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Emerging Role of MicroRNAs and Long Noncoding RNAs in Respiratory Disease

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The advent of techniques such as microarrays and high-throughput sequencing has revolutionized our ability to examine messenger RNA (mRNA) expression within the respiratory system. Importantly, these approaches have also uncovered the widespread expression of “noncoding RNAs,” including microRNAs and long noncoding RNAs, which impact biologic responses through the regulation of mRNA transcription and/or translation. To date, most studies of the role of noncoding RNAs have focused on microRNAs, which regulate mRNA translation via the RNA interference pathway. These studies have shown changes in microRNA expression in cells and tissues derived from patients with asthma, pulmonary fibrosis, cystic fibrosis, COPD, and non-small cell lung cancer. Although the evidence is currently limited, we review the work that has been carried out in cell and animal models that has identified the function and mechanism of action of a small number of these microRNAs in disease etiology. In addition to microRNAs, we assess the emerging evidence that long noncoding RNAs regulate respiratory phenotype. Because these investigations into long noncoding RNAs were performed almost exclusively in non-small cell lung cancer, future work will need to extend these into other respiratory diseases and to analyze how microRNAs and long noncoding RNAs interact to regulate mRNA expression. From a clinical perspective, the targeting of noncoding RNAs as a novel therapeutic approach will require a deeper understanding of their function and mechanism of action. However, in the short term, changes in miRNA and long noncoding RNA expression are likely to be of use as biomarkers for disease stratification and/or assessment of drug action.

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ABBREVIATIONS: CF = cystic fibrosis; CFTR = cystic fibrosis transmembrane conductance regulator; HASM = human airway smooth muscle; IPF = idiopathic pulmonary fibrosis; lncRNA = long noncoding RNA; MALAT1 = metastasis associated in lung adenocarcinoma transcript 1; MEG3 = maternally expressed gene 3; miRNA = microRNA; mRNA = messenger RNA; ncRNA = noncoding RNA; NSCLC = non-small cell lung cancer; SCAL1 = smoke and cancer-associated long noncoding RNA-1; TGF = transforming growth factor

Evidence is accumulating that shows that large sections of the genome are transcribed into noncoding RNAs (ncRNAs) (ie, RNA sequences that are not translated into

proteins). The exact proportions are still an area of controversy. Thus, the *Encyclopedia of DNA Elements* (the “ENCODE” project that aims to catalog all the functional

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elements in human DNA) has concluded that approximately 80% of DNA is functional and, importantly, that the majority (approximately 62%) is transcribed into RNA.¹ However, a number of investigators have questioned these conclusions, most notably whether ncRNA is indeed functional.²

Despite this controversy, there are many ncRNAs with demonstrated function. At the present time, ncRNAs are divided broadly into three groups: housekeeping RNAs, short ncRNAs (<200 nucleotides), and long ncRNAs (lncRNAs) (>200 nucleotides) (Fig 1).³⁻⁶ The housekeeping RNAs include ribosomal RNA, transfer RNA, small nucleolar RNA, and small nuclear RNA. By far the largest component is the ribosomal RNAs (5S, 18S, and 28S), which represent >90% of total RNA in mammalian cells and which interact with proteins and transfer RNAs to form the functional ribosome complex that is central to translation. The best characterized family of short ncRNAs is the microRNAs (miRNAs), which regulate the translation of messenger RNA (mRNA) into protein via the RNA interference pathway. Interestingly, recent sequencing studies have identified a tranche of new small RNA families that are commonly located at sites of active transcription; they include transcription initiation RNAs, splice site RNAs, and promoter-associated small RNAs.³ Finally, the least characterized but largest group by number is the lncRNAs, which are thought to regulate both mRNA transcription and/or translation. Given the paucity of information on the many thousands of lncRNAs, these are commonly subdivided

by sequence and position relative to protein coding genes into antisense lncRNAs, pseudogenes lncRNAs, intronic lncRNAs, and intergenic lncRNAs. To date, most studies of the role of ncRNAs in respiratory disease have concerned miRNAs, which will, therefore, form the focus of the current review. However, we also plan to highlight the emerging role of lncRNAs.

miRNAs and the RNA Interference Pathway

As implied by the name, miRNAs are small 21 to 23 nucleotide RNA sequences that are produced from primary miRNA transcripts by the sequential action of two RNase III endonucleases, Drosha and Dicer (Fig 2).⁷ The initial primary miRNA transcripts are essentially identical to mRNAs but lack the translational start codon (AUG) and fold to produce a characteristic hairpin structure. These are recognized by Drosha and the DiGeorge syndrome critical region gene 8 and cleaved in the nucleus to produce a precursor miRNA with a stem-loop structure and a length of approximately 70 to 90 nucleotides (Fig 2). The precursor miRNA is exported into the cytoplasm and is cleaved by Dicer to produce an approximately 22-nucleotide double-stranded RNA. This is subsequently unwound, and a single miRNA strand is loaded into the Ago2 protein, an essential component of the RNA-induced silencing complex. Within the RNA-induced silencing complex, miRNAs are able to induce mRNA degradation and/or inhibit the translation of target mRNAs by a mechanism titled RNA interference. This process

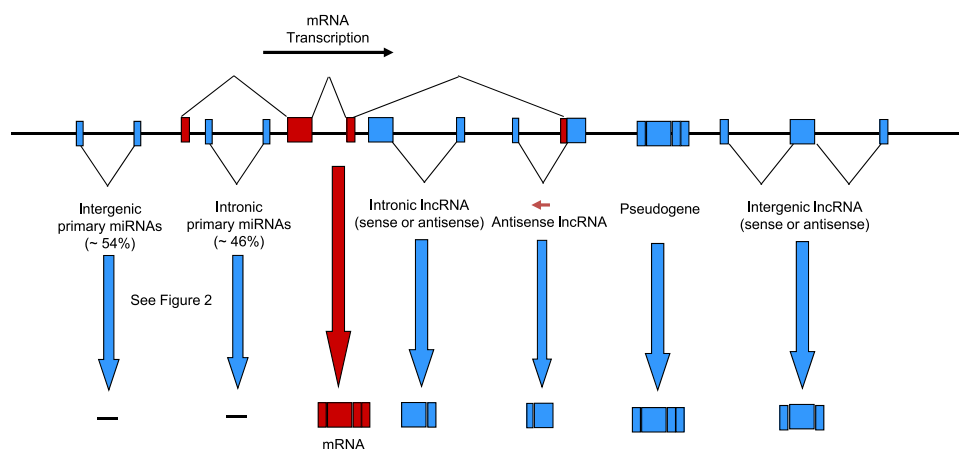


Figure 1 – Classification of miRNAs and lncRNAs. Noncoding RNAs (shown in blue) are commonly divided into housekeeping RNAs (ribosomal RNA, transfer RNAs, small nucleolar RNAs, and small nuclear RNAs [not shown]), small noncoding RNAs (<200 nucleotides), and lncRNAs (>200 nucleotides). The best characterized group of small noncoding RNAs is the miRNAs, which are produced from primary miRNAs transcripts by the sequential action of endonucleases (see Fig 2). Primary miRNAs are located within both intronic and intergenic regions of protein-coding mRNAs (shown in red). The lncRNAs are also classified by their structure and position relative to mRNAs and include intronic lncRNA, antisense lncRNA, intergenic lncRNA, and pseudogenes. lncRNA = long noncoding RNA; miRNA = microRNA; mRNA = messenger RNA.

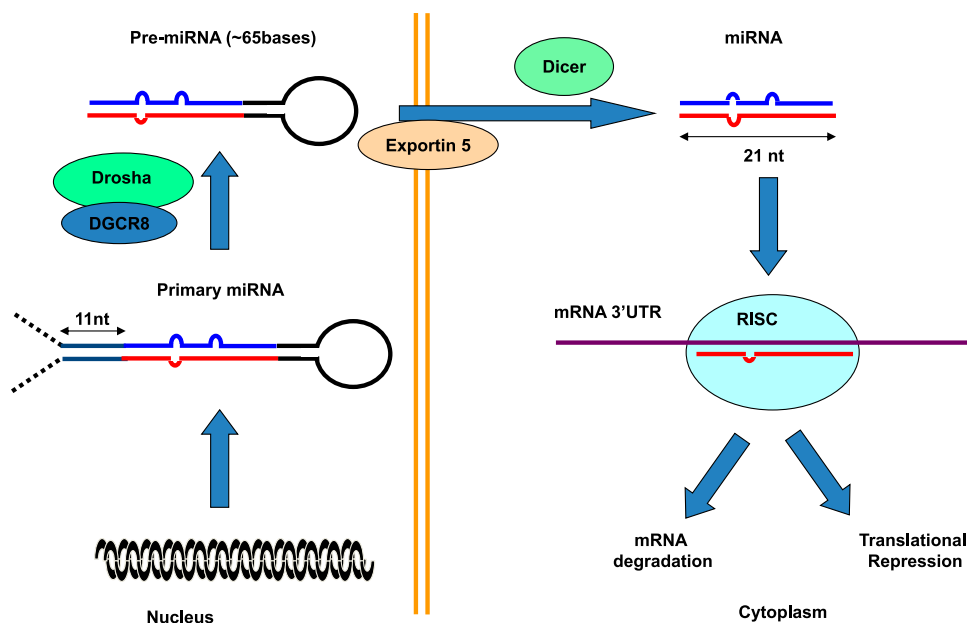


Figure 2 – Mechanism of miRNA-mediated RNA interference. Following polymerase II-mediated transcription, the primary miRNA transcript folds to produce a characteristic hairpin structure. This is recognized by Drosha and DGCR8, which positions the endonuclease Drosha to cleave the hairpin structure at 11 nucleotides from the base and release the pre-miRNA. The pre-miRNA is then transported to the cytoplasm, where the hairpin is removed by the second endonuclease, Dicer, and one strand of the resulting double-stranded miRNA is removed by Ago2 to produce the mature miRNAs. Bound within the Ago2 with multiple other proteins, the miRNA-Ago2 complex searches for noncomplementary sequences within the 3' UTR of mRNA. Once mRNA-miRNA binding occurs, this results in either mRNA degradation or blockage of mRNA translation. 3' UTR = 3'-untranslated region; DGCR8 = DiGeorge syndrome critical region gene 8; pre-miRNA = precursor miRNA; RISC = RNA-induced silencing complex. See Figure 1 legend for expansion of other abbreviations.

involves noncomplementary binding between the miRNAs, particularly the seed region at residues 2 to 7, and the 3'-untranslated region of the target mRNA. Importantly, the noncomplementary nature of this binding and the short length of the seed region mean that miRNAs can target multiple mRNAs, whereas individual mRNAs contain target sites for multiple miRNAs. Indeed, it is predicted that about 60% of mRNAs can act as targets for miRNAs.⁸

What Is the Physiologic Function of miRNAs in the Respiratory System?

Before examining the role of miRNAs in respiratory disease, it is important to understand their function under physiologic conditions. In general, analysis of multiple organs and cells indicates that miRNAs have a dual role as both regulators of development (in nondifferentiated cells) and in the maintenance of homeostasis in differentiated cells.^{9,10} In the respiratory system, their importance in the former is underlined by the widespread changes in miRNA expression observed during lung development¹¹ and in studies in Dicer knockout mice, in which miRNA processing is disrupted, that demonstrate a lethal phenotype resulting from impaired

lung growth.¹² Our understanding of the role of individual miRNAs during lung development is limited, although profiling studies have shown downregulation of the miR-154 family and the miR-17 to about 92 cluster (miR-17, -18a, -19a, -20a, 19b-1, and -92-1) and increases in the miR-29 family and miR-127.^{11,13} Blocking the reduction in expression of the miR-17 to about 92 cluster using transgenic mice resulted in abnormal (and lethal) lung development that was characterized by continued proliferation and impaired differentiation of epithelial cells.¹⁴

The centrality of miRNAs in the maintenance of homeostasis in the adult (differentiated) lung is supported by expression studies that demonstrate no change in miRNA expression during the process of lung aging.¹⁵ Examination of the profile of expression in the adult lung shows that this is dominated by a small number of miRNAs, with miR-21 being the most highly expressed at about 30% of the total (Table 1). Indeed, when added together, it was found that the 10 and 50 most expressed miRNAs represent approximately 68% and approximately 97% of the total, respectively (Table 1). Interestingly, although some miRNAs are expressed in a cell-specific manner, many of these highly expressed

TABLE 1] Relative Expression of miRNAs in Human Lung and Their Association With Respiratory Disease

miRNA	% Total miRNA Expression	Disease Association
mir-21	29.61	Asthma, IPF, NSCLC
mir-22	10.63	...
mir-143	6.15	...
mir-203	4.97	...
mir-148a	4.44	...
mir-99b	2.97	...
mir-10a	2.57	...
mir-103-1	2.31	...
mir-182	2.27	...
mir-30a	1.95	...
let-7b	1.92	Asthma, IPF, NSCLC
mir-10b	1.90	...
mir-92a-2	1.79	...
mir-205	1.67	...
mir-183	1.62	...
let-7a-2	1.38	Asthma, IPF, NSCLC
mir-101-1	1.34	CF
mir-200c	1.24	IPF
mir-25	1.21	Asthma
mir-30e	1.15	...
mir-93	0.93	...
mir-375	0.90	...
mir-30d	0.81	...
let-7f-2	0.69	Asthma, IPF, NSCLC
let-7a-3	0.69	Asthma, IPF, NSCLC
let-7a-1	0.69	Asthma, IPF, NSCLC
mir-29a	0.66	Lung development, IPF
mir-100	0.63	...
mir-28	0.56	...
mir-23a	0.55	...
mir-9-1	0.38	...
mir-9-2	0.38	...
mir-126	0.37	Asthma, CF
mir-151	0.35	...
mir-142	0.34	...
mir-199b	0.34	IPF
mir-210	0.31	...
mir-181a-1	0.29	...
mir-24-2	0.28	...
mir-1307	0.27	...
mir-199a-2	0.27	IPF, COPD
mir-27a	0.24	...

(Continued)

TABLE 1] (continued)

miRNA	% Total miRNA Expression	Disease Association
mir-27b	0.22	...
mir-29c	0.21	Lung development, IPF
mir-141	0.19	...
mir-26a-2	0.19	Asthma
mir-23b	0.19	...
mir-145	0.17	Asthma, IPF, CF
mir-199a-1	0.16	IPF, COPD
mir-181a-2	0.15	...

CF = cystic fibrosis; IPF = idiopathic pulmonary fibrosis; miRNA = microRNA; NSCLC = non-small cell lung cancer.

miRNAs, including let-7 and miR-21, are common among all differentiated cells/tissues (miRNA expression in specific cells and tissues can be found at <http://www.mirbase.org>). This has led to speculation that they regulate common phenotypes such as proliferation and apoptosis and that changes in their expression are associated with cancer (see the miRNAs and Non-small Cell Lung Cancer section).

Attempts to elucidate the role of miRNAs have primarily involved the measurement of their differential expression in cells/tissues obtained from patients with the relevant respiratory disease, whereas subsequent functional and mechanistic studies have involved overexpression and inhibition of these miRNAs in cell and animal models.¹⁶ We have, therefore, adopted this systematic approach to reviewing the role of miRNAs in various respiratory diseases.

miRNAs and Asthma

Asthma is characterized by chronic airway inflammation and reversible airflow obstruction, the latter commonly occurring in response to environmental challenges such as allergens and infections. Initial studies using airway biopsy specimens obtained from mild asthmatics were unable to show changes in miRNA expression, although it was speculated that this may have resulted from the mixed cell populations in the biopsy specimens and/or the phenotype of those with mild asthma.¹⁷ These problems were addressed subsequently by two studies using isolated bronchial epithelial cells, in which Jardim et al¹⁸ showed differential expression of 66 miRNAs in patients with mild asthma, and Solberg et al¹⁹ demonstrated changes in 217 miRNAs and 200 miRNAs in steroid-naive and steroid-using patients with asthma respectively. Of relevance, the variations in miRNAs observed by Solberg et al¹⁹ could be recapitulated by

exposing epithelial cells to IL-13, a cytokine implicated in the development of asthma.¹⁹

T cells are believed to be important orchestrators of the chronic inflammatory response in asthma. Circulating CD4⁺ T cells from mild and moderate asthmatics showed differential expression of miR-132, miR-223, miR-374a, and miR-1290, whereas CD3⁺ T cells from bronchial biopsy specimens of patients with mild asthma exhibited downregulation of miR-125b, miR-19a, miR-19b, and miR-106b.²⁰ Our own studies using circulating CD4⁺ and CD8⁺ T cells showed downregulation of miR-146a and miR-146b in patients with severe asthma, but not in those with nonsevere asthma.²¹ Given that miR-146a and miR-146b negatively regulate the immune response,²² we speculated that a reduction in their expression might contribute toward T-cell activation. In addition to studies of T cells, a number of reports have examined peripheral blood mononuclear cells and have shown miR-192 downregulation in mild asthmatics²³ and miR-211 and miR-485-3p upregulation in pediatric patients with asthma.^{24,25}

In addition to their role in airway constriction, human airway smooth muscle cells (HASMs) are thought to contribute to the airway thickening (hypertrophy) and the chronic inflammatory response that is characteristic of asthma. Indeed, isolated HASMs exhibit increased proliferation and release of inflammatory mediators in response to exogenous stimulation.²⁶ At the present time, no studies have examined miRNA expression in asthmatic samples, although there are reports on the function of individual miRNAs in isolated HASMs. As detailed in Table 2,²⁷⁻⁵⁵ this includes a role for miR-140-3p³⁵ and miR-133a³⁴ in contractility, miR-26a³¹ and miR-221²⁶ in proliferation, and miR-25³⁰ and miR-221²⁶ in the inflammatory response.

The functional relevance of individual miRNAs has also been assessed using a number of mouse models of allergic asthma, including those induced using ovalbumin, house dust mite, and IL-13 transgenics. Using intratracheal or intranasal delivery of antisense oligonucleotides targeted at individual miRNAs, these studies have identified potential roles for miR-21,⁵⁶ miR-126,^{33,57} miR-145,⁵⁸ miR-106a,³² miR-1,²⁹ let-7,²⁷ and miR-221²⁵ in the development of allergic asthma through the targeting of various cytokines and transcription factors (Table 2).

miRNAs and Idiopathic Pulmonary Fibrosis

Idiopathic pulmonary fibrosis (IPF) is localized to the lung interstitium, is characterized by fibrosis, and is

thought to result from the excessive production of extracellular matrix components by fibroblasts and myofibroblasts. It is speculated that this results from an aberrant wound-healing response linked to excessive transforming growth factor (TGF)- β production. Most studies of the role of miRNAs in IPF have used a combination of human lung samples, isolated lung fibroblasts, and the bleomycin-induced mouse model of lung fibrosis. A range of miRNAs, including let-7d,³⁶ miR-21,³⁸ miR-29,⁵⁹ miR-200,⁶⁰ miR-154,⁶¹ the miR-17 to about 92 cluster,³⁷ miR-145,⁴⁰ and miR-199-5p, have been shown to be differentially expressed in human IPF lung tissue, TGF- β -stimulated fibroblasts, or both.⁴¹ With the exception of miR-154,⁶¹ these changes in miRNA expression were confirmed in bleomycin-induced lung fibrosis. Crucially, inhibition of let-7d,³⁶ miR-21,³⁸ miR-29,³⁹ miR-200,⁶⁰ the miR-17 to about 92 cluster,³⁷ miR-145,⁴⁰ and miR-199-5p⁴¹ was also shown to impact bleomycin-induced lung fibrosis in mice, suggesting that these miRNAs may represent novel therapeutic targets.

As with other respiratory diseases, the mechanism of action of only a small proportion of these miRNAs is understood (Table 2). Interestingly, miR-29 is known to target a host of extracellular matrix proteins and has been implicated as a key regulator in a range of fibrotic diseases.⁶² However, in the case of IPF, miR-29 also appears to target expression of TGF- β , connective tissue growth factor, and Smad3.³⁹ Also of potential mechanistic relevance is the upregulation of the maternally imprinted miR-154 region.⁶¹ Having shown previously that this region is downregulated during lung development,¹¹ this has led to speculation that IPF represents a reversion to a fetal lung phenotype.⁶¹ Finally, the expression of many of these miRNAs has been linked to the activation of Smad3, a key transcription factor in the TGF- β signaling pathway.^{36,39}

miRNAs and COPD

COPD is driven predominantly by long-term exposure to cigarette smoke, leading to destruction of the lung parenchyma (emphysema) and associated reduction in lung function. Other symptoms include airway inflammation, as well as cough and mucus secretion (bronchitis). Unlike miRNAs in asthma, IPF, and lung cancer, virtually nothing is known regarding the role of miRNAs in COPD. A report by Ezzie et al⁶³ compared the miRNA expression in lung tissue derived from smokers with and without COPD and identified 70 miRNAs, including miR-223, miR-1274a, and miR-15b, that were differentially expressed in smokers without COPD,

TABLE 2] Function and Mechanism of Action of miRNAs in Respiratory Disease

Study	miRNA	Cell or Animal Model	Function and Mechanism
Asthma			
Kumar et al ²⁷	let-7	Primary T cells and IL-13 transgenic mouse model	let-7 regulates IL-13 expression
Polikepahad et al ²⁸	let-7	OVA-challenge mouse model	let-7a regulates IL-13 expression
Takyar et al ²⁹	miR-1	Endothelial cells in OVA-, dust mite- and transgenic IL-13 mouse models	VEGF-induced miR-1 expression targets Mpl in endothelial cells; Mpl regulates P-selectin expression
Kuhn et al ³⁰	miR-25	Human airway smooth muscle	IL-1 β , TNF- α , and IFN- γ -induced miR-25 expression regulates inflammatory response via targeting of Kruppel-like factor-4
Mohamed et al ³¹	miR-26a	Human airway smooth muscle	Mechanical stretch upregulates miRNA-26a, which induces human airway smooth muscle proliferation by suppressing glycogen synthase kinase-3 β
Sharma et al ³²	miR-106a	OVA-challenge mouse model	Increased miR-106a downregulates IL-10
Mattes et al ³³	miR-126	TH2 cells in house dust mite-challenge mouse model	Inhibition of miR-126 resulted in augmented expression of POU domain class 2 associating factor 1, which activates the transcription factor PU.1 via negative regulation of GATA3 expression
Chiba et al ³⁴	miR-133a	Human airway smooth muscle cells and OVA-challenge mouse model	miR-133a regulates RhoA expression and contractility
Jude et al ³⁵	miR-142-3p	Human airway smooth muscle	TNF- α -induced reduction in miR-142-3p regulates CD38 expression and contractility
Perry et al ²⁶	miR-221	Human airway smooth muscle	FCS and TGF- β -induced miR-221 expression regulates proliferation and IL-6 by targeting the cyclin-dependent kinase inhibitors, p21WAF1 and p27kip1
IPF			
Pandit et al ³⁶	let-7d	Bleomycin-induced mouse model of lung fibrosis and IPF lung	let-7d targeted downregulation of cadherin-2, vimentin, smooth muscle actin- α , and HMGA2 in epithelial cells.
Dakhllallah et al ³⁷	miR-17 to about 92	Bleomycin-induced mouse model of lung fibrosis and primary human fibroblasts	miR-17 to about 92 negatively regulated DNMT-1 and DNA methylation

(Continued)

TABLE 2] (continued)

Study	miRNA	Cell or Animal Model	Function and Mechanism
Liu et al ³⁸	miR-21	Bleomycin-induced mouse model of lung fibrosis and primary human fibroblasts	miR-21 targets the inhibitory Smad 7
Xiao et al ³⁹	miR-29	Bleomycin-induced mouse model of lung fibrosis	miR-29 negatively regulates TGF- β , connective tissue growth factor (CTGF), and Smad3
Yang et al ⁴⁰	miR-145	Primary human fibroblasts and miR-145 knockout mice	miR-145 increases smooth muscle actin- α by targeting KLF-4
Lino Cardenas et al ⁴¹	miR-199a-5p	Bleomycin-induced mouse model of lung fibrosis and primary human fibroblasts	miR-199a-5p regulates caveolin-1 (CAV-1)
CF			
Oglesby et al ⁴²	miR-126	Airway epithelial cells	miR-126 targets TOM1, which regulates the innate immune response
Ramachandran et al ⁴³	miR-138	Airway epithelial cells	miR-138 targets transcriptional regulatory protein SIN3A, leading to the upregulation of multiple proteins involved in the biosynthesis and regulation of CFTR
Bhattacharyya et al ⁴⁴	miR-155	Airway epithelial cells	miR-155 targets SHIP-1, leading to activation of the PI3K/Akt pathway and IL8 expression
NSCLC			
Johnson et al ⁴⁵	let-7	HeLa cells	let-7 targets expression of ras
Kumar et al ⁴⁶	let-7	Mouse lung tumor model and A549 cells	let-7g targets ras and high mobility group A (HMGA)
Bandi et al ⁴⁷	miR-15a and miR-16	H2009 and A549 epithelial cells	miR-15a and miR-16 target the cell cycle via Rb and G1 cyclins
Taguchi et al ⁴⁸	miR-17 to about 92 cluster	BEAS2B bronchial epithelium	miR-17 to about 92 targets hypoxia-inducible factor-1 α
Hatley et al ⁴⁹	miR-21	Transgenic miR-21 mouse	miR-21 targets sprout, inhibits negative regulators of Ras/MEK/ERK pathway including sprouty 1 (Spry1), sprouty 2 (Spry2), B-cell translocation gene 2 (Btg2), and programmed cell death 4 (Pdc4d4).
Acunzo et al ⁵⁰	miR-27a	A549 epithelial cells	miR-27a targets MET, EGF receptor, and sprouty
Liu et al ⁵¹	miR-31	Mouse cancer model and multiple epithelial cell lines	miR-31 targets large tumor suppressor 2 (LATS2) and PP2A regulatory subunit B α
Lin et al ⁵²	miR-135b	Mouse cancer cell lines	miR-135b regulates metastasis by components of the Hippo pathway

(Continued)

TABLE 2] (continued)

Study	miRNA	Cell or Animal Model	Function and Mechanism
Garofalo et al ⁵³	miR-221 and miR-222	Multiple cancer cell lines	miR-221 and miR-222 increase cell migration through targeting PTEN and TIMP3
Xi et al ⁵⁴	miR-487b	Multiple cancer cell lines	Epigenetic repression following exposure to cigarette smoke is mediated downregulation of miR-487b that targets SUZ12, BMI1, WNT5A, MYC, and KRAS
COPD			
Mizuno et al ⁵⁵	miR-34a and miR-199-5p	Pulmonary microvascular endothelial cells	miR-199a-5p and miR-34a target HIF-1 α expression

CFTR = cystic fibrosis transmembrane conductance regulator; FCS = fetal calf serum; IFN- γ = interferon- γ ; OVA = ovalbumin; TGF = transforming growth factor; TNF = tumor necrosis factor; VEGF = vascular endothelial growth factor. See Table 1 legend for expansion of other abbreviations.

whereas miR-34a and miR-199a-5p were increased in smokers with COPD. Only two studies have examined the role of miRNAs in isolated cells from patients with COPD. Using human lung fibroblasts, Sato et al⁶⁴ observed reduced miR-146a expression in patients with COPD and showed that this increased expression of PGE₂ through the targeting of COX-2⁶⁴ (Table 2). The second publication by Lewis et al⁶⁵ showed that miR-1 was downregulated in quadriceps muscles and speculated that this is linked to the muscle weakness observed in COPD.

miRNAs and Non-small Cell Lung Cancer

As with COPD, non-small cell lung cancer (NSCLC) is thought to result from long-term exposure to cigarette smoke and can be divided roughly equally between adenocarcinoma and squamous cell carcinoma, which together account for approximately 80% of total lung cancers. There are nearly 1,000 publications relating to miRNAs and lung cancer, and this short report will distill some of the major conclusions; readers are referred to more in-depth reviews.⁶⁶⁻⁶⁸

Expression studies have identified a number of miRNAs linked to NSCLC. Early studies demonstrated that reduced expression of the members of the let-7 family was associated with a poor prognosis.⁶⁹⁻⁷¹ Interestingly, let-7 acts as a tumor suppressor by targeting the downregulation of the tumor promoting proteins, ras, and HMGA2.^{45,46} Other miRNAs that have been identified as having a similar tumor-suppressing action include miR-15a,⁴⁷ miR-16,⁴⁷ miR-31,⁵¹ miR-487b,⁵⁴ and miR-4423⁷² by targeting myc and ras,⁵⁴ the cell cycle regulators Rb and G₁ cyclins,⁴⁷ the large tumor suppressor 2,⁵¹ and the epigenetic regulators, SUZ12, BMI1, and WNT5A⁵⁴ (Table 2). In contrast, the expression of miR-155,^{70,71} the miR-17 to about 92 cluster,⁴⁸ miR-221/222,⁵³ miR-21,⁴⁹ miR-146b,⁷¹ and miR-135b⁵² is reduced in NSCLC. In this case, these miRNAs are thought to promote tumor formation because their reduced levels result in increased expression of their proteins targets. These targets include many known tumor promoters including PTEN,⁵³ programmed cell death 4,⁴⁹ the sprouty⁴⁹ and hippo⁵² pathway, and hypoxia-inducible factor-1a⁴⁸ (Table 2).

miRNA and Cystic Fibrosis

As may be expected, the analysis of miRNA function in cystic fibrosis (CF) has focused exclusively on lung airway epithelial cells and has examined their role in regulating the expression of the cystic fibrosis transmembrane conductance regulator (CFTR) or in the innate immune response.

A number of miRNAs, including miR-101, miR-145, miR-223, miR-384, miR-494, miR-509-3p, and miR-1246, have been demonstrated to target CFTR expression.⁷³⁻⁷⁷ However, only the expression of miR-145, miR-223, miR-494, and miR-509-3p were upregulated in patients with CF and shown to be further increased in response to inflammatory mediators such as tumor necrosis factor- α , IL-1 β , and *Pseudomonas aeruginosa*.^{76,77} A report by Amato et al⁷⁸ identified a single nucleotide polymorphism within the 3'-untranslated region of a number of patients with CF and CFTR-related disorders that do not contain mutations within the coding sequencing. Because this single nucleotide polymorphism was located within the predicted binding site for miR-433 and miR-509-3p, they speculated that it may act as a mild CFTR mutation by enhancing miRNA affinity, leading to downregulation in CFTR expression. Interestingly, a screening-based approach by Ramachandran et al⁴³ showed that miR-138 knockout enhanced CFTR abundance and activity. Importantly, this action was mediated through knockdown of the transcriptional regulatory protein SIN3A, which relieved the repression of multiple genes involved in CFTR biosynthesis and regulation.⁴³

In addition to their actions on CFTR, changes in miRNA expression in patients with CF are thought to impact the immune response to infection. Oglesby et al⁴² found that decreased expression of miR-126 in CF epithelial cells resulted in the elevation of target of Myb1 (TOM1), a protein involved in regulating toll-like receptor- and tumor necrosis factor- α -induced signaling pathways. Similarly, Bhattacharyya et al⁴⁴ showed upregulation of miR-155 and speculated that targeting SHIP1 expression would in turn lead to activation of the PI3K/Akt pathway and the production of IL-8. In a subsequent report, Tsuchiya et al⁷⁹ showed that exposure of lung epithelial cells to *P aeruginosa* resulted in suppression of miR-155 and increased production of a range of miRNAs including miR-215, which may impact the survival and immune response in the epithelial cells.

lncRNA in Respiratory Disease

Emerging evidence suggests that changes in lncRNA expression are associated with the development of various types of cancer.⁸⁰⁻⁸² It is, therefore, unsurprising that, as with miRNAs, most studies of the role of lncRNAs in respiratory disease have been performed in NSCLC. Indeed, one of the first lncRNAs was discovered in a screen of genes associated with lung adenocarcinoma and was named metastasis associated in lung adenocarcinoma transcript 1 (MALAT1).⁸³ Interestingly,

this large noncoding transcript (> 8 kb) is expressed at high levels in most cells, and initial mechanistic studies indicated that the nuclear-localized MALAT1 regulated alternative splicing through an interaction with serine/arginine splicing factors.^{84,85} However, more recent examination of knockdown in cell and animals models has shown that MALAT1 regulates gene expression and not splicing, and specifically those genes involved in cell migration, colony formation, and metastasis.⁸⁶⁻⁸⁸

Two well-characterized and highly expressed lncRNAs whose levels are also increased in NSCLC are HOTAIR⁸⁹ and H19.⁹⁰ HOTAIR, which is known to regulate chromatin structure through an interaction with the polycomb response complex 2, has been shown to upregulate cell migration and anchorage-independent cell growth.⁸⁹ Interestingly, a possible interaction between lncRNA and miRNAs has been uncovered from studies showing that the increased expression of the paternally imprinted H19 lncRNA may act as a sponge for let-7, thereby explaining the downregulation of this miRNA in NSCLC.⁹¹

Expression changes in four additional lncRNAs have also been linked to NSCLC and/or COPD, including smoke and cancer-associated lncRNA-1 (SCAL1),⁹² GAS6-antisense 1,⁹³ maternally expressed gene 3 (MEG3),⁹⁴ and lncRNA low expression in tumor.⁹⁵ SCAL1, whose expression is driven by nuclear factor erythroid 2-related factor, is increased in NSCLC and following exposure to cigarette smoke in vivo and in vitro.⁹² A protective role for this lncRNA was indicated in studies that demonstrated increased cigarette smoke-induced toxicity following SCAL1 knockdown.⁹² In contrast, MEG3 expression was reduced in NSCLC, and overexpression of MEG3 increased MDM3 and p53 expression, resulting in increased proliferation and reduced apoptosis.⁹⁴ Interestingly, we have also shown reduced expression of MEG3 in the circulating CD8⁺ T cells of patients with severe asthma, as well as the differential expression of an additional 18 lncRNAs.²¹

miRNAs as Biomarkers of Respiratory Disease

In addition to their role in disease, there is now a growing literature to suggest that miRNAs obtained from either blood, tissue, or exhaled breath may provide reliable biomarkers for the diagnosis and prognosis of respiratory disease. A recent comparison of exhaled condensates showed consistent reduction in miRNA expression in asthmatic patients (miR-1248, let-7a, miR-155, miR-21, miR-328, and miR-133a) and patients with COPD (miR-21 and miR-328) compared with control subjects.⁹⁶ Examination of circulating exosomes,

which are vesicular structures released into the blood stream by cells, has also demonstrated differential expression of miRNAs, including let-7a and miR-21, in mild asthma⁹⁷ and increased expression of muscle-specific miRNA species (miR-1, miR-133, miR-206, and miR-499) in patients with COPD that was suggestive of muscle breakdown.⁹⁸ As may be expected, there is a considerable body of biomarker work in NSCLC,^{99,100} including reports showing that tissue and plasma miRNA signatures can be correlated with prognosis and disease classification.¹⁰¹ As a specific example, laser capture from bronchial brushings was able to identify two miRNAs that could discriminate between small cell lung cancer and NSCLC (miR-29a and miR-375) and adenocarcinoma and squamous cell carcinoma (miR-205 and miR-34a).¹⁰²

Conclusions

There is now increasing evidence that the expression of miRNAs is altered in the airways and lungs of patients with a broad range of respiratory diseases. Whether these are simply a reflection of the disease or an important mediator of the underlying pathology has yet to be determined. However, knockdown studies in animal models of asthma and IPF suggest that miRNAs are indeed important in the cause of disease and may offer potential therapeutic targets using approaches such as antisense oligonucleotides and short interfering RNAs. Although not related to respiratory disease, the targeting of the liver-specific miR-122 using an antisense-based approach is currently undergoing human trials for the treatment of the hepatitis C virus.¹⁰³ Given the accessibility of the lung to topical administration, this approach may also be of use in tackling respiratory disease.¹⁰⁴ In contrast to miRNAs, much less is known regarding the role of the many thousands of lncRNAs, and this is likely to provide an exciting and fertile new area of scientific discovery. However, given that therapeutic modulation of ncRNAs is a medium to long prospect (5-10 years), it is likely that their short-term application will be as biomarkers in disease classification and/or the assessment of novel drugs.

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