

Asthma diagnosis: MicroRNAs to the rescue



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Asthma is a general term describing a disease encompassing various phenotypes. Indeed, patients with asthma can be classified using several parameters along various axes such as their inflammatory profile (eg, eosinophilic, neutrophilic, or mixed granulocytic), atopic condition, triggers of attack (eg, exercise, allergens, and viruses), and age of disease onset. The recognition that asthma characteristics are different in distinct individuals suggests that diverse biological processes (termed “endotypes”) determine these observed phenotypes.¹ One particular area of interest is the identification of noninvasive biomarkers for asthma, which will enable the stratification of patients according to their specific phenotype and predict their response to therapy.

MicroRNAs (miRNAs) are evolutionary conserved, small single-stranded, noncoding RNAs. They regulate gene/protein expression through direct interactions between their 5′ region and the 3′ untranslated region of target mRNAs. Direct binding of miRNAs to a target mRNA may result in either mRNA degradation or inhibition of protein translation.² miRNAs may indirectly alter gene expression through global effects on methylation and targeting of transcription factors essential to gene expression. Computational analyses suggest that miRNAs may regulate up to one-third of all protein-coding genes in the human genome.³ Thus, miRNAs can bind and regulate hundreds of genes simultaneously. Indeed, miRNAs may regulate critical cellular processes and have been implicated in the pathogenesis of various diseases (eg, cancer).⁴ One of the unique characteristics of miRNAs is the finding that they can be secreted into plasma, bronchoalveolar space, and body fluids. Importantly, miRNAs

are resistant to degradation by nucleases because they are often enclosed in nanovesicles termed exosomes and associate with argonaute-2, a key protein in the RNA-induced silencing complex, which protects them. Collectively, this poses miRNAs as potential sensitive and noninvasive diagnostic and prognostic markers in various diseases including asthma.⁵

To assess the expression and roles of miRNAs in asthma, previous studies have used human and mouse models of asthma with specific emphasis on profiling single cells (eg, bronchial epithelial cells, smooth muscle cells, and T cells) or whole lung tissue. Additional studies in mouse models of asthma, including our own, have shown important roles for miR-126, miR-145, and miR-21 in regulating pathophysiology by governing T-cell function and their ability to produce cytokines that orchestrate the inflammatory response.^{6–8} Collectively, differential expression of more than 90 miRNAs has been reported in asthma. Notably, these previous studies assessed the expression and function of miRNAs in cells implicated in pathogenesis of asthma *per se*. What remained unknown was whether there were differences in the levels of miRNAs in the circulation or in respiratory secretions in patients, and if so could this help stratify these individuals into specific subpopulations.

In this issue, Maes et al⁹ and Panganiban et al¹⁰ report expression patterns of miRNAs in sputum supernatants and plasma, respectively. In the first study, Maes et al measured the expression profile of 755 miRNAs in sputum supernatants of 10 healthy subjects, 17 patients with mild-to-moderate asthma, and 9 patients with severe asthma. Comparison of miRNA expression in healthy subjects versus patients with severe asthma revealed 3 miRNAs that were significantly increased in those with severe asthma, namely, miR-629-3p, miR-223-3p, and miR-142-3p. These miRNAs were negatively associated with FEV₁/forced vital capacity and FEV, and their expression was further validated in an additional cohort of 60 individuals (10 healthy controls and 50 patients with asthma). Notably, expression of these miRNAs was closely linked to neutrophilic asthma (defined as 76% neutrophilic, 3% eosinophilic). In fact, correlation analyses comparing sputum cell counts and miRNA expression levels revealed that the expression of these 3 miRs was positively associated with the percentage of sputum neutrophils. Using *in situ* hybridization on bronchial biopsies, Maes et al⁹ demonstrated that the expression of miR-629-3p was confined to epithelial cells whereas the expression of miR-223-3p was localized to neutrophils. Subsequent *in silico* analyses predicted that these miRNAs target inflammatory pathways, which are linked to the development of asthma (eg, IL-1β and IL-8) and were negatively associated with FEV₁ and FEV₁/forced vital capacity. Importantly, IL-1β and IL-8 levels correlated significantly with increased sputum neutrophil numbers, and levels of miR-223-3p and miR142-3p correlated with the expression of IL-1β.

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Finally, the biological function of the identified miRNAs was investigated by transfecting primary human bronchial epithelial cells with miRNA mimics, which altered gene expression of predicted targets related to asthma, wound repair, and inflammation. For example, treatment with miR-629-3p mimic induced the expression of IL-8 and IL-6, whereas the expression of FBXW7 was decreased by miR-223-3p and miR-629-3p mimics.

In an independent study, Panganiban et al.¹⁰ assessed the expression levels of 135 miRNAs (detectable in the plasma) in 12 patients with allergic asthma and 12 healthy subjects, revealing 30 differentially expressed miRNAs. To identify miRNAs that might be associated with allergic rhinitis (AR), asthma, or both, the expression of these 30 miRNAs was subsequently profiled in a larger number of individuals ($n = 79$), which included 35 patients with asthma (29 of whom had AR), 25 subjects with AR but without asthma, and 19 healthy subjects. Expression levels of 30 miRNAs were found to be differentially expressed among the 3 groups and were clustered into 5 distinct groups.

Group 1 miRNAs exhibit changes in expression among patients with AR compared with healthy controls, and the magnitude of these changes was largely increased in those with asthma. Thus, mRNA transcripts targeted by group 1 miRNAs (eg, IL-13 and TGF- β receptors) may be common to AR and asthma. Group 2 contained miRNAs that were unique to asthma, because these were differentially expressed in asthma versus AR and the healthy groups, but were similarly expressed in subjects with AR and healthy subjects. These clustered miRNAs were predicted to regulate prominent inflammatory genes and signal transduction regulators, such as mitogen-activated protein kinases, nuclear factor kappa B components, and signal transducer and activator of transcription molecules, which are important for driving inflammation. Group 3 miRNAs were dysregulated by a similar magnitude in both AR and asthma. This suggested that group 3 miRNAs might be indicative of the common pathways that may be involved in AR and asthma, possibly providing a molecular basis for the “one airway-one disease” hypothesis.¹¹ Group 4 miRNAs were differentially expressed only in patients with AR but without asthma. Therefore, it is likely that targets of group 4 miRNAs are involved in AR-specific pathways. Finally, group 5 miRNAs demonstrated a greater magnitude of dysregulation among patients with AR than among patients with asthma.

Because the aim of the study was to determine whether miRNAs could serve as noninvasive biomarkers to identify subsets of those with asthma, an unsupervised cluster analysis was performed, which identified a signature of 20 miRNAs (including miR-223, miR-126, miR-21, and let-7b) that were

increased in patients with high levels of blood eosinophils. Strikingly, the authors were able to identify the crucial miRNAs that could predict disease status. Using the top 6 predictive miRNAs (miR-125b, miR-16, miR-299-5p, miR-126, miR-206, and miR-133b) and machine-learning algorithms, the authors were capable of correctly determining with an accuracy of 92.4% whether subjects were healthy, had AR, or suffer from asthma. This study is the first to establish that circulating miRNAs can be used to diagnose and differentiate between asthma phenotypes.

Although these investigations examined a small patient cohort, an interesting unifying “mechanistic theme” that emerges from both studies is the finding that differentially expressed miRNAs in sputum and plasma predominantly regulate inflammatory signaling pathways. Furthermore, the outcomes raise key questions for future research. For example, can we use miRNAs to predict drug resistance (eg, responsiveness to steroids) along with disease subtypes? Can we use miRNAs to classify asthma into specific endotypes? Can we better understand disease etiology through miRNA mining? Answering only some of these questions may pave the way to the development of biomarkers that may be diagnostic and prognostic of disease status.

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