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Today's Science - Tomorrow's Medicine

MicroRNAs in Lung Development and Disease

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EDUCATIONAL AIMS

- MicroRNAs (miRNAs) are small, non-coding RNAs that post-transcriptionally regulate gene expression and are increasingly recognized as crucial factors in development and disease.
- MiRNAs regulate aspects of the immune system and inflammation in cystic fibrosis (CF), including cytokines, proteases activity, and expression of the CF transmembrane conductance regulator (CFTR).
- In asthma, miRNAs regulate cytokines, the immune and inflammatory responses.
- Differentially expressed miRNAs have been identified in bronchopulmonary dysplasia (BPD), many of which might regulate late lung development and alveolarization.
- Research into the roles of miRNAs in pediatric lung disease and lung development is still in the early stages but has sometimes offered new insights into the pathogenesis and the potential for novel therapies.

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SUMMARY

MicroRNAs (miRNAs) are small (~22 nucleotides), non-coding RNA molecules that regulate gene expression post-transcriptionally by inhibiting target mRNAs. Research into the roles of miRNAs in lung development and disease is at the early stages. In this review, we discuss the role of miRNAs in pediatric respiratory disease, including cystic fibrosis, asthma, and bronchopulmonary dysplasia.

INTRODUCTION

MicroRNAs (miRNAs) are small, ~22 nucleotides-long, non-coding RNAs that inhibit translation of target mRNAs. *Lin-4* was first discovered in *Caenorhabditis elegans* as an inhibitor of the LIN-14 protein and was followed by the identification of let-7 [1,2]. Both of these miRNAs are potent regulators of developmental timing in *C. elegans*. Since then, more than 1,000 validated miRNA genes have been identified in the human genome [3]. Most of the human genome is predicted to be regulated by miRNAs, and multiple miRNAs may act cooperatively to silence the same target gene. One miRNA may regulate hundreds of targets [4]. MiRNAs influence the development of mammalian organ systems, including cell proliferation, migration, differentiation, and apoptosis [5]. Abnormally expressed miRNAs may in turn cause abnormal expression of their target genes, and influence the progression of human disease. Dysregulation of miRNAs can be caused by epigenetic phenomena, and in turn, some miRNAs can control epigenetic regulators [6]. Thus, miRNAs present as potential disease mechanisms, biomarkers, and therapeutic targets.

Herein we will review our current understanding of miRNAs in pediatric respiratory disease. Dysregulation of miRNAs has been identified in many diseases, such as cancer including the lungs [7], psychiatric diseases [8], idiopathic pulmonary fibrosis [9], cardiovascular diseases [10], and many childhood diseases [11]. Much remains to be learned about the role and biological relevance of miRNAs in pediatric respiratory diseases. We will discuss the body of miRNA lung research, the majority of which has focused on cystic fibrosis (CF), asthma, and bronchopulmonary dysplasia (BPD) (Table 1).

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Table 1
MicroRNA dysregulation in pediatric lung diseases.

Cystic fibrosis	
miR-126 [12]	Downregulated in airway epithelium and may regulate the innate immune response via TOM1
miR-31 [13]	Downregulated in airway epithelium and may mediate protease activity and inflammation through an IRF-1/CTSS-dependent pathway
miR-93 [14]	Downregulated in bronchial epithelial cells and leads to overexpression of IL-8 and induction of proinflammatory responses
miR-145, miR-223, miR-494 [15],	Upregulation of these miRNAs is associated with decreased ΔF508 CFTR expression
miR-138 [16]	Represses *SIN3A* expression, and treatment of cystic fibrosis airway epithelia with miR-138 mimics relieves ΔF508 CFTR biosynthesis
miR-101/miR-494 [17]	Synthetic miRNA mimics synergistically inhibit CFTR constructs
Asthma	
miR-221, miR-485-3p [18]	Upregulated in asthmatic children and murine asthma models, and Spred-2, a predicted target, is downregulated and negatively regulates allergen-induced airway inflammation and hyperresponsiveness
miR-3162-3p [19]	Upregulated in the circulating blood of juvenile asthma patients and downregulates β-catenin; treatment with miR-3162-3p antagomirs alleviates airway hyperresponsivess and inflammation in OVA-induced mice
miR-19a [20]	Upregulated in airway T cells in asthma patients and may cause increased T_H2 production in the airways and promote allergic inflammation
let-7 [21]	Downregulated in a murine allergic airway model, and has an anti-inflammatory role by inhibiting IL-13
miR-106a [22]	Upregulated in a short-term mouse asthma model, and inhibits the anti-inflammatory cytokine IL-10; miR-106a knockdown alleviates airway hyperresponsiveness and inflammation
miR-21 [23]	Upregulated in mice with allergic airway inflammation, and modulates IL-12 expression leading to polarization of T_H cells toward a T_H2 response
Bronchopulmonary dysplasia	
miR-29 [24]	Upregulated in a hyperoxia mouse model of BPD, and downregulates target mRNAs implicated in BPD-altered pathways
miR-152, miR-30a-3p [25]	Downregulated in the peripheral blood of preterm BPD patients
miR-133b, miR-7 [25]	Upregulated in the peripheral blood of preterm BPD patients
miR-206 [26]	Downregulated in BPD mice and human patients, and fibronectin 1 is upregulated and a direct target of miR-206, playing a role in extracellular matrix remodeling

CFTR: Cystic fibrosis transmembrane conductance regulator; **TOM1:** Target of Myb1; **Spred-2:** Sprouty-related protein with an EVH1 domain-2; **IRF-1:** interferon regulatory factor 1; **IL:** Interleukin; **CTSS:** Cathepsin S; **T_H:** T helper cell.

MICRORNA BIOGENESIS, SILENCING MECHANISM, AND FUNCTION

MiRNA biogenesis, silencing mechanisms, and its regulation have been studied extensively [5,27–29]. Human miRNA genes are typically transcribed by RNA polymerase II (pol II) [30] and are encoded within introns, but sometimes are contained within exonic regions [27]. The genes form a cluster if they are co-transcribed (polycistronic) as a single primary transcript [27]. This hairpin-containing transcript, the primary miRNA (pri-miRNA), can be up to several kilobases long and the single-stranded 5′ and 3′ tails are capped and polyadenylated, respectively [30]. A ~70-nt stem-loop precursor miRNA (pre-miRNA) is liberated from the pri-miRNA by the Microprocessor, which includes the RNase III endonuclease Drosha and its double-stranded-RNA-binding cofactor DGCR8 [31]. When intron splicing is substituted for canonical Microprocessor cleavage, the miRNA is termed a mirtron [32].

The karyopheran exportin-5 binds and mediates the translocation of the pre-miRNA from the nucleus to the cytoplasm through nuclear pore complexes in a Ran•GTP-dependent manner [33,34]. The RNase Dicer cleaves the pre-miRNA to produce a ~22-nt RNA duplex [35]. Formation of the miRNA effector molecule, the RNA-induced silencing complex (RISC), involves loading of the RNA duplex into Argonaute (AGO) proteins and unwinding of the duplex [36]. The RNA duplex separates, releasing the RNA passenger strand and retaining the guide (mature) miRNA strand [37]. Two-to-eight nucleotides (seed sequence) of the mature strand are used to recognize the target mRNA with strong complementarity, typically at the 3′-UTR, and together with the AGO protein the miRNA mediates translational repression and/or degradation of the mRNA [29,37].

In animals, miRNAs are a major class of small silencing RNAs and are distinguished from two other classes, small interfering RNA (siRNA) and PIWI-interacting RNA (piRNA). These classes differ in biogenesis, mechanism of action, and the processes they regulate [38]. Naming of miRNAs is inconsistent with the earlier discovered let-7 and lin-4, but it is currently recommended that miRNAs are named sequentially by the time of discovery [39]. Nearly identical orthologs can have the same number, and nearly identical sequences within a species can have a letter or number suffix [39]. The species name is assigned as a prefix in the miRNA name [39]. To refer to the mature miRNA that originates from either arm of the pre-miRNA, a -5p and -3p suffix is used. Previously, the -3p miRNA was denoted with an asterisk, and it was also sometimes used to refer to the minor miRNA product that exists at a lower physiologic concentration.

MICRORNAS AND PEDIATRIC RESPIRATORY DISEASE

The lungs begin to develop in the embryonic period, and after four weeks, two lung buds form that will repeatedly branch to create the bronchial tree. The signaling between the lung endoderm and supporting mesenchyme plays a crucial role in lung development. The entire air conducting zone of the bronchial tree is generated during the pseudoglandular stage between five through sixteen weeks of gestation. Multiple signals, including FGF10 and WNT, for example, drive branching morphogenesis [40]. Epithelial and mesenchymal differentiation occur during the following three stages—canalicular, saccular, and alveolar. During the canalicular stage, the respiratory zone, and air-blood barrier forms, between sixteen and twenty-five weeks of gestation. Surfactant within amniotic fluid from differentiated type II alveolar cells is detectable during the saccular stage, during which the air spaces also expand. This occurs from weeks twenty-four through thirty-six of gestation, after which the alveoli form through secondary septation in the alveolar stage and finishes up to eight years into childhood.

Lung development is orchestrated by a complex array of genes that must be temporally and spatially regulated. Currently, research into the role of miRNAs in lung development, homeostasis, and disease is in its early stages. Enrichment of *Ago1* and *Ago2* is localized to branching regions in the distal epithelium and mesenchyme, respectively, at sites undergoing dynamic changes in gene expression and rapid remodeling of the distal structures [41]. Three studies were used to show differential expression of 21 [42], 167 [43], and 198 [44] miRNAs during lung development, and