB and AP-1.[218,219] Overexpression of TOM1 decreases NF-κB activity even upon LPS stimulation. Likewise, knocking down TOM1 in LPS-stimulated cells increases NF-κB mediated IL-8 expression. Their studies indicate that increased expression of TOM1 via miR-126 downregulation may act in an anti-inflammatory role and counter the effects of other proinflammatory regulators in CF lungs.[217] Fabbri et al.[220] analyzed the microRNA profile in CF bronchial epithelial cells infected with *Pseudomonas aeruginosa*. In that study, they showed that *P. aeruginosa* infection decreases the expression of miR-93 in CF, in parallel with overexpression of pro-inflammatory IL-8. They identified a potential target site in the 3′ UTR region of IL-8 mRNA. Downregulation of miR-31 in CF airway epithelial cells promotes increased production of cathepsin S.[221] Cathepsin S is a potent elastase that promotes remodeling of the extracellular matrix via its proteolytic activity and is reported in CF lungs, along with cancer and heart disease.[222] Weldon et al. showed that transcription factor IRF-1 is the target for miR-31, and increased levels of IRF-1 due to the downregulation of miR-31 results in overexpression and secretion of cathepsin B by CF airway epithelial cells.

miRNA-Targeted Interventions as Therapy in Respiratory Diseases

Identifying clinically relevant miRNA is important for exploiting their therapeutic potential. Given that miRNA expression profiles are similar for both human and mouse lung, in most cases mouse models can be used to study the effects of aberrant microRNAomes in lung diseases while also identifying therapeutic leads to reverse the downstream effects of the dysregulated miRNA (Figure 3).[59] By using nucleic acid-based inhibitors such as small interfering RNAs (siRNAs), miRNA mimics, and miRNA inhibitors, researchers are trying to restore the normal microRNAome and improve clinical outcomes. The mechanism of cellular uptake of antisense oligonucleotide (ASO) depends on the structure of ASO and the cell type.[223] Various energy-dependent and non-energy-dependent entry pathways are believed to be involved in oligonucleotide internalization.[224] However, effective delivery of the oligonucleotides to their intracellular site of action remains a major challenge, and therapeutic applications can be limited because of problems associated with *in vivo* delivery of these therapeutic oligonucleotides and possible off-target effects.[225] The airway system uniquely consists of pulmonary surfactants, which are zwitterionic lipids that possess cationic properties at the pH of the respiratory tract.[226] Moschos et al.[227] demonstrated that anionic oligonucleotides are designed in a way to be absorbed by the respiratory surfactant and efficiently taken up by the cells. Moreover, the miRNA mimics, siRNAs, or antagomiRs can stimulate the immune system or saturate the post-transcriptional gene silencing mechanism.[228] Several strategies such as SNPs in the miRNA gene, miRNA 3′ tailing, editing, and methylation are being designed to minimize off-target effects, enhance uptake, and increase their stability.[229]

Therapies Using Mimics to Restore MicroRNA Levels

Earlier efforts for delivery of mimics focused on direct intratumoral injections (in case of cancers) or by viral vectors. Unfortunately, using modified viral vectors as therapeutic vehicles has some limitations and is considered controversial due to the risk of integration of viral DNA into transcriptionally active sites in host genome possibly dysregulating the expression of oncogenes or imparting excessive immunogenicity.[230] Lately, liposome and nanoparticle-based drugs have been used to facilitate the delivery and uptake of miRNA mimics or inhibitors and siRNAs. Trang et al.[231] explored therapeutic delivery of lipid-based let-7 and miR-34 formulations to show tumor-suppressive effects in a KRAS mouse model for lung cancer, and Rai et al.[232] showed that a miR-7 expressing plasmid has anti-proliferative effects against EGFR oncogene addicted lung cancer cells using liposomal delivery. Also, Chen et al.[233] found that GC4 single-chain variable fragment (scFv)-targeted nanoparticles containing miR-34a actively reduce the tumor size as well as survivin expression, an inhibitor-of-apoptosis protein, by targeting the MAPK pathway in lung metastasis. MRX34 is the first microRNA (miRNA) mimic encapsulated in a liposomal nanoparticle system to facilitate target cellular uptake to be tested in a clinical setting.[234] However, researchers are trying to overcome the liposome-based therapies due to charged molecules in liposomes and low pH sensitivity.[235] On the other hand, Xiao et al.[236] identified one small molecule activator of miR-34a called Rubone, which can upregulate the miR-34a expression in hepatocellular carcinoma. Young et al.[237] reported that small-molecule activator induces the expression of miR-122 in liver cancer cells and promotes the apoptosis through caspase activation. Chen et al.[238] identified a small-molecule activator derived from the photoreaction of naphthalene-1,4-dione and acetylenes and demonstrated its application is rescuing levels of miR-1 and miR-122 miRNA which are involved in tumorigenesis.[239] For the treatment of pulmonary diseases, miRNA-based therapeutics can be formulated as aerosols and delivered through inhalation that might decrease systemic exposure and reduce the possible toxicity and off-target effects.[240]

Therapies Targeting miRNA

Anti-sense oligonucleotide-based techniques (antagomirs, locked nucleic acid [LNA], and miRNA sponges) have also been designed to inhibit onco-miRs in lung cancer.[240] Chemical modifications like 2′-O-methyl group in antagomir gives the required stability against nucleases, and insertion of cholesterol moiety into the passenger strand facilitates cellular uptake. Antagomirs, also known as anti-miRs, are chemically synthesized oligonucleotides complementary to the miRNA and designed to bind to and interfere with their

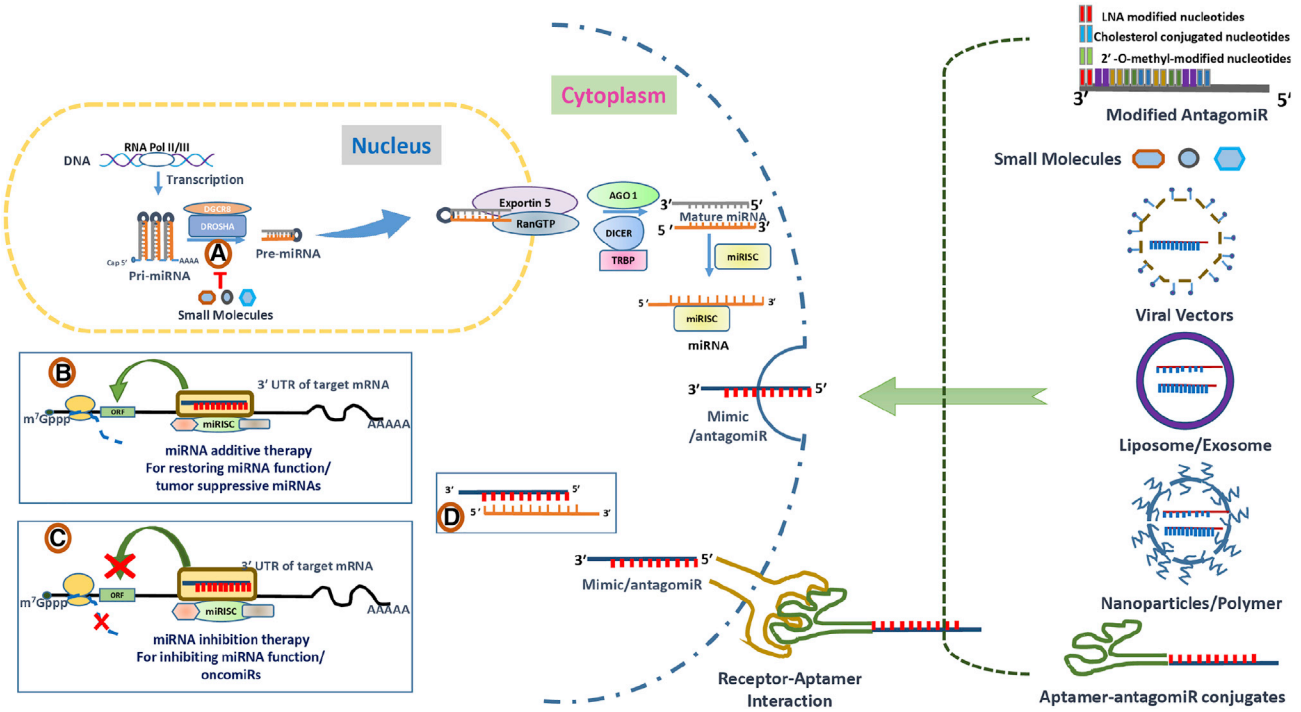

Figure 3. Therapeutic Approaches to Rescue miRNA Dysfunction

Exosome/liposome, viral vectors (lentivirus [LV], adeno-associated virus [AAV], adeno, and plasmid), nanoparticles/polymers, aptamer-mediated antagomiR, and miRNA mimic delivery into the pulmonary cells. (A) Small molecules bind to Drosha and Dicer processing sites of human miRNAs that are disease associated and inhibit their biogenesis. (B) miRNA mimics function like endogenous miRNAs restoring the activity of a miRNA. (C and D) Binding of single-stranded antagomiRs having complementary sequences to the target endogenous miRNA genome sequence and inhibiting the synthesis of disease-causing miRNAs (C), and antagomiRs having seed sequence sequesters the endogenous free miRNA target inhibiting the activity (D). AGO, Argonaute proteins; DGCR8, DiGeorge syndrome critical region gene 8; m7G cap, 7-methylguanosine; miRISC, miRNA-induced silencing complex; miRNA, microRNA; pre-miRNA, miRNA precursor; pri-miRNA, primary miRNA; RAN-GTP, Ras-related nuclear protein coupled with guanosine-5′-triphosphate; T, inhibitory effect; TRBP, transactivating response RNA-binding protein.

function (Figure 3D).[241] We have shown that CFTR and SLC26A9 suppression in primary human bronchial epithelium redifferentiated *ex vivo* can be rescued by miR-145 antagonism with the consequent restoration of chloride efflux.[100] An antagomir targeting miR-9 rescues protein phosphatase 2A (PP2A) activity with the consequent restoration of dexamethasone (DEX)-induced GR nuclear translocation and restores steroid sensitivity in AHR.[242] Use of LNA-based anti-miRs in which the ribose sugar ring in each nucleotide is "locked" with a methylene bridge between 2′-O and the 4′-C groups confers high affinity to target the miRNA sequence and improves resistance to nucleases. "Miravirsen," an LNA-based drug, effectively inhibits miR-122, which plays a crucial role in hepatitis C virus (HCV) replication.[243] Of note, multiple miRNA "sponges," considered as transgenes, have been suggested that encode RNA transcripts consisting of several tandem repeats of the miRNA target sequence, serving as decoys to compete with native mRNA targets for miRISC binding, thereby lowering sequestering of the miRNA to prevent it from binding to its cellular target sites.[244]

On the other hand, high-throughput screening and reporter based assays have identified several small molecules from a small-molecule drug library that act by either inhibiting the formation of active RNA-induced silencing complex (RISC) or preventing the processing of pri-miRNA to mature miRNA (Figure 3A).[245,246] Gumireddy et al.[247] reported that azobenzene inhibits the expression of miR-21, an anti-apoptotic factor that is elevated in various cancers such as breast, ovarian, and lung cancer, as well as glioblastomas.[248] Later on, several studies subsequently identified other diverse small-molecule modifiers that can act as activators or inhibitors of miRNA-mediated post-transcriptional gene silencing. Shi et al.[249] reported that AC1MMYR2, a potent and selective inhibitor of miR-21, reverses EMT and suppresses tumor growth and progression. Young et al.[237] also discovered one small-molecule inhibitor that suppresses the HCV replication in the liver cells by targeting miR-122, and thereby functions as a novel treatment approach in HCV infection.

Aptamers, an emerging class of therapeutics, are high-affinity single-stranded nucleic acid ligands that exhibit specificity and avidity comparable with or exceeding that of antibodies, and can be generated against most targets.[250–252] Unlike antibodies, aptamers can be synthesized chemically and hence offer significant advantages in terms of production cost, more straightforward regulatory approval, and lower immunogenicity when administered in preclinical doses 1,000-fold higher than those used for animal and human therapeutic

application.[253,254] Aptamers are highly specific and can discriminate between related proteins that share common sets of structural domains.[255,256] Nucleic acid aptamers are already approved for use in humans (e.g., Macugen).[257,258] Different strategies have been employed to develop cell-specific aptamers for the delivery of oligonucleotide-based therapies.[259,260] Upon receptor-mediated uptake, miRNA cargo is processed by DICER and incorporated in the RISC, and finally binds to the target of interest (Figures 3A and 3B).[235] MUC1 aptamer functionalized as nanoparticles and coupled with miR-29b has demonstrated selective delivery of miRNA-29b to lung tumor cells and tissues.[261] Likewise, aptamers conjugated to miR-34c and miR-212 have been shown to suppress proliferation of NSCLC or promote susceptibility of NSCLC cells to TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis.[262,263] Also, Esposito et al.[264] characterized a selective RNA-based aptamer (GL21.T) that is conjugated with tumor suppressor let-7g miRNccA sequence and binds with high affinity to the oncogenic tyrosine kinase receptor, Axl. They found that specific delivery of this multifunctional conjugate complex to the Axl-expressing cancer cells and suppression of the let-7g targeting gene expression resulted in the inhibition of cancer cell progression and invasion, as well as reduction of tumor growth, in a xenograft model of lung adenocarcinoma.[265] Hence aptamer-miRNA conjugates can function as novel tools with the therapeutic potential to inhibit cancer cell survival and migration *in vitro* and *in vivo* in lung cancer.

Conclusions

The field of miRNA in lung health and disease is ever evolving. Indeed, the lung is continuously exposed to different stresses such as chemical irritants, free radicals, and air pollutants, so it is likely that miRNA play a permanent role in the host defense and cellular responses against/under these external stresses. Even though significant studies have been made in determining the pathological (or therapeutic) role of miRNA in lung diseases, much remains to be done. Rising evidence supports the hypothesis that deregulation of protein expression because of abnormal unique miRNA expression signature is directly or indirectly linked to the pathogenesis of pulmonary disorders. The major challenge for researchers is in identifying a defined molecular pathway involving a particular miRNA because each mRNA can regulate multiple genes, and multiple miRNA can regulate a single gene. Although the study of the microRNAome itself can identify molecular pathways in lung health and disease, characterization of genes involved in post-transcriptional gene silencing, such as DICER1, Argonaute, TRBP, and so forth, can provide additional information in the pathophysiology of an aberrant microRNAome. Of note, the peripheral lung clock has been implicated in several lung pathologies, and several reports have mentioned the role of miRNA in regulating the molecular clock.[266–269] Several miRNA have been known to modulate genes involved in the lung peripheral molecular clock.[270–272] Although the lung can provide a unique inhalation-based delivery route for these therapeutics, epithelial barrier functions coupled with the mucociliary escalator can result in decreased bioavailability of these therapeutics. Hence efforts to improve therapeutic formulations that can increase residence time and release, for instance, mucoadhesive nanoparticles, can open new avenues for various lung diseases and improve the therapeutic outcomes in patients.

AUTHOR CONTRIBUTIONS

R.K.D.: manuscript outline, preparation of the draft manuscript, and preparation of figures and the table. S.C.: critical reading and editing of the draft manuscript. H.U.: critical reading and editing of the draft manuscript and writing of the introduction section. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no competing interests.

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