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Diagnostic, functional, and therapeutic roles of microRNA in allergic diseases

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Activity Objectives

1. To identify microRNA (miRNA) profiles involved in the pathogenesis of asthma, eosinophilic esophagitis, allergic rhinitis, and atopic dermatitis.
2. To understand the role of miRNAs in regulating eosinophils and their development.
3. To identify perspectives for future investigation and clinical utility of miRNAs.

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Allergic inflammation is accompanied by the coordinated expression of a myriad of genes and proteins that initiate, sustain, and propagate immune responses and tissue remodeling. MicroRNAs (miRNAs) are a class of short single-stranded RNA molecules that posttranscriptionally silence gene expression and have been shown to fine-tune gene transcriptional networks because single miRNAs can target hundreds of genes. Considerable attention has been focused on the key role of miRNAs in regulating homeostatic immune architecture and acquired immunity. Recent studies have identified miRNA profiles in multiple allergic inflammatory diseases, including asthma, eosinophilic esophagitis, allergic rhinitis, and atopic dermatitis. Specific miRNAs have been found to have critical roles in regulating key pathogenic mechanisms in allergic inflammation, including polarization of adaptive immune responses and activation of T cells (eg, miR-21 and miR-146), regulation of eosinophil development (eg, miR-21 and miR-223), and modulation of IL-13–driven epithelial responses (eg, miR-375). This review discusses recent advances in our understanding of the expression and function of miRNAs in patients with allergic inflammation, their role as disease biomarkers, and perspectives for future investigation and clinical utility. (J Allergy Clin Immunol 2013;132:3-13.)

Key words: *Allergy, microRNA, noncoding RNA, asthma, eosinophilic esophagitis, atopic dermatitis, allergic rhinitis, eosinophils, inflammation, biomarkers*

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Allergic inflammatory diseases encompass a wide range of conditions, including asthma, allergic rhinitis, atopic dermatitis,

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Abbreviations used
BALF: Bronchoalveolar lavage fluid
CTLA-4: Cytotoxic T lymphocyte–associated protein 4
HDM: House dust mite
IGF1R: Insulin-like growth factor 1 receptor
LNA: Locked nucleic acid
miRNA or miR: MicroRNA
MyD88: Myeloid differentiation primary response gene–88
OVA: Ovalbumin
STAT: Signal transducer and activator of transcription
TLR: Toll-like receptor
Treg: Regulatory T
TSLP: Thymic stromal lymphopoietin
UTR: Untranslated region

and, more recently, eosinophilic esophagitis.[1-6] Each of these diseases involves sustained inflammation that is associated with marked histologic, as well as molecular, changes in gene and protein expression. The pathways used in regulating and fine-tuning these processes represent a particularly attractive area for microRNA (miRNA) studies.

miRNAs are single-stranded RNA molecules of 19 to 25 nucleotides in length that mediate posttranscriptional gene silencing of target genes[7] and are highly conserved throughout evolution.[8,9] miRNAs were initially discovered in *Caenorhabditis elegans* in 1993 as silencers of genes that regulate developmental timing.[10] Subsequently, miRNAs were recognized as a distinct class of small regulatory RNAs in multiple species that regulate a wide variety of functions, such as cell proliferation, differentiation, apoptosis, stress response, and immune response.[11-13] miRNAs directly suppress gene expression by base pairing to the 3′ untranslated region (UTR) of target mRNA. Depending on the level of complementarity to the target sites, target mRNA degradation, translational repression, or both occur, with imperfect base pairing favoring translational repression.[14] A single miRNA can target hundreds of genes, and individual genes are typically targeted by multiple miRNAs, adding complexity to the network. miRNAs can also exert global effects on gene expression by either affecting epigenetic mechanisms, such as DNA methylation or histone acetylation, or targeting transcription factors.[15-19] Therefore miRNAs are a particularly promising class of molecules that might be well positioned to regulate allergic inflammatory processes.

Recently, miRNAs have been shown to be detectable in cell-free body fluids, such as serum and plasma samples.[20,21] The circulating miRNAs are protected from blood RNAses either by existing in cell membrane–derived vesicles, such as exosomes, or by forming a complex with lipid protein carriers, such as high-density lipoprotein.[22-24] The majority of miRNAs in plasma form a complex with the Argonaute-2 protein, which is part of the RNA-induced silencing complex responsible for gene silencing; this association protects the miRNAs from degradation.[25-27] These circulating miRNAs could be ideal blood biomarkers because of their disease-specific dysregulation and their relative stability compared with mRNAs. This possibility is especially attractive for diseases, such as eosinophilic esophagitis, for which current modalities for diagnosis and follow-up involve invasive endoscopic procedures.[28,29]

Herein we review and discuss the connections between miRNAs and allergic inflammatory diseases, including asthma, eosinophilic esophagitis, atopic dermatitis, and allergic rhinitis. We focus on specific miRNAs that are dysregulated in 1 or more allergic diseases, leading to inappropriate expression of mRNA targets that contribute to disease pathology, and identify a core set of miRNAs involved in the pathogenesis of allergic inflammation.

ALLERGIC ASTHMA

Asthma is a common chronic disorder of the airways that involves a complex interaction of airflow obstruction, bronchial hyperresponsiveness, and underlying inflammation. Allergic asthma is the most common form of asthma, with symptoms triggered by inhaled allergens.[30]

miR-21 regulates polarized adaptive immune responses in allergic asthma models

We and others have found that miR-21 is upregulated in multiple experimental asthma models, including ovalbumin (OVA)–, *Aspergillus fumigatus*–, house dust mite (HDM)–, and lung-specific IL-13–induced murine models.[31,32] Although the methods of experimental asthma induction are different in each of these systems, all of these models share similar phenotypes, including airway eosinophilia, T_H2-associated inflammation, mucus production, and airway hyperreactivity.[32-39] Notably, miR-21 is among the top overexpressed miRNAs in the inflamed lung tissue.[31] The highest expression levels of miR-21 are localized to macrophages and dendritic cells.[31] The IL-12p35 3′UTR harbors a highly evolutionarily conserved target sequence for miR-21 (Fig 1).[31] Because IL-12 is a key molecule involved in T_H1 polarization in adaptive immune responses,[40] this finding suggests that miR-21 could potentially regulate T_H1 versus T_H2 balance by regulating IL-12p35 expression. Indeed, by using a luciferase reporter system, IL-12p35 has been identified as a molecular target of miR-21.[31] Pre–miR-21 dose-dependently inhibits cellular expression of the luciferase reporter vector harboring the 3′UTR of IL-12p35, and mutating miR-21 binding sites in the IL-12p35 3′UTR abrogate miR-21–mediated repression.[31] Furthermore, miR-21–deficient dendritic cells produce increased levels of IL-12 compared with wild-type dendritic cells after equivalent LPS stimulation. By using the OVA-induced asthma model, miR-21–deficient mice had reduced lung eosinophilia after allergen challenge and significantly increased levels of the T_H1 cytokine IFN-γ and decreased levels of T_H2 cytokine IL-4 in the bronchoalveolar lavage fluid (BALF).[41] Notably, the IL-12/IFN-γ pathway was the most prominently affected pathway in the lungs of miR-21–deficient mice, as identified by using whole-genome microarray analysis, adding significance to the finding that one of miR-21's major functions is likely to regulate immune polarization, at least in the setting of immune hypersensitivity responses.[41] Additionally, miR-21–deficient $CD4^+$ T cells produced increased IFN-γ and decreased IL-4 levels. Conversely, by using a T_H1-associated delayed-type hypersensitivity model, miR-21 deficiency significantly enhanced delayed-type hypersensitivity responses.[41] These findings are not just of academic interest because miR-21 is upregulated in multiple human T_H2-associated diseases, including eosinophilic esophagitis, atopic dermatitis, and ulcerative colitis.[42-44] In addition, miR-21 has been reported to be upregulated in human airway epithelial cells in response to IL-13 treatment and suppresses Toll-like receptor (TLR) 2 signaling in an animal model of asthma.[45] AntagomiRs or anti-miRs are a class of chemically engineered nucleotides

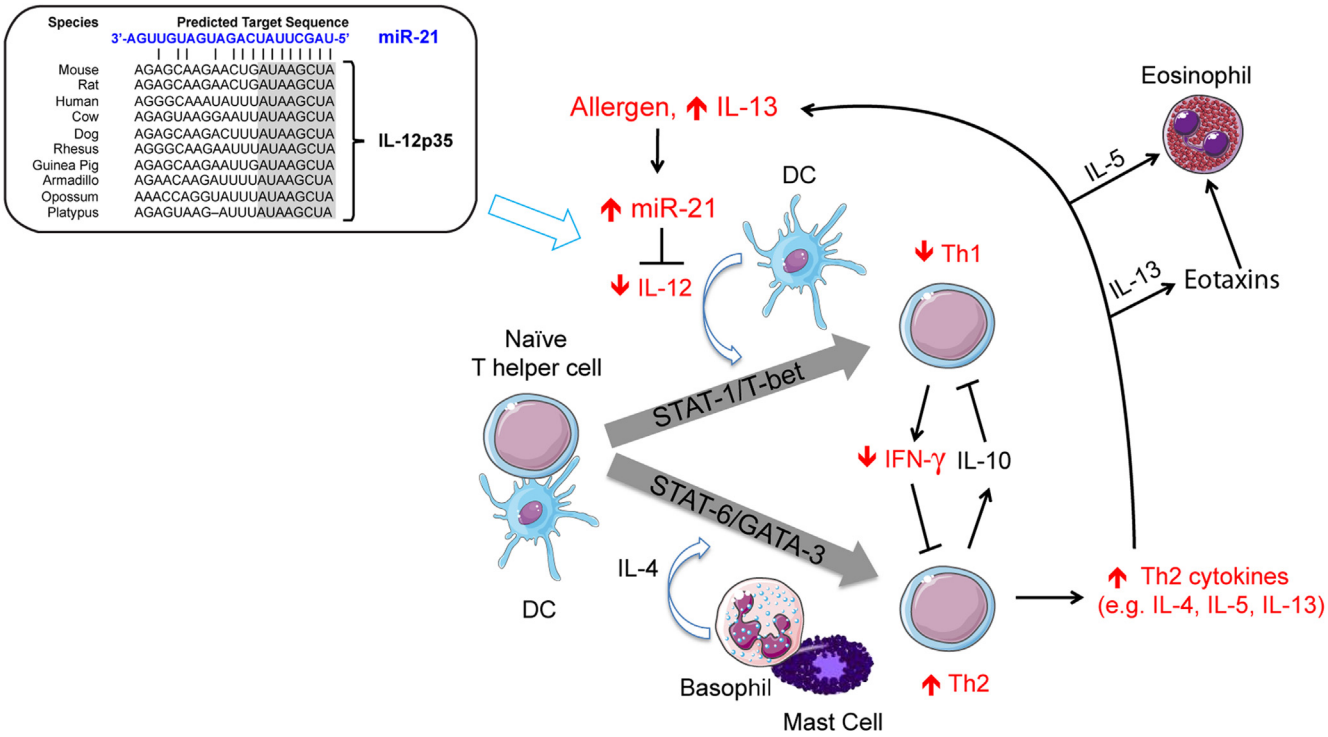

FIG 1. Schematic showing regulation of the adaptive immune system by miR-21 in patients with allergic inflammatory responses. T_H1 and T_H2 cells exist in a balanced state. Allergen or IL-13 upregulates miR-21 expression, which represses the target gene IL-12 and in turn inhibits T_H1 differentiation. Decreased production of the T_H1 cytokine IFN-γ causes unopposed T_H2 activation and increased production of T_H2 cytokines. The T_H2 cytokines might further amplify the miR-21–mediated responses. A highly conserved miR-21 seed sequence in the 3′UTR of IL-12p35 is shown in the insert at the *top left corner*; the seed sequence is *shaded in gray*. *DC*, Dendritic cell.

that silence miRNA expression *in vitro*, *in vivo*, or both.[46,47] Although a recent report by Collison et al[48] found that intranasal anti–miR-21 administration starting 1 day before allergen challenge in an HDM model of asthma had no significant effect on eosinophil recruitment or T_H2 cytokine production,[48] the anti–miR-21 was administered after the intranasal sensitization phase of the protocol, when the T_H1 versus T_H2 balance had likely already been established. This might suggest that miR-21 has its most significant role in the early sensitization stage. Alternatively, the antagomiRs might not recapitulate the phenotype of miR-$21^{-/-}$ mice because of either the type of antagomiR used or the mode and frequency of administration, as indicated by the lack of agreement in recent antagomiR and gene-deficient murine studies.[49,50] These results demonstrate that small perturbations in miRNA levels can have profound effects on adaptive immunity.

miR-126 regulates the effector function of T_H2 cells and the allergic inflammatory response in experimental asthma

miR-126 has been found to be upregulated in the airway wall in an acute HDM-induced experimental asthma model.[32] The upregulation was dependent on the TLR4 and myeloid differentiation primary response gene–88 (MyD88) pathways. miR-126 was not upregulated in either TLR4- or MyD88-deficient mice after HDM challenge. Inhibition of miR-126 by antagomiRs abrogated the asthmatic response, as demonstrated by reduced inflammation, airway hyperreactivity, T_H2 cytokines (eg, IL-5 and IL-13), airway eosinophilia, and mucus production.[32] However, in a chronic model of experimental asthma, miR-126 was initially upregulated after 2 weeks of allergen challenge but decreased to near baseline levels after 6 weeks of allergen challenge.[51] Inhibition of miR-126 by antagomiRs in the chronic asthma model reduced recruitment of intraepithelial eosinophils in the conducting airways but had no effect on mucus cell hyperplasia or subepithelial fibrosis. The authors concluded that sustained changes in miRNAs might not be essential for perturbation of chronic asthma.[51]

Let-7 regulates IL-13 expression and the allergic inflammatory response in experimental asthma

The let-7 family includes let-7a through let-7k.[52] Originally discovered in *C elegans*, let-7 was subsequently found as the first known human miRNA.[52] The let-7 family members are highly conserved across species. However, let-7h, let-7j, and let-7k are not expressed in mice or human subjects.[52] An initial report by Polikepahad et al[53] demonstrated that IL-13 is a direct target of let-7 using a luciferase reporter system. They subsequently demonstrated that T_H1 cells have significantly higher let-7a expression compared with T_H2 cells. The authors inhibited let-7a expression by using locked nucleic acid (LNA) antagomiRs, which are short antisense RNAs with a modified ribose moiety resistant to endonucleases and exonucleases.[54] Inhibition of let-7a by LNA antagomiRs significantly upregulated IL-13 mRNA expression in T cells. By using an anti–let-7 LNA antagomiR that

targets let-7a, let-7b, let-7c, and let-7d, the authors' *in vivo* findings were opposite of their *in vitro* findings. They found that anti–let-7 LNA antagomiR alleviated experimental asthma *in vivo*, with reduced BALF inflammatory cell infiltration and downregulation of IL-4, IL-5, and IL-13 levels.[53] A subsequent report by Kumar et al[55] demonstrated downregulation of let-7 family members including let-7a, let-7b, let-7c, let-7d, let-7f, let-7g, and let-7i in the asthmatic lungs after OVA challenge. They found that let-7 inhibited IL-13 secretion in phorbol 12-myristate 13-acetate/PHA–stimulated T cells. Using intranasal delivery of a let-7 mimic, the authors found that let-7 attenuated experimental asthma, with reduced inflammatory cell infiltration, mucus secretion, airway fibrosis, and airway hyperreactivity.[55]

Several differences could potentially explain the discrepancies between these 2 reports. First, Polikepahad et al[53] used an antagomiR that inhibited only 4 members of the let-7 family. It is possible that there was a compensatory upregulation of other let-7 family members.

Second, in the study by Polikepahad et al,[53] the mice received 2 intravenous doses of LNA antagomiRs; the first dose was given the day before, and the second dose was given the day after the first intranasal allergen challenge. In contrast, Kumar et al[55] administered the 2′-O-Methyl antagomiR intranasally 30 minutes before every intranasal allergen challenge. The different types of antagomiRs and the route and frequency of challenge could partially account for the disparate results.

Third, both the let-7 mimic and the let-7 inhibitor could have off-target effects that contribute to the observed *in vivo* phenotype. An additional report by Collison et al[48] found upregulation of let-7b in an HDM model of experimental asthma without any effect of anti–let-7b on the asthmatic phenotype. Given the discrepancies reported in the literature, future studies are needed to better define the role of let-7 in allergic inflammation.

Antagonism of miR-145 inhibits experimental allergic airway inflammation

miR-145 was recently found to be upregulated in the airway wall in an HDM model of experimental asthma.[48] The authors found that anti–miR-145 significantly attenuated eosinophil infiltration, mucus production, T_H2 cytokine production, and airway hyperreactivity if the anti-miR was administered intranasally every other day starting before the first HDM challenge. Notably, the effects of anti–miR-145 were comparable with dexamethasone treatment. However, once the inflammation was established, both anti–miR-145 and dexamethasone demonstrated limited attenuation of the asthma phenotype. Administration of a single dose of anti–miR-145 before the last HDM challenge attenuated airway hyperreactivity and mucus production but had no effect on eosinophil infiltration.[48] Future studies are needed to identify the precise molecular targets of miR-145 that mediate its anti-inflammatory effects.

Antagonism of miR-106a decreases experimental asthma severity

miR-106a has been found to be upregulated in the lungs of an OVA/alum model of experimental asthma.[56] miR-106a is expressed in macrophages, T cells, B cells, and epithelial cells, with the highest level of expression in macrophages.[57] miR-106a regulates IL-10 expression by directly targeting the 3′UTR of IL-10.[57] Anti–miR-106a administered after sensitization and intranasal allergen challenge significantly reduced inflammatory cell infiltration, T_H2 cytokine levels (eg, IL-4, IL-5, and IL-13), OVA-specific IgE levels, goblet cell metaplasia, airway fibrosis, and airway hyperreactivity.[56] These findings are notable because anti–miR-106a decreased experimental asthma severity after the asthma phenotype had been firmly established. Thus anti–miR-106a could be highly relevant to the treatment of clinical diseases and provide further rationale for the development of miRNA therapeutics.

miRNAs in smooth muscle cells of asthmatic subjects

Smooth muscle cells are the main effector cells of airway hyperreactivity and have a key role in asthma pathogenesis.[58] miR-133a, a muscle-specific miRNA, has been found to be downregulated in cultured human airway smooth muscle cells after IL-13 stimulation.[59] Anti–miR-133a increased the expression of the small GTPase RhoA at baseline, and pre–miR-133a significantly attenuated IL-13–induced RhoA expression.[59] Because the Rho kinases have been shown to mediate smooth muscle contraction, the authors proposed that synthetic miR-133a could suppress airway hyperresponsiveness by targeting RhoA expression.[60] A separate study found that miR-140-3p is downregulated in human asthmatic airway smooth muscle cells after TNF-α stimulation but not in nonasthmatic airway smooth muscle cells.[61] miR-140-3p modulates CD38 expression both by directly targeting the CD38 3′UTR and by means of indirect activation of p38 mitogen-activated protein kinase and nuclear factor κB.[61] These findings are of interest because the CD38 pathway is important for calcium mobilization and smooth muscle contractility.[62]

miRNA expression in human asthmatic subjects

Although most of our current understanding of the role of miRNA in asthma pathogenesis comes from experimental allergic asthma models, several studies have attempted to identify the role of miRNA in human asthmatic subjects. An initial study by Williams et al[63] found no significant difference in the expression of 227 miRNAs in the airway biopsy specimens obtained from 8 patients with mild asthma (FEV_1, 83% ± 4% of predicted value) compared with 8 healthy subjects (FEV_1, 95% ± 4%). Several possibilities could explain the lack of changes in the miRNA expression profile. The authors proposed the possibility that the inflammatory changes were too mild and that changes in miRNA expression might be more evident in patients with more severe asthma. Another possibility is that the samples were not taken during an episode of asthma exacerbation. Studying the airways after an allergen challenge that is known to intensify allergic airway inflammation might have revealed miRNAs that are critical in mediating the allergic inflammatory response. A subsequent study by Liu et al[64] identified upregulation of miR-221 and miR-485-3p in the peripheral blood of 6 pediatric patients with asthma admitted to the hospital compared with 6 control subjects. They subsequently confirmed the upregulation of miR-221 in the peripheral blood of an additional 4 pediatric patients with asthma compared with 4 additional control subjects in a separate study.[65] They also found upregulation of miR-221 and miR-485-3p in the lungs in an OVA-induced experimental asthma model. Administration of anti–miR-221 with a nebulizer reduced the total cell and eosinophil numbers in the BALF compared with administration of

scrambled control.[65] The authors proposed that this effect is potentially mediated by targeting sprouty-related, EVH1 domain containing 2 (SPRED2), which is a regulator of allergen-induced airway inflammation and hyperresponsiveness in murine asthma models.[64] In a separate study comparing human airway bronchial epithelial cells from 16 patients with asthma with those from 16 healthy control subjects, the authors found 24 differentially expressed miRNAs, with miR-203 being the most downregulated.[66] Because miR-203 has been reported to regulate skin cell differentiation, the authors proposed that miR-203 might be involved in regulating the differentiation of lung epithelial cells.[66]

miRNAs have been found to be crucial for the development and function of T cells.[67] $CD8^+$ T-cell development is dependent on the miRNA-processing enzyme DICER, and $CD4^+$ T cells deficient in DICER preferentially differentiate into T_H1 cells.[67] A recent study compared the miRNA expression in $CD4^+$ and $CD8^+$ T cells from 12 patients with severe asthma compared with 8 healthy control subjects. For patients with severe asthma, miR-146a and miR-146b were reduced in both $CD4^+$ and $CD8^+$ T cells, whereas miR-28-5p was downregulated in $CD8^+$ T cells only.[68] The downregulation of miR-146a might partially mediate the severe asthma phenotype because T cells lacking miR-146a have been found to be hyperactive in both acute and chronic inflammatory states.[69] On the other hand, a recent animal study using an experimental OVA-induced asthma model showed that miR-146a, miR-146b, miR-150, and miR-181a levels were upregulated in splenic $CD4^+$ T cells after allergen challenge.[70] Further studies are needed to determine whether miR-146a and miR-146b have proinflammatory or anti-inflammatory effects in asthmatic subjects and whether their roles are context and timing dependent.

Exosomal miRNA expression in BALF of human asthmatic subjects

Recently, exosomes have been implicated in the pathogenesis of asthma. In a preliminary report Levanen et al[71] isolated exosomes from the BALF of subjects with mild intermittent asthma and healthy control subjects and measured the miRNA profiles of the exosomes. At baseline, 24 miRNAs were differentially expressed in subjects with asthma compared with control subjects, with the majority of the altered miRNAs being downregulated. The expression profile of these 24 miRNAs was correlated with the FEV_1 of the subjects ($R^2 = 0.74$). Exposure to air pollution did not significantly alter the exosomal miRNA expression in either group. Pathway analysis showed that the differentially regulated miRNAs are implicated in regulating cytokines and inflammatory responses.[71] Further studies are needed to elucidate the function of differentially expressed exosomal RNAs.

EOSINOPHILIC ESOPHAGITIS

Eosinophilic esophagitis is an emerging allergic disease characterized by intense eosinophil infiltration in the esophagus that is unresponsive to acid-suppressive therapy. The incidence and prevalence of eosinophilic esophagitis has been steadily on the increase, with the disease now reported in every continent except Africa.[28,29] Eosinophilic esophagitis represents a special opportunity to identify which miRNAs truly participate in human T_H2-associated inflammatory responses because the allergic tissue is readily available and amenable to molecular analysis due to esophageal biopsies being standard procedures during routine endoscopy for disease monitoring. By using esophageal biopsy samples from patients, miRNA signatures that distinguish noneosinophilic forms of esophagitis from patients with eosinophilic esophagitis have been identified.[44,72] The expression of these differentially regulated miRNAs was largely reversible in patients who responded to glucocorticoid treatment.[44] In 2 independent studies miR-21 has been identified as one of the most upregulated miRNAs in human patients with eosinophilic esophagitis and to directly correlate with esophageal eosinophil levels.[44,72] Notably, esophageal miR-21 levels inversely correlated with esophageal IL-12p35 levels. By using bioinformatic pathway analysis, coregulated miR-21 target genes in patients with eosinophilic esophagitis were found to be significantly enriched in the regulation of T-cell polarization and IFN-γ production.[44] These data provide the first evidence to substantiate a connection between human allergic disease and the IL-12/IFN-γ axis, adding credence to the preclinical studies that implicated miR-21 as a key regulator of IL-12 and immune polarization (Fig 1). Analysis of esophageal eosinophil levels and a myriad of esophageal transcripts for correlation with miR-21 demonstrated impressive correlations of miR-21 with esophageal eosinophil counts, cell-specific markers for eosinophils, and CCL26 (eotaxin-3), which is functionally involved in eosinophil recruitment.[44,73]

Both miR-146a and miR-146b have been found to be upregulated in patients with eosinophilic esophagitis.[44] A previous report by Lu et al[74] found that miR-146a could selectively suppress T_H1 responses. By using miR-146a–deficient mice, the authors showed that miR-146a deficiency selectively impaired regulatory T (Treg) cell–mediated suppression of T_H1 responses, leaving the suppression of T_H2 and T_H17 responses intact. The inhibition of Treg cell–mediated suppression of T_H1 responses was mediated in part by targeting signal transducer and activator of transcription (STAT) 1 expression.[74] Therefore upregulation of miR-146a could prevent differentiation of IFN-γ–producing T_H1 cells. Because IFN-γ promotes T_H1 responses and inhibits the differentiation of T_H2 and T_H17 cells, miR-146a–mediated suppression of T_H1 responses could lead to unopposed T_H2 activation. By using plasma samples from patients with eosinophilic esophagitis, 3 of the differentially regulated miRNAs in the esophageal biopsy specimens, miR-146a, miR-146b, and miR-223, were also found to be differentially regulated in the plasma.[44] Because circulating miRNAs often exist in exosomes, which can be taken up by other cells,[22,25,75,76] the circulating plasma miR-146a could be taken up by the Treg cells and further propagate or help to maintain the T_H2 responses by suppressing T_H1 activation. In addition, miR-146b levels remain increased in patients with eosinophilic esophagitis that responds to pharmacologic treatment and is in remission. Although the specific role of miR-146b in regulating adaptive immune responses has not been investigated, miR-146a and miR-146b have an identical seed sequence that is critical for miRNA-mediated target gene expression. It is plausible that miR-146b could also regulate STAT1 expression and suppress T_H1 responses. Perhaps increased miR-146b levels might predispose eosinophilic esophagitis in disease remission to relapse. Current diagnostic criteria for eosinophilic esophagitis are based on esophageal biopsy histology.[28,29] An esophageal biopsy specimen needs to be obtained every time a diagnosis is made and on subsequent follow-up to assess therapeutic response. The circulating plasma miRNAs, including miR-146a and miR-223, could potentially serve as noninvasive biomarkers

for diagnosis or assessment of therapeutic response alone or in combination with other biomarkers.[77]

One of the most downregulated miRNAs in patients with eosinophilic esophagitis is miR-375.[44] Notably, miR-375 is downregulated after IL-13 stimulation in both human bronchial and human esophageal epithelial cells.[78] In addition, miR-375 has been reported to be downregulated in patients with T_H2-associated diseases, including atopic dermatitis and ulcerative colitis.[43,79] Interestingly, in patients with eosinophilic esophagitis, miR-375 expression inversely correlates with the degree of allergic inflammation, as measured by esophageal eosinophil levels and gene expression levels of the T_H2 cytokines IL-5 and IL-13 and the mast cell–specific enzymes carboxypeptidase A3 and tryptase α/β 1 (TPSAB-1). Notably, when miR-375 is overexpressed in human esophageal epithelial cells, it regulates IL-13–induced genes, particularly in immunoinflammatory pathways. Regulated genes include those involved in extracellular matrix organization, formation of cell junctions, and the inflammatory response. However, it is important to note that Biton et al[80] found that IL-13 stimulation induces miR-375 expression in HT-29 human colon adenocarcinoma cells. Notably, the induction of miR-375 in these cells was only seen after 2 hours of treatment and subsequently returned to baseline or below-baseline levels. It is interesting that miR-375 regulated production of thymic stromal lymphopoietin (TSLP) in HT-29 cells,[80] especially because TSLP is an important epithelium-derived cytokine that is involved in the pathogenesis of allergic inflammation and is genetically linked with eosinophilic esophagitis susceptibility.[81,82] Despite this interesting mechanistic connection, miR-375 has not been found to regulate TSLP expression in esophageal epithelial cells.[78] Notably, miR-375 is downregulated in samples from patients with multiple T_H2-associated diseases,[43,44,79] as well as in samples from patients with hyperproliferative diseases, including esophageal squamous carcinoma.[83,84] These data suggest that the activity of miR-375 might be dependent on its cellular context, as indicated by previous reports.[85,86] Although the collective data already draw strong attention to the role of miR-375 in regulating epithelial cell responses in T_H2 immunity, additional studies are needed to define its exact role and importance, especially with regard to TSLP production.

ATOPIC DERMATITIS

Atopic dermatitis is a hyperproliferative cutaneous disorder associated with a defective skin barrier and a mixed T_H1/T_H2 inflammatory response resulting in susceptibility to cutaneous infections and prominent pruritis.[87] Notably, miRNAs upregulated in patients with other allergic disorders, including miR-21, miR-146, and miR-223, are also upregulated in the skin of patients with atopic dermatitis.[42,88] In addition, Sonkoly et al[79] found that miR-155 was one of the most upregulated miRNAs in skin biopsy specimens from patients with atopic dermatitis compared with healthy control subjects. Topical exposure of nonlesional skin of patients with atopic dermatitis with relevant allergens could induce miR-155 expression. miR-155 is expressed by skin T cells, dendritic cells, and mast cells. Upregulation of miR-155 is observed after T-cell differentiation into both T_H1 and T_H2 lineages.[79] Functionally, miR-155 has been shown to suppress cytotoxic T lymphocyte–associated protein 4 (CTLA-4), a negative regulator of T-cell function. This CTLA-4 suppression in turn enhances the T-cell proliferative response because CTLA-4 has known antiproliferative function in activated T cells.[79] In macrophages miR-155 has been shown to target IL-13 receptor α1.[89] Downregulation of miR-155 favors the development of a pro-T_H2 M2 type of macrophage and reduces IL-12p70 production in monocyte-derived dendritic cells.[89,90] Given the critical role of miR-155 in regulating innate and adaptive immunity,[90-93] more detailed studies on the regulation of miR-155 and its interactions with the immune system in the context of atopic dermatitis are needed to further elucidate its role in pathogenesis and its potential to serve as a novel therapeutic target.

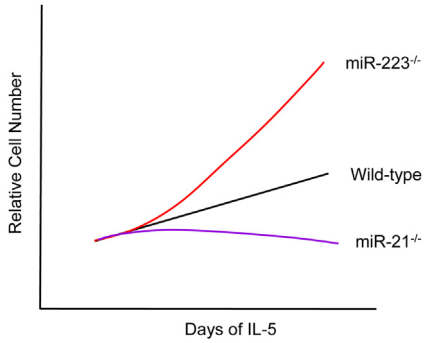

FIG 2. Schematic showing miR-21 and miR-223 have opposite effects in eosinophil progenitor proliferation. Relative cell number of eosinophil progenitor cultures derived from wild-type *(black line)*, miR-223$^{-/-}$ *(red line)*, and miR-21$^{-/-}$ *(purple line)* mice is shown. Targeted ablation of miR-21 decreases eosinophil progenitor proliferation. Targeted ablation of miR-223 increases eosinophil progenitor proliferation.

ALLERGIC RHINITIS

The evaluation of miRNA expression and function in patients with allergic rhinitis has received relatively little attention. A report by Shaoqing et al[94] compared the miRNA expression profile of the nasal mucosa from patients with allergic rhinitis and nonallergic control subjects who underwent surgery for nasal obstruction. They found 9 miRNAs with more than a 2-fold change between the allergic rhinitis group and control group. They subsequently verified the downregulation of miR-143, miR-187, and miR-224 by using quantitative RT-PCR. A report by Zhang et al[95] found that miR-125b is overexpressed in epithelial cells of the sinonasal mucosa in patients with chronic eosinophilic rhinosinusitis with nasal polyps. miR-125b was found to enhance type I interferon production by targeting eIF-4E binding protein 1.[95] A preliminary report by Chen et al[96] screened miRNA levels in cord blood samples with increased cord blood IgE levels. Eight miRNAs were downregulated in cord blood samples with increased IgE levels. Downregulation of miR-21 in the cord blood samples was associated with increased TGF-β receptor 2 expression on cord blood leukocytes. The authors also examined peripheral blood monocytes in 6-year-old children and found that miR-21 and miR-126 remained significantly downregulated in monocytes from children with allergic rhinitis compared with those from nonallergic control subjects. The authors proposed that downregulation of miR-21 in peripheral blood persisted from the neonatal stage to childhood and could potentially be an early predictor of allergic rhinitis.[96] Mechanistically, the association of miR-21 downregulation with subsequent development of atopy is not

TABLE I. List of a core set of miRNAs involved in allergic inflammation, the direction of their change in expression, and their mRNA targets

miRNA	Asthma	Eosinophilic esophagitis	Atopic dermatitis	mRNA targets in allergic inflammation
Upregulated or downregulated in all 3 diseases				
let-7c	↓	↓	↓	IL-13
miR-21	↑	↑	↑	IL-12p35
miR-142-5p	↑	↑	↑	
miR-142-3p	↑	↑	↑	
miR-146a	↑	↑	↑	STAT1
miR-193b	↓	↓	↓	
miR-223	↑	↑	↑	IGF1R
Upregulated or downregulated in 2 diseases				
let-7a	↓	ND	↓	IL-13
let-7b	↓	ND	↓	IL-13
let-7d	↓	ND	↓	IL-13
miR-146b	↑	↑	ND	
miR-155	↑	ND	↑	CTLA4
miR-365	ND	↓	↓	
miR-375	ND	↓	↓	

ND, Difference in expression not significantly different from control value.

consistent with its known role in regulating IL-12.[31,41] Further studies are needed to investigate the potential role of miR-21 and other miRNAs in patients with allergic rhinitis.

EOSINOPHIL DEVELOPMENT

Significant attention has been focused on the role of miRNAs in regulating eosinophils and their development. Eosinophils differentiate from a common myeloid progenitor and then through an eosinophil lineage–committed progenitor that is CD45 and IL-5 receptor α positive.[97,98] The cytokine IL-5 is particularly important in eosinophil lineage development because it promotes the selective differentiation of eosinophils and the release of mature eosinophils from the bone marrow.[99] In an *ex vivo* culture model of bone marrow–derived eosinophils, both miR-21 and miR-223 were upregulated during the differentiation of eosinophil lineage–committed progenitors to mature eosinophils. miR-21 deficiency reduced eosinophil progenitor cell growth, whereas miR-223 deficiency accelerated eosinophil growth (Fig 2).[100,101] miR-21–deficient eosinophil progenitor cultures have increased apoptosis during differentiation of mature eosinophils from progenitor cells. The miR-21–deficient mice have reduced eosinophil levels in the blood and reduced eosinophil colony-forming unit capacity in the bone marrow.[101] Although microarray analysis of differentially regulated genes between miR-21$^{+/+}$ and miR-21$^{-/-}$ eosinophil progenitor cultures identified only 1 gene *(Psrc1)* as a predicted target of miR-21, functional enrichment analysis identified an overall functional effect in the pathways associated with the observed phenotype and known role of miR-21 in other systems.[102,103] Thus it is likely that miR-21 exerts modest effects on direct targets that then synergistically interact to ultimately regulate eosinophilopoiesis.

miR-223–deficient eosinophil progenitors had the opposite phenotype of miR-21–deficient eosinophil progenitors. miR-223 deficiency resulted in increased eosinophil progenitor proliferation compared with that seen in wild-type cells. Mechanistically, miR-223 can exert its effect by regulating insulin-like growth factor 1 receptor (IGF1R), at least in part. Indeed, IGF1R is a target of miR-223 and is expressed on eosinophil progenitors.[100] Notably, IGF1R is upregulated in miR-223–deficient eosinophil progenitors. Treatment with an IGF1R inhibitor attenuated the proliferation of both miR-223$^{+/+}$ and miR-223$^{-/-}$ eosinophil progenitors.[100] Whole-genome microarray analysis of miR-223–deficient eosinophil progenitor cultures identified an alteration in eosinophil cell growth and hematologic development as the most affected biological functions, substantiating a key role for miR-223 in controlling eosinophilopoeisis.[100] Indeed, miR-223$^{-/-}$ eosinophil progenitors have been shown to have a delay in the upregulation of CCR-3, likely indicating a delayed maturation *in vitro*.[100] It is likely that miR-21 and miR-223 cooperatively regulate the proliferation and differentiation of eosinophil lineage–committed progenitors, with upregulation of miR-21 preventing premature arrest in eosinophil progenitor proliferation and the later upregulation of miR-223 preventing overproduction of eosinophils and promoting eosinophil maturation.

T_H CELL DIFFERENTIATION AND ACTIVATION

T lymphocytes are a major driver of allergic diseases. Early studies used global miRNA deletion in T cells by deleting the miRNA-processing enzyme DICER. DICER-deficient T_H cells proliferated poorly on stimulation and preferentially expressed the T_H1 cytokine IFN-γ.[67] More recent studies focusing on the roles of individual miRNAs have shown critical roles of miRNA in regulating the development and activation of T_H cells.

During T-cell development, miR-181a has been shown to augment the sensitivity of T cells to peptide antigens. Inhibiting miR-181a expression in immature T cells reduces antigen sensitivity and significantly impairs T-cell selection.[104] T-cell apoptosis is important in regulating both the length and strength of T-cell responses. The miR-17-92 cluster has been shown to promote a lymphoproliferative disease phenotype by targeting phosphatase and tensin homolog (PTEN) and Bim.[105] MiR-21 has been shown to be upregulated during T-cell activation and suppresses apoptosis in activated T cells.[105]

The development of polarized T_H cells is central to the pathogenesis of allergic inflammation because allergic inflammation is predominately a T_H2 response. Several of the miRNAs upregulated in patients with allergic inflammation, including miR-155, miR-21, and miR-146a (Table I), have been shown to regulate polarized T-cell responses. miR-155–deficient T_H cells exhibit a T_H2 bias *in vitro* in part due to upregulation of the miR-155 target cMaf.[91,92] MiR-155 positively regulates both Treg and T_H17 cell differentiation *in vitro* by targeting suppressor of cytokine signaling 1.[106] Upregulation of miR-21 has been shown to promote the T_H2 response both by repressing IL-12p35 expression in dendritic cells and through a T-cell intrinsic pathway.[31,41,107] MiR-146a has been found to selectively target Treg cell–mediated suppression of T_H1 responses by targeting STAT1.[74] It is likely that additional miRNAs are involved in T-cell development and activation during allergic inflammatory responses, and this represents an exciting area for future investigation.

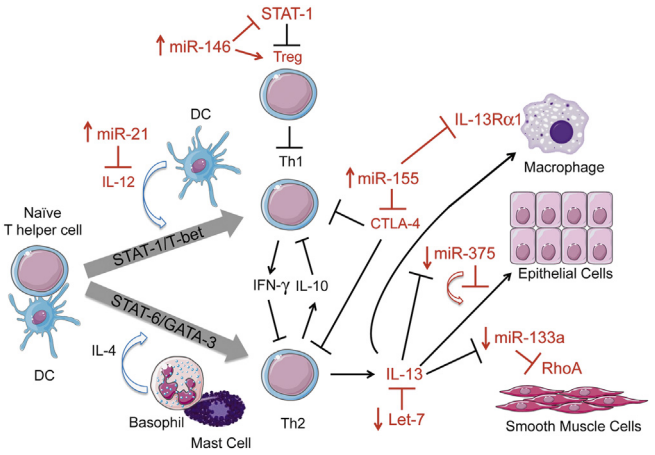

FIG 3. Schematic showing the known roles of a commonly regulated set of miRNAs and their targets in allergic inflammation. T_H1 and T_H2 cells exist in a balanced state and inhibit each other's differentiation. miR-21 inhibits IL-12, which in turn inhibits T_H1 differentiation. miR-146 promotes Treg cell–mediated suppression of T_H1 cells, likely by targeting STAT-1. The suppression of the T_H1 response promotes a T_H2-dominant response with production of the T_H2 cytokine IL-13. Let-7 inhibits IL-13 production, and downregulation of let-7 likely further amplifies the T_H2 response. In T cells miR-155 promotes both the T_H1 and T_H2 responses by inhibiting the inhibitor CTLA-4. In macrophages miR-155 attenuates IL-1–mediated responses by downregulating IL-13 receptor α1 *(IL-13Rα1)*. IL-13 inhibits miR-375 expression in epithelial cells, and miR-375 can both potentiate and inhibit IL-13–mediated responses in epithelial cells. IL-13 inhibits miR-133a in smooth muscle cells, and this increases the expression of the small GTPase RhoA that mediates smooth muscle contraction. The miRNAs and their direct targets are shown in *red*. *DC*, Dendritic cell.

MAST CELLS

Mast cells are key effector cells in allergic inflammation that release potent inflammatory mediators on allergen exposure. miRNAs have been shown to regulate the development of mast cells. miR-221 and miR-222 are significantly upregulated after mast cell activation. Overexpression of miR-221 and miR-222 led to an increased number of cells in the G1/G0 phase and fewer cells in the G2/M phase.[108] In addition, miR-221 overexpression in mast cells led to increased degranulation, decreased migration, and increased adherence.[109] Another miRNA upregulated upon mast cell activation, miR-146a, was shown to increase mast cell apoptosis.[110] In a separate study it was shown that miR-132 was the most upregulated miRNA upon mast cell activation and regulates the heparin-binding EGF-like growth factor.[111] During mast cell differentiation, miR-126 has been shown to be downregulated, and it positively regulates mast cell proliferation by targeting the negative regulator of mast cell proliferation SPRED1.[112] Although miRNAs have shown promising roles in regulating mast cell proliferation and activation, the current data are preliminary, and future investigations are needed to further define the functions of miRNAs in mast cells.

IDENTIFICATION OF A CORE SET OF miRNAs INVOLVED IN ALLERGIC INFLAMMATION

To date, miRNA profiles have been identified in patients with allergic asthma,[31,32,48,51,53,55,63,64,66,113] eosinophilic esophagitis,[44,72] atopic dermatitis,[42,79] and allergic rhinitis.[94] Excluding allergic rhinitis, which does not have an overlapping miRNA profile, perhaps because of the preliminary analysis reported to date, a core set of miRNAs involved in allergic inflammation is becoming apparent. These miRNAs and their direction of regulation are listed in Table I, which includes miRNAs that are upregulated or downregulated in at least 2 of the following 3 allergic diseases: allergic asthma, eosinophilic esophagitis, and atopic dermatitis. Although several of these miRNAs, such as miR-193b and miR-365, have no known reports on their roles in allergic inflammation, the roles of many of the other major allergy-related miRNAs and their molecular targets have been investigated (Fig 3). These commonly regulated miRNAs could be particularly important for the initiation and/or maintenance of allergic inflammatory processes.

TABLE II. Potential utility of miRNAs in allergic diseases

1. Coordinately targeting key pathways, such as the polarization of adaptive immune responses, by several miRNAs
2. Reversal of an established disease phenotype
3. Combinatorial targeting of a key mRNA by several miRNAs
4. Regulating the epigenetic machinery
5. Serving as biomarkers for disease diagnosis, stratification, and prognosis
6. Development as potential therapeutic targets

FUTURE PERSPECTIVES

miRNAs are potentially of critical importance in the pathogenesis of allergic inflammation. Although our knowledge about the miRNA regulation of allergic inflammation has considerably advanced over the last several years, multiple areas warrant future investigation. One exciting possibility is the ability of multiple miRNAs to coordinately target a common pathway. In particular, the polarized T_H responses could be regulated by multiple miRNAs targeting different components of the T-cell polarization pathways. The miRNAs miR-21 and miR-146a are upregulated, and the let-7 family members are downregulated in all of the allergic inflammatory diseases profiled to date. Upregulation of miR-21 appears to promote T_H2 and attenuates T_H1 responses by targeting IL-12 expression. miR-146a has been found to be required for Treg cell–mediated suppression of T_H1, but not T_H2, responses. Upregulation of miR-146a could potentially enhance the Treg cell–mediated suppression of T_H1 responses and result in unopposed T_H2 activation. The let-7 family members appear to target IL-13 expression; downregulation of let-7 could enhance T_H2 responses by upregulating IL-13 expression. Because T_H1 and T_H2 responses exist in a balanced state, miR-21, miR-146a, and let-7 might work additively or synergistically to initiate and/or maintain an exaggerated T_H2 response by targeting different components of the T_H cell polarization pathway. The coordinated regulation of a pathway by multiple miRNAs might lead to much greater activation or repression than that mediated by a single miRNA.

A second area worthwhile of future investigation is the reversibility of an established disease phenotype. Most of the studies to date have used either gene-targeted mice or delivery of antagomiRs before allergen challenge. In one study, delivery of an antagomiR after allergic inflammation was established resulted in very limited attenuation of the disease phenotype compared with delivery of the antagomiR before allergen challenge.[48] The importance of miRNAs in reversing disease phenotype needs to be

further investigated, perhaps by delivering several miRNA mimics or inhibitors in combination.

A third area worth future investigation is a stronger focus on the roles of miRNAs in human studies. Most of our knowledge of the functions of miRNAs in the setting of allergic inflammation to date is based on cell cultures and murine models, except for a few early studies in human allergic diseases. Further elucidating the roles of miRNAs in the human context will likely improve our understanding of miRNAs in the pathogenesis, diagnosis, and prognosis of allergic diseases and lay the foundation for the development of miRNA-based therapies.

A fourth area worth future investigation is the combinatorial targeting of a key mRNA by several miRNAs or the targeting of multiple mRNAs by a key miRNA. Most studies have focused on the targeting of a single mRNA by one miRNA. Although this approach has given us important insight into the role miRNAs play in the pathogenesis of allergic inflammation, combinatorial targeting could lead to even greater target mRNA repression or identification of new gene networks and gene-gene interactions.

A fifth area worth future investigation is the interplay between miRNAs and epigenetics. miRNAs have been reported to be epigenetically regulated and to regulate the epigenetic machinery.[17] DNA methylation and histone modifications have been shown to regulate the expression of multiple miRNAs. Conversely, miRNAs have been shown to regulate epigenetic modulators, including DNA methyltransferases and histone deacetylases.[17] Uncovering the role of miRNAs in the epigenetic regulation of allergic inflammation could help us to understand both the chronicity of the disease and the high relapse potential observed in patients.

A sixth area worth future investigation is the relationship between miRNAs dysregulated in patients with helminth infections and the allergy-related miRNAs. Helminth infection has been proposed to induce a modified T_H2 response that favors survival of both the host and the parasites. This modified T_H2 response is characterized by high levels of IL-10 expression and is proposed to be protective against allergy.[114] Currently, very little is known about the host miRNA response to parasitic infection, except that suppression of let-7 produces significantly lower parasite burden.[115] Comparing helminth-induced miRNAs and allergy-induced miRNAs could further refine the role of miRNAs in the development of T_H2-type inflammation and allergies.

A seventh area worth future investigation is the potential for miRNAs to serve as biomarkers for disease diagnosis, stratification, and monitoring and as an alternative to current invasive measurements. Most of the studies to date are preclinical studies focused on cancer diagnosis and prognosis.[116,117] However, it is likely that miRNAs have both diagnostic and prognostic value in allergic inflammation. The recent discovery of circulating miRNAs in the serum/plasma, particularly in a form that is relatively stable compared with mRNA and even protein, offers the exciting possibility that miRNAs might serve as noninvasive biomarkers. Lastly, the development of improved technologies, including new bioinformatic algorithms, proteomics, next-generation sequencing, new miRNA mimics/antagomiRs, and novel small-molecule miRNA inhibitors, will help us define novel pathways regulating or regulated by miRNAs and to facilitate the development of miRNAs as potential therapeutic targets (Table II).

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What do we know?

- **miRNAs have critical roles in regulating the pathogenic mechanisms involved in allergic inflammation.**
- **miRNAs have been demonstrated to regulate T_H1 versus T_H2 polarization in multiple allergic inflammatory diseases.**
- **miRNAs have been shown to regulate cell types critical to the allergic inflammatory response, including eosinophils, T cells, mast cells, and basophils.**
- **An esophageal miRNA signature could distinguish eosinophilic esophagitis from noneosinophilic forms of esophagitis.**
- **Proof-of-principle preclinical studies with gene knockout strategies and anti-miRNA–based therapeutics have demonstrated the potential clinical utility of modifying miRNAs in patients with allergic disease.**
- **Collectively, these data establish miRNAs as fundamental regulators of allergic inflammation; as such, miRNAs have potential importance as diagnostic and therapeutic targets for disease.**

What is still unknown?

- **What is the function of some of the commonly dysregulated miRNAs in patients with allergic diseases?**
- **Could multiple dysregulated miRNAs cooperatively target a common pathway in allergy?**
- **Could miRNAs reverse an established disease phenotype?**
- **What is the therapeutic potential of miRNA mimics and inhibitors?**
- **How do miRNAs regulate the epigenetic machinery, and how does the epigenetic machinery regulate miRNA expression in patients with allergic diseases?**
- **Could miRNAs be developed into biomarkers for the diagnosis, stratification, and prognosis of allergic diseases?**
- **What are the cell-specific action and mode of action of individual miRNAs?**

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