

Diagnostic Potential of Extracellular MicroRNA in Respiratory Diseases

Dhamotharan Pattarayan¹ · Rajesh K. Thimmulappa² · Vilwanathan Ravikumar³ · Subbiah Rajasekaran¹

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Abstract Lack of markers of subclinical disease state and clinical phenotype other than pulmonary function test has made the diagnosis and interventions of environmental respiratory diseases a major challenge. MicroRNAs (miRNAs), small non-coding single stranded RNAs, have emerged as potential disease-modifier in various environmental respiratory diseases. They can also be found in various body fluids and are remarkably stable. Because of their high stability, disease-specific expression, and the ease to detect and quantify them have raised the potential of miRNAs in body fluids to be useful clinical diagnostic biomarkers for lung disease phenotyping. In the present review, we provide a comprehensive overview of progress made in identifying miRNAs in various body fluids including blood, serum, plasma, bronchoalveolar lavage (BAL) fluid, and sputum as biomarkers for a wide range of human respiratory diseases such as acute lung injury/acute respiratory distress syndrome (ALI/ARDS), idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and asthma. Finally, we discuss several challenges remain to be concerned and suggest few disease-specific and non-specific miRNAs to become part of future clinical practice.

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✉ Subbiah Rajasekaran
rajasekarphd@gmail.com

¹ Department of Biotechnology, BIT-Campus, Anna University, Tiruchirappalli, Tamil Nadu, India

² Department of Biochemistry, Jagadguru Sri Shivarathreeswara University, Mysuru, Karnataka, India

³ Department of Biochemistry, School of Life Science, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India

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Introduction

Environmental lung diseases are major cause of human morbidity and mortality in both the developed and developing countries [1] and its prevalence, particularly COPD and asthma, is dramatically increasing globally. One of the major challenges in the management and treatment of lung diseases is the lack of reliable, accurate, and easily measurable screening strategies. Pulmonary function test (PFT) remains a standard way of diagnosing and assessing the severity of airway diseases (COPD, asthma, and idiopathic pulmonary fibrosis (IPF)); however, PFT may not detect subclinical diseases state, when the pathological processes are more amenable for therapeutic interventions [2]. Likewise, IPF [3, 4], acute lung injury (ALI), and acute respiratory distress syndrome (ARDS) are clinically under-diagnosed [5], causing enormous challenge for the management of disease. Lately, imaging has revolutionized the diagnosis of non-infectious lung diseases. For instance, National Lung Cancer Screening Trial reported that annual low-dose computerized tomographic (LDCT) screening was highly sensitive and more effective in reducing mortality of patients with lung cancers when compared to traditional annual chest X-ray screening. However, elevated screening costs, potential health risks, and high false positive rates are associated with LDCT [6]. Undoubtedly, there is an urgent need for molecular biomarkers for detecting subclinical and severity of disease state of lung diseases, which could aid in more personalized approaches for the treatment of patients with respiratory diseases [7, 8].

An ideal biomarker is reported to fulfill a number criterion such as measurable with minimal invasiveness, a high degree

of specificity and sensitivity, allowing for rapid and accurate detection, a long half-life within the sample, as a predictor of normal versus disease state or of pharmacological responses, and minimal discomfort to the patients. In this context, miRNAs present in body fluids have been reported to fulfill a number of those criteria and used as diagnostic markers or predictive biomarkers of therapeutic responses in many areas, such as various cancers, heart diseases, diabetes, and liver diseases [9–13].

A growing body of literature reports deregulation of intracellular miRNA expression in the pathogenesis of many lung diseases such as IPF, COPD, ALI/ARDS, asthma, cystic fibrosis (CF), and pulmonary artery hypertension (PAH) [14–16]. More importantly, miRNAs are secreted by the cells in body fluids such as blood, saliva, and urine, although the secretory mechanisms and biological functions remain largely enigmatic. Though serum contains ribonuclease, the circulatory miRNA is shown to be stable [17] and holds great potential for repetitive, non-invasive, and real-time molecular-based monitoring of disease progression as well as treatment effects [18]. Therefore, in this review, we will discuss the current knowledge about miRNAs present in body fluids such as blood, serum, plasma, bronchoalveolar lavage (BAL) fluid, and sputum as putative biomarkers for few human non-cancerous lung diseases, including ALI/ARDS, IPF, COPD, and asthma. Further, we will discuss the issues that need to be considered while taking into account the novel role of miRNAs as biomarkers in a clinical setting. Finally, we will also suggest few miRNAs as potential candidates for future clinical validation and application.

Biogenesis and Biological Functions of miRNAs

miRNAs are a class of small endogenous, non-coding RNAs, whose length is an approximately 19–25 nucleotides. The process of miRNA biogenesis involves a series of well-orchestrated steps. In the nucleus, miRNA transcripts are generated as a capped and polyadenylated transcript, known as primary miRNA (pri-miRNA) [19] by RNA polymerase II or in a few cases by RNA polymerase III. Then, RNaseIII endonuclease, Drosha, and its cofactor, DiGeorge syndrome critical region 8 (DGCR8), process Pri-miRNAs into 60–100 nt stem-looped structures known as precursor miRNAs (pre-miRNAs) [20, 21]. The nuclear transport receptor exportin-5 and the nuclear protein Ran-GTP transport Pre-miRNAs into the cytoplasm, where they are further processed by Dicer to produce 25 nt mature miRNA duplexes (miRNA-miRNA*) [22, 23]. The miRNA* strand is degraded, whereas the miRNA strand is preferentially retained and loaded into the Argonaute (AGO-2) protein subfamily and for RNA-induced silencing complex (RISC). The enzyme complex

regulates the expression of transcriptome either inhibition of translation or degradation of its target mRNAs [24]. Detailed information on the biogenesis of miRNA by different pathways (canonical and non-canonical) has been reviewed elsewhere [25].

Many mammalian miRNAs are expressed in a cell- and tissue-specific manner while some are ubiquitously expressed [26]. Gain and loss of function studies in model organisms (*Caenorhabditis elegans*, *Drosophila*, different cell lines, and mice) have demonstrated the role of specific miRNA in embryonic development, stress signaling, cell adhesion, proliferation, angiogenesis, apoptosis, immune response, inflammation, and tissue repair and disease progression [27–29]. Although miRNAs have been shown to be involved in the lung development and tissue homeostatic processes such as the immune response and metabolism [30], miRNA dysregulation is reported in several pulmonary diseases such as cancer, IPF, COPD, asthma, bronchopulmonary dysplasia (BPD), and ALI/ARDS [14–16]. Therefore, miRNA could serve as a biomarker for the development and progression of pulmonary diseases.

Origin, Extraction, and Quantification of Cell-Free miRNAs

A growing body of evidence suggests that miRNAs obtained from either blood, plasma, serum, tissues, BAL fluid, sputum, or exhaled breath condensate (EBC) may serve as reliable biomarkers for diagnosis, phenotyping, and prognosis of pulmonary diseases. These miRNAs may present in both freely or associated with lipoproteins (HDL), ribonucleoprotein complexes such as *NPM-1* (Nucleophosmin-1) and AGO2-miRNA, and packaged within extracellular vesicles including exosomes, apoptotic bodies, and microvesicles [31–33]. miRNA secretion is selective and dynamic with the progression of disease [34]. However, the source of miRNAs and their biological role in extracellular fluids remains a contentious issue [35]. miRNA release into extracellular space could be a by-product of cellular biological process or cell death and could act as chemical messengers between cells [36]. To implement recovery of miRNAs from body fluids, several commercial kits such as miRNeasy mini kit (Qiagen, CA), the total RNA purification kit (Norgen Biotek Corporation, Canada), Nucleospin miRNAs kit (Macherey-Nagel, Duren, Germany), and miRCURY RNA isolation kit-Biofluids (EXIQON) are available. Similarly, a wide range of methods has been used for the profiling of miRNAs such as Northern blotting, qRT-PCR, Taqman low-density arrays (TLDA), Affymetrix GeneChip microarrays, Agilent Oligonucleotide microarrays, Exiqon MiRCURY microarrays, NanoString

nCounter assay, and Next Generation Sequencing. The extracellular functions, different methods of isolation, and profiling of extracellular miRNAs have recently been reviewed [37].

Implications of miRNAs as Biomarkers for ALI/ARDS

ALI and its serious form ARDS results from various conditions such as sepsis, trauma, pneumonia, acute pancreatitis, and aspiration of gastric contents and are characterized by an influx of excessive neutrophils, diffuse alveolar injury, pulmonary edema, surfactant dysfunction, excessive release of inflammatory and cytotoxic mediators, and a local pro-coagulant state [38, 39]. Together, these pathologies compromise the gas exchange function of the lung [40] leading to respiratory failure. Despite growing evidence implicating miRNAs in the pathogenesis of ALI/ARDS [15], there are relatively very few publications highlighting miRNAs in body fluids as potential biomarkers for the diagnosis of ALI/ARDS (see Table 1).

In a small study of 16 patients with ARDS, Guo et al. [41] reported that serum miR-125b expression was significantly decreased and inversely correlated with the disease severity of patients with ARDS. This study further evaluated the potential therapeutic significance of this miRNA in a mouse model of ARDS and found that up-regulation of miR-125b expression dampened pulmonary inflammation and improved survival in LPS-induced ARDS mice. These results indicate that miR-125b-based therapy may improve survival of ARDS patients. Along this line, Yang et al. [42] explored the possible relationship between miRNAs change and ALI caused by cardio-pulmonary bypass (CPB) in humans. microRNA microarray was performed in the arterial blood samples of patients collected at five different time points, including T0 (after thoracotomy, but before CPB), T1 (1 h after reperfusion), T2 (4 h after reperfusion), T3 (8 h after reperfusion), and T4 (16 h after reperfusion). Compared with T0 time point, in T4, 11 miRNAs levels were significantly altered (6 up-regulated and 5 down-regulated). These changes were more obvious in miR-499 (0.25 folds) and in miR-320 (3.56 folds). The blood tumor necrosis factor- α (TNF- α) level and the data of the respiration index (RI) positively correlated with the miR-320 level, whereas oxygenation index (OI) negatively associated with miR-320. Further, in vitro model of reperfusion injury demonstrated that miR-320 significantly elevated in A549 cells following injury. These results suggested that higher circulating miR-320 level might be a valuable biomarker to indicate poor respiratory functions in ALI patients.

miRNAs as Biomarkers in IPF

IPF is a progressive, irreversible, and devastating disorder characterized by interstitial fibrosis and deterioration of respiratory function. Although the causes are unknown, the therapeutic options are with limited success [43]. The contribution of miRNAs in the pathogenesis of pulmonary fibrosis is beginning to emerge despite the majority of studies being limited to human cell culture system and animal models of diseases [14]. Several studies have reported significant differential expression of miRNAs between the lungs of patients with IPF and control subjects [14]. However, only few studies have proposed that miRNAs in biological fluids of patients could be ideal for the prediction and prognosis of IPF (see Table 1). The first analysis of circulating miRNAs in IPF patients was performed by Li et al. [44]. According to their findings, serum miR-21 and miR-155 levels were significantly higher in patients with IPF ($n=65$) compared with healthy control subjects ($n=65$). Higher serum miR-21 levels were also significantly correlated with the severity of pulmonary fibrosis. The same research group later in 2014 compared miRNAs expression profiles in serum of 76 IPF and 73 control subjects by miRNA microarray analysis [45]. They identified a unique profile made of 60 differentially expressed (8 up-regulated and 52 down-regulated) miRNAs allowing the distinction of IPF patients from control subjects. Then, the authors combined the results of qRT-PCR and microarray and found that the changes in the expression of up-regulated miRNAs (miR-21 and miR-155) and down-regulated miRNA (miR-101-3p) were more apparent than those of other miRNAs. Moreover, the expression levels of these miRNAs correlated well with the reduced forced vital capacity (FVC) and degree of lung damage. Collectively, these results suggested that miR-155, miR-21, and miR-101-3p may play a significant role in the development and progression of lung fibrosis.

A recent study has evaluated the potential role of miRNAs in lung fibrogenesis [46]. They performed qRT-PCR to measure miR-486-5p concentration in serum samples of 60 patients with silicosis ($n=20$ per stage I, II, III), an occupational lung fibrosis caused by inhalation of dust containing free crystalline silica and in the lung tissues of patients with either silicosis or IPF. Compared to control subjects, circulating levels of miR-486-5p was lower in silicosis patient as well as in the lung tissue samples from patients with silicosis and IPF. Two different murine models (silica and bleomycin) of fibrosis also exhibited decreased miR-486-5p expression. Subsequently, the same authors further explored the potential significance of this miR-486-5p in a mouse model of lung fibrosis. Wherein, intratracheal administration of miR-486-5p significantly dampened the distribution and severity of fibrotic lesions in the lungs of bleomycin-treated mice, suggesting a potential application for miR-486-5p as a biomarker and therapeutic options in clinical view.

Table 1 Circulating miRNAs as biomarkers in ALI and IPF

S. no.	Population/ no. region	Cohort size		Sample	Method	Normalization	Total no. of miRNAs analyzed	Differentially expressed miRNAs		Significance	Ref.
		Patient	Control					Up	Down		
ALI											
1	China	16	16	Serum	TaqMan microRNA assays		1		miR-125b	Expression level inversely correlated with the disease severity [41]	
2	China	45		Blood	MicroRNA microarray qRT-PCR	U6		miR-320 ^a , miR-494, miR- 200c, miR-205, miR-187, miR-192	miR-451, miR-499 ^a , miR-133, miR-143, miR-214	Higher miR-320 level is described as an indicator of poorer lung function [42]	
IPF											
1	China	65	65	Serum	qRT-PCR	U6	2	miR-21, miR-155		Biomarkers for diagnosis and assessment of prognosis of patients with IPF [44]	
2	China	76	73	Serum	MicroRNA microarray qRT-PCR	U6 snRNA	2549	miR-3675-3p ^a , miR-21 ^a , miR- 1229, miR-155 ^a , miR-18b- 3p, miR-636, miR-3646, miR-574-5p (≤2.5 fold)	miR-142-5p ^a , miR-3652, miR- 4322, miR-557 ^a , miR-187- 5p ^a , miR-1224-5p, miR- 484, miR-3682-3p, miR- 3131, miR-148b-3p, miR- 874, miR-150-5p, miR- 1305, miR-663a, miR-101- 3p ^a , miR-516a-5p, miR-328, miR-877-5p, miR-3137, miR-483-3p, miR-23b-3p (≤2.5 fold)	miR-21, miR-155, and miR- 101-3p levels were associated with clinicopathological characteristics of IPF patients [45]	
3	China	60	20	Serum	qRT-PCR	Cel-miR-39	1		miR-486-5p	Lower in serum of IPF patients [46]	
4	China	24	12	Serum	TaqMan microRNA assays	Mamm U6	366	miR-21, miR-9, miR-10a, miR-15a, miR-25, miR-26b, miR-98, miR-199a-5p, miR- 99b, miR-144*, miR-125a- 5p, miR-125b, miR-135a, miR-137, miR-140-3p, miR-200b, miR-200c, miR- 152, miR-154, miR-181a	miR-187, miR-31, Let-7a, Let-7b, miR-542-5p, Let- 7c, miR-190, miR-193a-5p, miR-203, miR-218, miR- 301, miR-302b, miR-329, miR-330, miR-331-5p, miR-335, miR-338-3p, miR-362, miR-363, miR- 365, miR-367, miR-372, miR-374, miR-374-5p, miR-381, miR-410, Let-7d	Levels altered in serum of IPF patients [47]	

^aThese microRNAs were validated by qRT-PCR

Yang et al. [47] analyzed serum miRNAs in rapidly progressive ($n = 12$) and slowly progressive ($n = 12$) IPF patients compared to control subjects ($n = 12$) by TaqMan microRNA assays. Of 366 human miRNAs analyzed, 47 miRNAs were significantly differentially expressed (20 up-regulated and 27 down-regulated) in rapid or slowly progressive IPF patients compared to controls. Bioinformatic analysis revealed enrichment of several biological processes, including transforming growth factor-beta (TGF- β) signaling implicated in the pathogenesis of fibrosis among differentially expressed serum miRNAs. In agreement with the TaqMan microRNA assay results, independent serum samples from a newly recruited cohort of 20 rapid progressive, 24 slowly progressive IPF patients, and 20 healthy controls also showed miR-21, miR-199a-5p, and miR-200 levels were significantly increased, while miR-31, let-7a, and let-7d levels were significantly under expressed in IPF patients compared to healthy controls.

miRNAs as Biomarkers in COPD

COPD comprising of chronic bronchitis and emphysema is the fourth-leading cause of death in the USA that is primarily caused by chronic exposure to irritants, including cigarette smoke and air pollutants [48]. Few studies to date have implicated miRNAs in the pathogenesis of COPD using cell culture and animal models [49] and revealed clinical application of miRNAs in biological fluids as biomarkers in COPD (see Table 2).

Van Pottelberge et al. [50] reported significant down-regulation of eight miRNAs (miR-218, let-7c, miR-34c, miR-34b, miR-125a-5p, miR-30e-3p, miR-342-3p, and miR-125b) in sputum of patients with COPD compared with never-smokers as analyzed by single qRT-PCR reaction system. By gene set enrichment analysis (GSEA), it confirmed that tumor necrosis factor receptor II (TNFR-II), a key mediator in COPD pathogenesis, is a predicted target gene of let-7c. In agreement with bioinformatic analysis, let-7c levels in sputum samples of COPD patients negatively correlated with the levels of TNFR-II. In another matched case-control study, serum samples were used to perform miRNA profiling in 20 COPD patients and 12 healthy controls smokers by qRT-PCR method [51]. Among 72 miRNAs examined, 4 miRNAs (miR-20a, miR-28-3p, miR-34c-5p, and miR-100) were found to be significantly down-regulated and miR-7 level was elevated compared with controls.

To discriminate patients with heart failure, with COPD, other breathless patients, and healthy controls, Ellis et al. [52] found four miRNAs namely miR-324-5p, miR-185, miR-1909, and miR-150 were differentially expressed in COPD patients compared to healthy controls. Donaldson et al. [53] reported that analysis of muscle-specific miRNAs in plasma of patients with COPD reflects skeletal muscle weakness, indicating COPD-associated muscle dysfunction. In this study, miRNA profiles of COPD patients ($n = 103$)

were compared with control subjects ($n = 25$) and it was found that miR-1, miR-499, miR-133, and miR-206 levels were higher in COPD patients compared with controls. This differential expression was perhaps as a consequence of increased muscle wasting in COPD subjects. Although this study did not offer a biological function for the circulating miRNAs, provided a non-invasive marker for COPD-associated muscle dysfunction. In another study, serum miRNAs profiling by qRT-PCR in 41 healthy subjects, 40 asymptomatic heavy smokers, and 49 COPD patients, Xie et al. [54] identified two-miRNA signature, miR-21 and miR181a, that may be valuable for COPD phenotyping in heavy smokers. The authors found that miR-21 were significantly elevated, while the levels of miR-181a were significantly down-regulated in the COPD patients and asymptomatic heavy smokers than in the healthy subjects. Based on the previously known functions of these miRNAs, the authors went on to suggest that a high ratio of serum miR-21 to miR-181a expression may be a prognostic marker for early onset of COPD in heavy smokers.

As both asthma and COPD are characterized by reversible airway obstruction, Wang et al. [55] performed miRNAs microarray in plasma samples to identify distinctive miRNAs that may predict the severity of disease. The authors observed significant differential expression of miR-145-5p, miR-338-3p, and miR-3620-3p in plasma of patients with COPD as compared with asthma and healthy individuals. Further, these miRNAs reported to target genes involved in inflammation, cell proliferation, and differentiation and oxidative stress, the underlying pathological events in COPD.

Due to the lack of specific and robust non-invasive biomarkers, the diagnosis as well as distinction of non-small cell lung carcinoma (NSCLC) from COPD remains highly challenging. A recent study conducted specific miRNA profiling in human blood samples to differentiate between patients with lung cancer and COPD [56]. With a high degree of accuracy, specificity, and sensitivity, 14 miRNAs levels (miR-26a, miR-92a, miR-93*, miR-328, miR-383, miR-369-5p, miR-513b, miR-636, miR-641, miR-662, miR-675, miR-940, let-7d*, and miR-1224-3p) were differentially regulated between healthy controls, lung cancer, and COPD patients, which suggest that the circulating miRNA signatures can be used to identify patients with COPD [56]. The authors also identified 424 putative target genes for those 14 miRNAs according to the microcosm database. This study proved that peripheral miRNA expression profile could distinguish lung cancer patients from COPD patients with high accuracy. Sanfiorenzo et al. [57] performed circulating miRNA profiling in 52 patients with NSCLC, 10 with COPD, and 20 healthy subjects for predicting the prognosis of NSCLC as well as to distinguish between NSCLC and COPD patients. Ten plasma miRNAs (miR-155, miR-20a, miR-25, miR-296, miR-126, miR-223, miR-199a, miR-152, miR-145, and let-7f) significantly differentiated healthy individuals from NSCLC patients

Table 2 miRNAs as biomarkers in biological fluids of patients with COPD

S. no.	Population/ region	Cohort size		Sample	Method	Normalization	Total no. of miRNAs analyzed	Differentially expressed miRNAs		Significance	Ref.
		Patient	Control					Up	Down		
1	Belgium	12	10	Sputum	qRT-PCR	Mean miRNA expression per sample	627	miR-1233, miR-106a, miR-20a, miR-20b, miR-1291, miR-377, miR-124, miR-216b, miR-24-1*, miR-646, miR-548b, miR-631, miR-518b, miR-216a, miR-182*, miR-188-5p, miR-492, miR-662, miR-200a*	miR-34c, miR-218, miR-34b, Let-7c, miR-342-3p, miR-125a-5p, miR-30e-3p, miR-125b	Let-7c levels were inversely correlated with the levels of TNFR-II, a protein implicated in the pathogenesis of COPD Useful to differentiate healthy controls, lung cancer, and COPD patients	[50]
2	Germany	24	19	Blood	MicroRNA microarray		863	miR-1233, miR-106a, miR-20a, miR-20b, miR-1291, miR-377, miR-124, miR-216b, miR-24-1*, miR-646, miR-548b, miR-631, miR-518b, miR-216a, miR-182*, miR-188-5p, miR-492, miR-662, miR-200a*	miR-23b, miR-888*, miR-641, miR-184, miR-95, miR-224, miR-369-5p, miR-140-5p, miR-613, miR-513c	Useful to differentiate healthy controls, lung cancer, and COPD patients	[56]
3	Turkey	20	12	Serum	qRT-PCR	U6 snRNA	72	miR-7 (>7 fold)	miR-20a, miR-28-3p, miR-34c-5p, miR-100 (>7-fold)	Expression altered in COPD patients	[51]
4	France	10	20	Plasma	qRT-PCR	Mean of miR-192-5p and miR-16-5p levels	17	miR-155-5p, miR-199a-5p, miR-191-5p, miR-145-5p	miR-20a-5p, miR-126-3p, Let-7f-5p	Useful to discriminate healthy controls, NSCLC, and COPD patients	[57]
5	New Zealand	15	14	Plasma	qRT-PCR	Reference miRNAs (miR-20a; miR-106b; miR-363; miR-140-3p)	742	miR-1909, miR-29a, miR-632, miR-720, miR-605, miR-342-3p, miR-99a, miR-132, miR-940, miR-497, miR-29c, miR-424, miR-222, miR-22*, miR-144, miR-142-5p, miR-150 ($P < 0.05$)	miR-186, miR-324-5p, miR-185, miR-19b, miR-425, miR-584, miR-652, miR-191, miR-151-3p, Let-7i, miR-423-5p, miR-93*, miR-423-3p, miR-339-3p, Let-7b, miR-25, miR-24, miR-18b, miR-181a, miR-191, miR-151-5p, Let-7d, miR-30e, miR-223, miR-331-3p, miR-320a, miR-140-3p, miR-1974, miR-484, miR-374b, miR-486-5p, miR-425* ($P < 0.05$)	Differentially expressed between healthy controls, heart failure, COPD, and dyspnea patients	[52]
6	UK	103	25	Plasma	qRT-PCR	Spiked <i>Caenorhabditis elegans</i>	7	miR-1, miR-499, miR-133, miR-206		Biomarker for the diagnosis of COPD-associated muscle dysfunction	[53]
7	China	49	41	Serum	qRT-PCR	U6	6	miR-21	miR-181a	High ratio of serum miR-21 to miR-181a is associated with early pathogenic process of COPD	[54]

Table 2 (continued)

S. no.	Population/region	Cohort size		Sample	Method	Normalization	Total no. of miRNAs analyzed	Differentially expressed miRNAs		Significance	Ref.
		Patient	Control					Up	Down		
8	Spain	23	16	BAL fluid	qRT-PCR	MammU6	381	miR-132, miR-145, miR-212, miR-223, miR-342-5p, miR-422a, miR-423-5p, miR-486-3p, miR-15b, miR-425	miR-29b, miR-34a, miR-98, miR-146b-5p, miR-193a-5p, miR-218, miR-324-5p, miR-342-3p, miR-365	Levels altered in BAL fluid	[58]
9	China	25	6	Plasma	MicroRNA microarray		234	miR-3620-3p	miR-145-5p, miR-338-3p	Biomarkers of COPD	[55]

with 87.9 % accuracy, 85 % sensitivity, and 82.9 % specificity. Only a small set of miRNAs (miR-152, miR-145, miR-199a, miR-24, miR-20a, and miR-25) distinguished NSCLC from COPD with 94.4 % accuracy, 90.9 % sensitivity, and 83.3 % specificity. On the other hand, seven circulating miRNAs (miR-155-5p, miR-199a-5p, miR-191-5p, miR-20a-5p, miR-126-3p, miR-145-5p, and let-7f-5p) were differentially expressed between COPD patients and healthy controls. Together, this study reported clinical importance of circulating miRNA profiles for predicting the patient outcome with resectable NSCLC as well as to distinguish NSCLC and COPD patients. Another study by Molina-Pinelo et al. [58] assessed miRNA by performing TaqMan Human MicroRNA A Array v2.0 analysis of BAL fluid samples obtained from patients with COPD, adenocarcinoma alone, COPD with adenocarcinoma, and control subjects. The study found significant up-regulation of one miRNA cluster (miR-132-212) only in patients with COPD, which could potentially discriminate adenocarcinoma from COPD.

miRNAs as Diagnostic Markers in Asthma

Asthma is an airway inflammatory disease characterized by airway hyper responsiveness, inflammation, and reversible airway obstruction [59]. miRNAs have been linked to the overall pathogenesis of asthma through several mechanisms [60]. Several studies reported changes in the levels of miRNAs in biological fluids of patients with asthma (see Table 3), implicating the role of miRNAs in the overall pathogenesis of asthma by regulating diverse mechanisms. In a small study of six asthmatic children, Liu et al. [61] performed a microRNA microarray analysis to detect the differences in miRNA expression levels in blood lymphocytes. Thirty-six miRNAs were significantly up-regulated, while 45 significantly down-regulated in the asthmatic patients compared to the control subjects. Among up-regulated miRNAs, miR-221 and miR-485-5p were reported to increase in a mouse model of asthma and also predicted to bind to Spred-2 mRNA (EVH1 domain-2 protein), a negative regulator of inflammation and air hyperresponsiveness in murine asthma models by regulating interleukin-5 (IL-5) signaling [61]. Hence, up-regulation of miR-221 and miR-485-5p may regulate the pathogenesis of human asthma.

Panganiban et al. [62] measured the expression of 10 miRNAs (let-7a, let-7d, miR-21, miR-133a, miR-1248, miR-26a, miR-328, miR-126, miR-146a, and miR-98) by qRT-PCR in the serum of 10 asthmatics and 10 control subjects. Expression of miR-1248 was increased, whereas let-7a, let-7d, and miR-26a expressions were down-regulated in asthmatics relative to control. Further, using several experimental approaches, the authors showed that miR-1248 physically interacts with the 3'-untranslated region and serves

Table 3 miRNAs as biomarkers in asthma

S. no.	Population/ no. region	Cohort size		Sample	Method	Normalization	Total no. of miRNAs analyzed	Differentially expressed miRNAs		Significance	Ref.
		Patient	Control					Up	Down		
1	China	6	6	Blood lymphocyte	MicroRNA microarray qRT-PCR	U6 snRNA	1957	miR-22, miR-106b, miR-320a, miR-615-3p, miR-891a, miR-877, miR-937, miR-196a, miR-492, miR-485-3p ^a , miR-640, miR-675, miR-554, Let-7b, miR-551b, miR-320c, miR-513b, miR-320d, miR-605, miR-523, miR-665, miR-1260, miR-3202, miR-224, miR-221 ^a , miR-4288, miR-4300, miR-491-3p, miR-4306, miR-4268, miR-3171, miR-1246, miR-620, miR-938, miR-1280, miR-483-3p	miR-126, miR-140-5p, Let-7i, miR-142-3p, miR-148a, miR-182, miR-193a-3p, miR-29b, miR-3607-5p, miR-335, miR-98, miR-96, miR-195, miR-143, miR-660, miR-532-5p, miR-192, miR-4301, miR-362-3p, miR-15b, miR-744, miR-15a, miR-30e, miR-7, miR-199a-5p, miR-374a, miR-125b, miR-576-5p, miR-324-5p, miR-20b, miR-20a, miR-451, miR-138-1, miR-424, miRPlus-1874, Let-7g, miR-3614-3p, miR-23b, miR-3926, miR-33a, Let-7d, miR-30a, Let-7a, miR-27b, miR-4284 Let-7d, miR-26a, Let-7a	miR-221 and miR-485-3p may regulate the pathogenesis of asthma by targeting Spred-2, a negative regulator of allergen-induced airway inflammation	[61]
2	Pennsylvania (USA)	10	10	Serum	qRT-PCR	Cel-miR-39	10	miR-1248	Let-7d, miR-26a, Let-7a	Levels altered in serum	[62]
3	San Francisco (USA)	10	10	BAL (exosomes)	MicroRNA microarray Nano-scale qPCR		894	miR-1972, miR-665, miR-658 ^a , miR-483-5p, miR-1268 ^a , miR-203, miR-130a	Let-7c, Let-7b, miR-141, miR-200b, Let-7d, Let-7a ^a , miR-21 ^a , miR-27a, Let-7e, miR-34c-5p, miR-34b-5p, miR-19b, miR-0022, miR-24 ^a , miR-0026a, miR-99a ^a , miR-200c ^a	Levels altered	[63]
4	China	8	6	Plasma	MicroRNA microarray		167	miR-145-5p, miR-338-3p	miR-3620-3p	Levels altered	[55]
5	Pennsylvania (USA)	10	10	Serum: exhaled breath condensate (EBC)	qRT-PCR	SnoRD 44	1		miR-570-3p (not significant in serum)	Expression levels inversely correlated with the lung function	[64]
6	Indiana (USA)	8	8	Serum	qRT-PCR	U6 miR	2	miR-21		Higher level present in the serum of asthmatic patients	[65]
7	Pennsylvania (USA)	35	19	Plasma	qRT-PCR	Copy numbers	420	miR-125b, miR-126, miR-21, Let-7b, Let-7c, Let-7e, miR-16, miR-223, miR-148a, miR-146a, miR-150, miR-145, miR-422 (>2 fold)	miR-1, miR-299-5p, miR-570, miR-133b, miR-133a, miR-26b, miR-1248, miR-330-5p, miR-29, miR-1291, miR-144, miR-106a, miR-155 (>2 fold)	Biomarkers to characterize asthma and other allergic diseases	[66]
8	Egypt	95	80	Serum	qRT-PCR	SNORD68	1	miR-21			[67]

Table 3 (continued)

S. no.	Population/ region	Cohort size		Sample	Method	Normalization	Total no. of miRNAs analyzed	Differentially expressed miRNAs		Significance	Ref.
		Patient	Control					Up	Down		
9	Belgium	50	10	Sputum	qRT-PCR	Stably expressed miR-20a, miR-30b	171	miR-629-3p, miR-223-3p, miR-142-3p		Useful biomarker to differentiate ICS sensitive from ICS resistant asthmatic patients Positively associated with neutrophilic airway inflammation in patients with severe asthma	[68]
10	China	72	35	Plasma	qRT-PCR	Cel-miR-39	1		miR-181b-5p	Levels were inversely correlated with airway eosinophilia in asthmatic patients	[69]

^a These microRNAs were validated by qRT-PCR

as a positive regulator of IL-5 expression, a key regulator of allergic airway disease and other eosinophilic esophagitis. Collectively, this study implicated the pathogenic role of miR-1248 in asthma by regulating IL-5 levels.

For the first time, Levanen et al. [63] revealed the presence of miRNA in the exosomes isolated from BAL fluid of asthmatic patients ($n = 10$) and healthy control subjects ($n = 10$). Exosomal miRNA profiling by microarray analysis revealed 24 miRNAs including members of the let-7 and miRNA-200 families in asthmatics both at baseline and after the subway particulate matter (PM) exposure. The majority of differentially expressed miRNAs were down-regulated and correlated well with poor lung function (FEV₁) in the asthma group at baseline. Pathway analysis revealed the role of these miRNAs in the regulation of pathogenic mediators of asthma, including interleukin-13 (IL-13), interleukin-10 (IL-10), IL-6, and interleukin-8 (IL-8). Collectively, this seminal study demonstrated significant differences in exosomal miRNA profiles between healthy controls and asthmatic patients and highlighted the applications of exosomal miRNA as biomarkers of obstructive airway diseases. To discriminate between COPD and asthma phenotypes, Wang et al. [55] identified miR-145-5p, miR-338-3p, and miR-3620-3p expressions were significantly different between COPD and asthmatic patients.

In an investigation by Roff et al. [64], expression of endogenous miR-570-3p was found to be significantly lower in EBC from asthmatic patients compared to control subjects. However, miR-570-3p was found to be inversely correlated with lung function (FEV₁) of asthmatic patients, with the highest miR-570-3p levels present in subjects with the lowest FEV₁. However, in multiple in vitro experiments using human normal bronchial epithelial cells and the human malignant airway epithelial cell line A549, the authors were able to show that miR-570-3p mimic up-regulated numerous cytokines and chemokines (CCL4 (Chemokine (C-C motif) ligand 4), CCL5 (Chemokine (C-C motif) ligand 5), TNF- α , and IL-6) and also enhanced their expression in response to inflammatory stimuli. Further, the authors identified that the RNA-binding protein (HuR) is a direct target of miR-570-3p, which regulates numerous inflammatory mediators. As decreased miR-570-3p level in EBC has a negative correlation with lung function in asthmatics, the authors speculate that miR-570-3p levels will increase as lung function decreased. In a recent study, an elevated level of serum miR-21 was associated with asthmatic patients, but it did not correlate with the presence of allergen-specific IgE (i.e., atopy) [65]. However, further studies are warranted to study the functional role of miR-21 in this allergic disease.

Panganiban et al. [66] identified miRNAs that are differentially expressed in plasma of patients with allergic rhinitis (AR), asthmatic, and healthy subjects. Of the 26 miRNAs differentially expressed, the circulating levels of miR-125b, miR-16, miR-299-5p, miR-126, miR-206, and miR-133b

were most predictive of allergic and asthmatic status. Further, the expression profiles of circulating miRNAs in asthmatic patients define two subtypes that differed by high or low circulating eosinophils, suggesting the potential use of these miRNAs for diagnosing eosinophilic asthma.

Elbehidy et al. [67] performed a large population-based study (175 children) to substantiate serum miR-21 level as biomarker to predict response to therapy in asthma. The study found significantly higher expression of miR-21 in serum of inhaled corticosteroid (ICS) sensitive (SS) asthmatic patients, steroid-resistant (SR) asthmatic, and patients without ICS therapy as compared to healthy controls. On the other hand, serum IL-12 p35 levels, a target of miR-21, was significantly lower in SS, SR, and patients without ICS therapy as compared to healthy controls. Receiver operating characteristic (ROC) analysis provided sensitivity of 97.5 % and specificity of 86.7 % for identifying ICS responders in asthmatic patients. Hence, this study underscores the potential use of serum miR-21 levels as a biomarker to predict ICS responses in asthma.

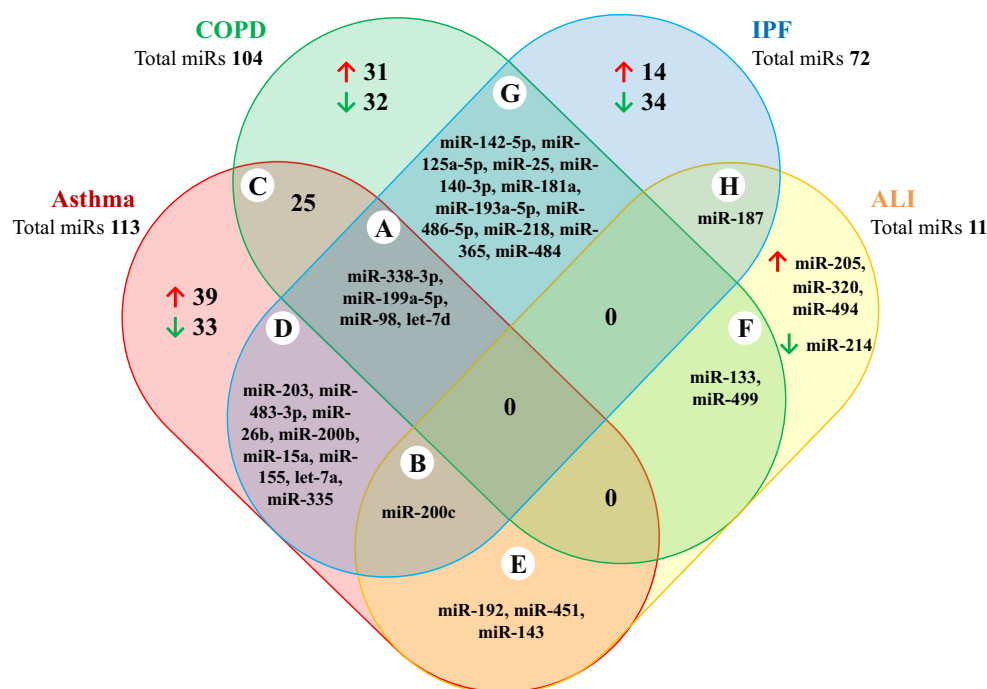
Maes et al. [68] provided an evidence that some miRNAs (miR-629-3p, miR-223-3p, and miR-142-3p) are increased in sputum of patients with neutrophilic asthma compared to control subjects. Further, *in situ* hybridization confirmed the expression of miR-629-3p in bronchial epithelium of patients with asthma and also suggested its possible role in the pathogenesis of neutrophilic asthma by regulating expression of neutrophil chemoattractant IL-8. In conclusion, the study identified these miRNAs as biomarkers in identifying patients with severe neutrophilic asthma.

Similarly, a recent study by Huo et al. [69] illustrated that decreased plasma and epithelial miR-181b-5p expression is associated with eosinophilic asthma and also served as a biomarker to predict response to inhaled corticosteroids treatment in asthma. This study further concluded that miR-181b-5p regulates IL-13-induced interleukin-1 β (IL-1 β) and CCL-11 (Eotaxin-1) expression by suppressing the expression of secreted phosphoprotein-1 (SPP-1), an extracellular matrix protein. This study suggests that miR-181b-5p may play a role in eosinophilic airway inflammation by regulating pro-inflammatory cytokines expression through regulating SPP-1.

Uniquely and Commonly Expressed miRNAs Among Different Populations in Different Lung Diseases

We provided Venn diagram to show uniquely expressed as well as miRNAs that were shared between two or more pathological conditions among different populations (see Fig. 1) in different samples. The sample details and expression pattern of uniquely and commonly expressed miRNAs have been presented in Supplemental Tables 1 and 2, respectively. Uniquely expressed miRNAs results showed that three miRNAs (miR-205, miR-320, and miR-494) were present in high levels, whereas miR-214 was present in low level in blood of ALI patients (see Supplemental Table 1). In addition, there were a total of 48, 63, and 72 miRNAs which were uniquely present in different samples of IPF, COPD, and

Fig. 1 Venn diagram shows number of uniquely expressed miRNAs as well as miRNAs that were shared between two or more pathological conditions in different samples among different populations. The information about samples, miRNAs, and their expression pattern could be found in the supplemental data



asthma patients, respectively (see Supplemental Table 1). However, commonly expressed miRNAs were differently altered in different disease conditions (see Supplemental Table 2). Further, we presented the known biological functions of some of these commonly expressed miRNAs in animal and cell culture models with respect to lung diseases (see Supplemental Table 3).

Promising miRNAs for Future Clinical Validation and Application in Respiratory Diseases

We analyzed and listed (see Table 4) disease-specific and non-specific miRNAs which could be further validated and developed into biomarker candidates for diagnostic and prognostic purpose. Five miRNAs namely miR-21, let-7d, miR-150, miR-492, and let-7a were found to be non-specifically expressed in two or more disease types. The expression pattern of individual miRNA remained same in all disease types irrespective of samples used and populations. Next, we suggest a few disease-specific miRNAs for ALI (miR-320 and miR-214), IPF (miR-3675-3p, miR-1229, miR-18b-3p, miR-3652, miR-557, and miR-150-5p), COPD (miR-132 and miR-34), and asthma (miR-665 and miR-26a). We suggest that the combined assessment of both disease-specific and non-specific miRNAs may provide an attractive signature to diagnose specific disease type described here.

Conclusion and Perspectives

Although studies have shown that miRNAs are differentially enriched in body fluids and suggested the feasibility of using miRNAs as potential diagnostic biomarkers for different lung diseases, their clinical application is yet to be tested. A major challenge in the advancement of this field is that most studies performed to date are evaluated with only small patient cohorts (<100). Larger patient cohort studies not only will identify the diagnostic performance of these miRNAs, but will also help in monitoring the response to therapy, as suggested for miR-21, which is reduced in serum of patients with asthma upon ICS therapy [67]. Similarly, a decreased plasma levels of miR-181b-5p in asthmatic patients is elevated in response to ICS therapy [69].

It is further demonstrated that disease susceptibility and gene expression, including miRNAs, exhibit population differences. For instance, miR-26 expression was found to be expressed differently in prostate cancer cell lines derived from African American men compared to those derived from Caucasians [70]. Further, miRNAs levels differ between different body fluids. Hence, panels of specific miRNAs to each type of lung diseases among different ethnicity and body fluids will also be required.

Furthermore, several important technical limitations including the lack of an established protocol for miRNA isolation, endogenous miRNA control to normalize for miRNA levels in the body fluids, and lack of knowledge about best biological samples to be used for specific disease type required further

Table 4 Promising miRNA candidates for future clinical validation and application

S. no.	miRNA	Model and regulation	Samples	Ref.
Non-specific miRNAs				
1	miR-21	IPF ↑, COPD ↑, asthma ↑	Serum and plasma	[44, 45, 47, 54, 65–67]
2	Let-7d	IPF ↓, COPD ↓, asthma ↓	Serum, plasma, blood (lymphocytes), and BAL (exosomes)	[47, 52, 61–63]
3	miR-150	COPD ↑, asthma ↑	Plasma	[52, 66]
4	miR-492	COPD ↑, asthma ↑	Blood and blood (lymphocytes)	[56, 61]
5	Let-7a	IPF ↓, asthma ↓	Serum, blood (lymphocytes), and BAL (exosomes)	[47, 61–63]
Specific miRNAs				
1	miR-320	ALI ↑	Blood	[42]
2	miR-214	ALI ↓	Blood	[42]
3	miR-3675-3p, miR-1229, miR-18b-3p,	IPF ↑	Serum	[45]
4	miR-3652, miR-557, miR-150-5p	IPF ↓	Serum	[45]
5	miR-132	COPD ↑	Plasma and BAL fluid	[52, 58]
6	miR-34	COPD ↓	Sputum and BAL fluid	[50, 58]
7	miR-665	Asthma ↑	Blood (lymphocytes) and BAL (exosomes)	[61, 63]
8	miR-26a	Asthma ↓	Serum and BAL (exosomes)	[62, 63]

investigation. Finally, though understanding of biological functions of altered miRNAs in specific disease type is needed to improve their use as biomarkers, altered miRNAs present in the body fluids and in EBC are an alternative to current invasive measurement, readily detectable and measurable and have the great potential to aid in the diagnosis as well as to monitor the response to therapy of various lung diseases.

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Compliance with Ethical Standards

Conflict of Interest All authors have read the journal's policy on disclosure of potential conflicts of interest and have none to declare.

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Supplemental Table 1: Uniquely expressed miRNAs in different lung diseases among different samples and populations

Model	Regulation	Sample	microRNAs
ALI	Up (n=3)	Blood	miR-205, miR-320, miR-494
	Down (n=1)	Blood	miR-214
IPF	Up (n=14)	Serum	miR-9, miR-10a, miR-18b-3p, miR-99b, miR-135a, miR-137, miR-152, miR-154, miR-574-5p, miR-636, miR-1229, miR-3646, miR-3675-3p, miR-144*
	Down (n=34)	Serum	miR-23b-3p, miR-31, miR-101-3p, miR-148b-3p, miR-150-5p, miR-190, miR-187-5p, miR-301, miR-302b, miR-328, miR-329, miR-330, miR-331-5p, miR-362, miR-363, miR-367, miR-372, miR-374, miR-374-5p, miR-381, miR-410, miR-516a-5p, miR-542-5p, miR-557, miR-663a, miR-874, miR-877-5p, miR-1224-5p, miR-1305, miR-3131, miR-3137, miR-3652, miR-3682-3p, miR-4322
Asthma	Up (n=39)	Blood	miR-106b, miR-196a, miR-320c, miR-320d, miR-491-3p, miR-513b, miR-523, miR-551b, miR-554, miR-615-3p, miR-620, miR-640, miR-665, miR-675, miR-877, miR-891a, miR-937, miR-938, miR-1246, miR-1260, miR-1280, miR-3171, miR-3202, miR-4268, miR-4288, miR-4300, miR-4306, miR-485-3p, miR-221
		Plasma	miR-16, miR-146a, miR-422
		BAL	miR-1972, miR-665, miR-658, miR-483-5p, miR-1268, miR-130a
		Sputum	miR-629-3p, miR-223-3p
	Down (n=33)	Blood	Let-7g, miR-27b, miR-30a, miR-33a, miR-96, miR-138-1, miR-182, miR-195, miR-193a-3p, miR-362-3p, miR-374a, miR-532-5p, miR-576-5p, miR-660, miR-744, miRPlus-1874, miR-3607-5p, miR-3614-3p, miR-3926, miR-4284, miR-4301
		Plasma	miR-299-5p, miR-570, miR-133b, miR-133a, miR-181b-5p, miR-330-5p, miR-29
		Serum	miR-26a
		BAL	miR-26a, miR-27a, miR-34b-5p, miR-141
		EBC	miR-570-3p
COPD	Up (n=31)	Blood	miR-124, miR-188-5p, miR-200a*, miR-216a, miR-216b, miR-377, miR-518b, miR-548o, miR-631, miR-646, miR-662, miR-1233, miR-182*, miR-24-1*
		Plasma	miR-155-5p, miR-191-5p, miR-1909, miR-29a, miR-632, miR-720, miR-132, miR-940, miR-497, miR-29c, miR-222, miR-22*, miR-206
		BAL	miR-132, miR-212, miR-342-5p, miR-422a, miR-486-3p

	Down (n=32)	Blood	miR-95, miR-184, miR-369-5p, miR-513c, miR-613, miR-641, miR-888*
		Plasma	Let-7f-5p, miR-18b, miR-20a-5p, miR-126-3p, miR-151-3p, miR-151-5p, miR-185, miR-186, miR-191, miR-331-3p, miR-339-3p, miR-374b, miR-423-3p, miR-584, miR-652, miR-1974, miR-425*, miR-93*
		Serum	miR-28-3p, miR-100
		BAL	miR-34a, miR-146b-5p
		Sputum	miR-30e-3p, miR-34b, miR-34c

Supplemental Table 2: miRNAs that were shared between two or more lung diseases in different samples among different populations

#	Regulation in disease models	n =	microRNAs
A	Asthma ↑ COPD ↓ IPF ↓	1	miR-338-3p
	Asthma ↓ COPD ↑ IPF ↑	1	miR-199a-5p
	Asthma ↓ COPD ↓ IPF ↑	1	miR-98
	Asthma ↓ COPD ↓ IPF ↓	1	Let-7d
B	ALI ↑ Asthma ↓ IPF ↑	1	miR-200c
C	Asthma ↑ COPD ↑	4	miR-605, miR-145, miR-492, miR-150
	Asthma ↑ COPD ↓	2	miR-320a, miR-224
	Asthma ↓ COPD ↑	10	miR-1, miR-20b, miR-7, miR-424, miR-3620-3p, miR-106a, miR-15b, miR-1291, miR-144, miR-99a
	Asthma ↓ COPD ↓	9	Let-7i, miR140-5p, miR-23b, miR-29b, miR-30e, miR-324-5p, miR-19b, miR-34c-5p, miR-24
D	Asthma ↑ IPF ↓	2	miR-203, miR-483-3p
	Asthma ↓ IPF ↑	4	miR-26b, miR-200b, miR-15a, miR-155
	Asthma ↓ IPF ↓	2	Let-7a, miR-335
E	ALI ↑ Asthma ↓	1	miR-192
	ALI ↓ Asthma ↓	2	miR-451, miR-143
F	ALI ↓ COPD ↑	2	miR-133, miR-499
G	COPD ↑ IPF ↓	1	miR-142-5p
	COPD ↓ IPF ↑	4	miR-125a-5p, miR-25, miR-140-3p, miR-181a
	COPD ↓ IPF ↓	5	miR-193a-5p, miR-486-5p, miR-218, miR-365, miR-484
H	ALI ↑ IPF ↓	1	miR-187

Supplemental Table 3: Function and mechanism of action of miRNAs in cell culture and animal models of lung diseases

Model	miRNA	Function and Mechanism	Ref.
ALI	miR-150	Dampens pulmonary inflammation by targeting interleukin-1R associated kinase-2 downstream of focal adhesion kinase	1
		Exacerbates hyperoxia-induced neonatal lung injury by targeting Glycoprotein nonmetastatic melanoma protein B (GPNMB)	2
		Promotes resolving of LPS-induced vascular injury through suppression of Angiopoetin-2 generation	3
	miR-15b	Augments the DNA damage following radiation in the lung through suppression of p53-induced phosphatase gene	4
	miR-23b	Inhibits the expression of inflammatory factors (NF-kB, TNF- α , IL-6, ICAM1, E-Selectin, and VCAM-1) in vascular endothelial cells during sepsis	5
	miR-19b	Promotes epithelial-mesenchymal transition via down-regulation of Phosphatase and tensin homolog (PTEN) in response to mechanical stretch	6
	miR-26b	Enhanced LPS-induced pro-inflammatory cytokines and chemokines expression in alveolar macrophages by modulating NF-kB pathway through down-regulation of PTEN expression	7
	miR-155	LPS-induced Triggering receptors expressed on myeloid cell-1 (TREM-1)-mediated lung inflammation is dependent on miR-155 expression	8
		Promotes <i>Staphylococcal enterotoxin B</i> (SEB)-mediated inflammation and lung injury through suppressor of cytokine signaling 1 (SOCS1) suppression	9

IPF	miR-199a-5p	Promotes proliferation, migration, invasion and differentiation of fibroblasts by targeting Caveolin-1	10
	miR-98	Suppresses TGF- β 1-induced epithelial-mesenchymal transition (EMT) by down-regulating pSTAT3 and STAT3 (Signal transducer and activator of transcription 3) levels.	11
	Let-7d	Attenuates EMT through suppression of HMGA2 (High-mobility group AT-hook 2)	12
		Reverse fibrotic phenotype in fibroblasts	13
		Lin28B induces the process of EMT by inhibiting let-7d	14
	miR-200c	Attenuates EMT and fibrogenic activity in fibroblasts	15
	miR-145	Promotes fibroblast differentiation through targeting Kruppel-like factor 4 (KLF4)	16
	miR-424	Promotes myofibroblast differentiation during EMT by potentiating the TGF-signaling pathway likely through SMURF2 (SMAD ubiquitination regulatory factor 2)	17
	miR-142-5p	Promotes pro-fibrogenic program in macrophages of lungs by targeting SOCS1.	18
	miR-29b	Prevents bleomycin-induced pulmonary fibrosis	19
	miR-155	Triggers bleomycin-induced lung fibrosis	20
	miR-486-5p	Decreases the severity of lung fibrosis	21
COPD	miR-199a-5p	Modulates the adaptive immune balance in favour of a Th1 and Th17 response in COPD patients	22
		Contributes to the pathogenesis of COPD and may also affect the hyperoxia-inducible factor -1 α -dependent lung maintenance	23
		Regulates unfolded protein responses in COPD and α 1-Antitrypsin deficiency	24

	miR-203	Inhibits NF- κ B through repression of TAK1 (Transforming growth factor beta-activated kinase 1) and PIK3CA (PHOSPHATIDYLINOSITOL 3-KINASE, CATALYTIC, ALPHA) and its up-regulation may contribute to the COPD initiation	25
	miR-34c	Modulates Serpin Peptidase Inhibitor, Clade E (Nexin, Plasminogen Activator Inhibitor Type 1), Member 1 (SERPINE 1) expression in COPD lung and also associated with the severity of COPD	26
	miR-218-5p	Protects from cigarette smoke-induced inflammation and COPD	27
Asthma	Let-7d	Inhibition alleviated the features of asthma	28
	miR-106a	Promotes allergic asthma probably through inhibiting IL-10 (interleukin-10) expression	29
	miR-23b	Inhibits TGF- β 1-induced airway smooth muscle cell proliferation through inactivation of TGF β R2/p-SMAD3	30
	Let-7	Inhibits airway inflammation and reduction in airway hyperresponsiveness through suppression of IL-13 (interleukin-13) levels	31
	miR-155	Increases the secretion of inflammatory cytokines by human bronchial epithelial cells through suppression of Src homology 2 domain-containing inositol 5 phosphatase 1 (SHIP1) production and subsequent activation of JNK signaling	32
		Promotes T(H)2-mediated allergic airway inflammation through modulating the transcription factor PU.1	33

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