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microRNAs in asthma: potential therapeutic targets.

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REVIEW

microRNAs in asthma: potential therapeutic targets

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Purpose of review
Asthma is a global disease affecting millions of people. Current treatments are largely symptomatic and, although often effective, can be associated with various side effects. microRNAs (miRNAs/miRs) are regulatory RNAs that affect protein synthesis. They represent new therapeutic targets, and medicines that target specific miRNAs may have potential in the treatment of asthma.

Recent findings
There have been a number of studies in the field of miRNA that implicate specific miRNAs in the pathophysiology of asthma. For example, studies using mouse models have identified miRNAs that are altered in response to allergen challenge. Certain miRNAs that are involved in the regulation of interleukin-13 and the T_H2 response, key components of the asthmatic response, have been shown to be amenable to modulation by premiRs and antimiRs. Other studies have identified miRNAs that are implicated in bronchial smooth muscle hyperresponsiveness and proliferation. Single-nucleotide polymorphisms in miRNA responsive elements within asthma susceptibility genes, and also in miRNAs themselves, can also contribute to the asthma phenotype.

Summary
Developing miRNA-based medicines to treat the pulmonary manifestations of asthma could yield therapeutics with new properties that have the potential to treat both the inflammation and hyperresponsivesness associated with this disease.

Keywords
asthma, microRNA, therapeutics

INTRODUCTION

Asthma is the reaction to inhaled antigens such as respiratory viruses, allergens, or air pollutants that results in airway inflammation, exaggerated airway hyperresponsiveness (AHR), and reversible airflow obstruction. Asthma afflicts both developed and developing nations, and as of 2004, over 300 million people were affected worldwide [1]. In the United States, 8.4% of the population have asthma, and it is responsible for approximately 500 000 hospitalizations and 1.9 million emergency room visits, totaling $56 billion annually [2].

Current treatments are primarily glucocorticoids (e.g., dexamethasone) and inhaled short-acting β-2 agonists, such as salbutanol. Although these have yielded major improvements to patient well being via their ability to inhibit multiple inflammatory processes and prevent contraction of the bronchial smooth muscles (BSMs), respectively, they are associated with side effects ranging from dysphonia and thrush to effects on the hypothalamic–pituitary–adrenal system and cardiac arrhythmia [3]. Some individuals are 'glucocorticoid resistant'. Thus, there is a clear need for new therapeutics that can effectively treat asthma and have fewer side effects.

microRNAs (miRNAs/miRs) are small noncoding RNAs that regulate protein synthesis by way of RNA silencing. miRNAs have been shown to have a key role in cancer [4,5], viral infections [6], and in pulmonary inflammatory diseases [7], such as cystic fibrosis [8]. Herein, we outline the current knowledge of miRNA function in asthma and how miRs could be targets for future therapeutics.

ASTHMA

Individuals with asthma experience respiratory stress that results from bronchial inflammation and smooth

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KEY POINTS

- Current asthma treatments are hampered by potentially serious side effects and glucocorticoid resistance, indicating a need for new therapeutics without these associated problems.
- miRNAs have emerged as major cellular regulators, the expression of which can be modulated by miR-based medicines. Targeting miRNAs represents a new therapeutic strategy for the treatment of asthma.
- Medicines based on over expressing let-7, miR-146, and miR-133, or inhibiting expression of miR-126, miR-21, miR-155, and miR-145 may be worthy if developed.

muscle contraction. Symptoms include reversible airflow obstruction, AHR/bronchospasms, mucus hypersecretion, and a poor forced expiratory volume$_1$ percentage predicted. Multiple cellular changes occur – the most significant being airway remodeling, characterized by smooth muscle hyperplasia, subepithelial cell fibrosis, goblet cell hyperplasia, and neovascularization. Pulmonary inflammation is also a major component of asthma involving influx of mast cells, neutrophils, T_H2 cells, and most notably eosinophils. There is also a change in the smooth muscle response wherein BSM cells become hyperresponsive to acetylcholine stimulation.

The physiological changes that occur are due to T_H2-driven inflammation in response to allergen exposure, resulting in increased T_H2 cytokine levels [interleukin (IL) 4, IL-5, IL-9, and IL-13] and decreased anti-inflammatory cytokines (e.g., IL-10). Together, these further promote the T_H2 response but also cause activation of natural killer cells, dendritic cells and eosinophils, promote eosinophila, increase matrix metalloproteinase activity, increase serum immunoglobulin E concentrations, and promote smooth muscle hypercontractiveness.

Both environmental exposure to air pollutants and genetic predisposition can cause the heightened T_H2 response. Genes involved in inflammation [e.g., IL-10, STAT6, IL-4, IL-13, tumor necrosis factor (TNF), IL-4R] or with a role in building immune tolerance during fetal development (HLA-DRB1, HLA-DQB1) have been implicated as genetic factors. Given the importance of miRNAs in the regulation of expression of up to 60% of the transcriptome, it is worth considering their potential as new therapeutic targets for asthma.

MICRORNAS

miRNAs are small noncoding RNAs that regulate protein synthesis by blocking translation or by targeting mRNA transcripts for degradation. miRNA regulation is complicated because a single mRNA can be regulated by many miRNAs, and a single miRNA can potentially regulate many mRNA transcripts. miRs were first identified in *Caenorhabitis elegans*, with the discovery of lin-4 [9].

Mature miRNAs are bound by the RNA-induced silencing complex (RISC) in the cytosol to form miRISC. This is guided to the appropriate mRNA via the complementary binding between the 6–8 nucleotide seed region, unique to each miRNA and miRNA recognition elements (MRE) within the 3′untranslated region of the target mRNA. mRNA translation is then prevented by blocking the ribosome reading the mRNA or by directing the mRNA to p-bodies wherein the mRNA is degraded.

miRs represent a new class of drug target and their therapeutic modulation is possible by the use of over expression or antisense inhibition approaches [10]. AntimiRNA oligonucleotides (antimiRs) anneal to miRNAs and inhibit their function. Other antimiR methods include locked nucleic acids [11] and cholesterol-conjugated so-called ‘antagomiRs’. miRNAs that are downregulated in disease can be transiently replaced using double-stranded miRNA mimetics called premiRs, or stably using plasmids encoding primary, premiR or mature miRNA. Plasmid or viral vectors expressing short-hairpin RNAs can also be used to overexpress miRs and have the potential for more persistent expression as this approach can facilitate the expression of multiple miRs from one transcript [12]. Blocking miRNA function in asthma via these approaches may provide a new nonsteroidal anti-inflammatory approach to treatment.

MICRORNA AND ASTHMA STUDIES

Although relatively few studies have been carried out to date, there is evidence that miRs are implicated in the asthmatic disease process (summarized in Fig. 1). For example, profiling studies of lung tissue from murine models of acute, intermediate, and chronic asthma identified eight candidate miRs likely to be implicated in asthma [13]. Data from human miRNA profiling studies by Tsitsiou *et al.* [14] identified selective downregulation of miR-28–5p and miR-146a/b in $CD8^+$ and $CD4^+$ cells from individuals with severe asthma that were associated with widespread changes in mRNA expression.

microRNAs that affect cytokines and inflammation

A selection of reports has described the role of certain miRs in regulation of cytokine expression and inflammation in asthma.

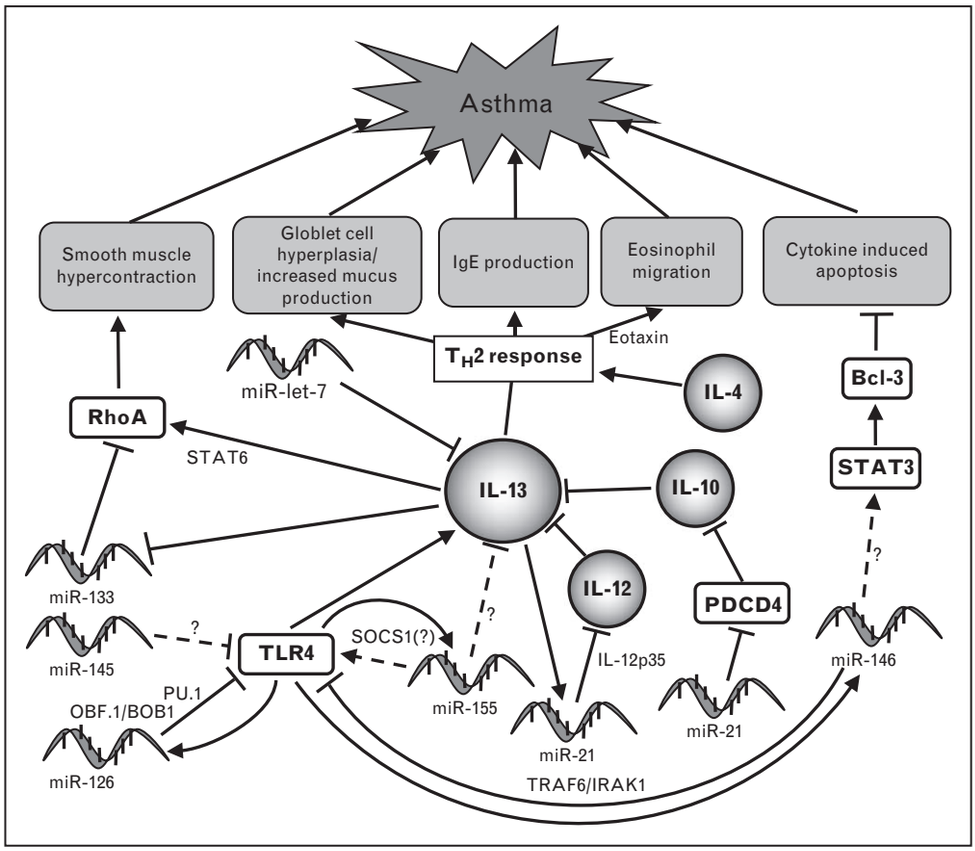

FIGURE 1. Overview of microRNA role in processes leading to asthma. Central to the progression of asthma is the cytokine IL-13 and many microRNA directly or indirectly regulate its production. IL-13 is produced in response to TLR4 signaling that is also regulated by microRNAs. Some of these serve as effectors of TLR4 activation, whereas others function as negative feedback regulation. IgE, immunoglobulin E; IL, interleukin; SOCS, suppressor of cytokine signaling; TRAF6, TNF receptor-associated factor 6; IRAK1, IL-1 receptor-associated kinase 1.

miR-126

Using a mouse house dust mite (HDM) model of asthma, Mattes *et al.* [15] identified changes in miRNA expression in the airways. Three miRNAs were significantly upregulated in the HDM model: miR-16, miR-21, and miR-126. The increase in miR-126 expression did not occur in Toll-like receptor (TLR) $4^{-/-}$ and $MyD88^{-/-}$ mice, suggesting that lipopolysaccharide (LPS) activation is required for the amplification of miR-126 levels. Knockout mice experienced less AHR and less migration of immune cells into the lungs in reaction to HDM, whereas treatment of wild-type mice with antimiR-126 before HDM exposure resulted in less AHR and mucus, and fewer eosinophils/neutrophils being recruited. AntimiR-126 also decreased levels of the T_H2 cytokines IL-5 and IL-13. Subsequent antimiR-126 studies in an ovalbumin (OVA) model showed a similar effect on the T_H2 response [16]. Whether the effects were uniquely due to effects on T_H2 cytokines could not be determined, given that eotaxin expression was also decreased in the airways possibly as a consequence of lower TLR4 expression.

Let-7

The first attempt at identifying the role of the let-7 family in asthma showed that let-7 members, specifically let-7a, had a proinflammatory role [17]. Kumar *et al.* [18] disputed these findings by reporting that increases in IL-13 in allergen-challenged mice were unrelated to let-7a and later went on to conduct work demonstrating that members of the let-7 family are anti-inflammatory and directly inhibit IL-13 expression [19▪]. Intranasal delivery of a let-7 mimic to lungs of mice with allergic inflammation resulted in a decrease in IL-13 levels, resolution of airway inflammation, reduction in AHR, and attenuation of mucus metaplasia and subepithelial fibrosis. Thus, let-7 miRs inhibit IL-13 expression and represent a major regulatory

mechanism for modulating IL-13 secretion in IL-13-producing cell types, and thereby T_H2 inflammation. Therefore, let-7 would, thus, appear to be anti-inflammatory, although an independent repeat of these results would be needed to confirm this conclusion.

miR-21

Lu *et al.* [20] provided the first significant insight into the role of miR-21 in asthma by showing that miR-21 expression could be stimulated by IL-13 and that miR-21 targets IL-12p35 mRNA and lowers IL-12 protein expression. IL-12p35 plays a role in balancing the T_H2/T_H1 skewing of T-helper cells; less IL-12 expression would partially account for the exaggerated T_H2 response seen in asthma. This work was expanded upon by studying the immune response of miR-21$^{-/-}$ mice [21]. Following OVA stimulation, these mice produced greater interferon (IFN) γ and IL-12, whereas there was significantly less IL-4 compared with normal OVA-treated mice. Consequently, these mice had less influx of eosinophils into the lung but experienced greater T_H1 hypersensitivity. Thus, Lu *et al.* have created a valuable narrative of how IL-12 controls the T_H1/T_H2 balance and how miRNA-21 in turn regulates IL-12 and promotes T_H2 skewing.

miR-21's role in IL-12 regulation is not the only proposed activity for this miR. Sheedy *et al.* [22] have proposed that miR-21 can have an anti-inflammatory/negative feedback regulatory role by targeting PDCD4 [22]. PDCD4 is a proinflammatory protein that downregulates IL-10 production. This dual role for miR-21 demonstrates the complex nature of miRNA regulation, and both studies make the case for miR-21 being an immunomodulating molecule.

miR-155

Another miRNA that could have a role in asthma but has yet to be studied in an asthma model is miR-155. miR-155 has a significant proinflammatory role in response to LPS [23▪▪]. The anti-inflammatory action of glucocorticoids has been shown to be dependent on the downregulation of miR-155 activity, and miR-155 expression is nuclear factor kappa B (NFκB)dependent. This would indicate that miR-155 is a proinflammatory effector produced in response to TLR4 signaling. Interestingly, miR-155 promotes T_H1 differentiation, as miR-155$^{-/-}$ mutants have enhanced T_H2 cytokine production. Although miR-155 has been studied in the context of other disease states such as arthritis [24] and endotoxin shock, its well proven role in inflammation warrants further study in the context of asthma, particularly in how it pertains to T_H2 differentiation.

miR-145

Although there is not extensive work done on a role for miR-145 in asthma, Collison *et al.* [25] have provided an insight into its proinflammatory role. miR-145 is highly expressed in the airways, and its expression is upregulated upon HDM stimulation in mice [25]. Levels of miR-145 were decreased by antimiR-145 administration in conjunction with the HDM stimulation. Concomitant decreases in goblet cells hyperplasia, peribronchial eosinophils, and production of IL-5 and IL-13 were also evident. The markedly anti-inflammatory effect of antimiR-145 in this model was as striking as dexamethasone treatment. Although no direct target of miR-145 was demonstrated that could explain these effects, miRNA-145 is likely to be a negative regulator of the proinflammatory machinery, much like miR-126 targeting of BOB.1/OBF.1.

miR-146

Similar to miR-155, miR-146 is an NFκB-dependent miRNA that is transcribed upon LPS stimulation. In contrast, however, miR-146 has been shown to be a negative regulator of the TLR4 pathway, participating as part of a negative feedback loop to prevent an overexuberant response to LPS stimulation. miR-146 is upregulated upon LPS stimulation and based upon luciferase reporter assays it targets TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1), both of which are adaptor proteins involved in TLR signaling [26]. Indeed miR-146 mimics can decrease IL-6 and IL-8 expression by lowering IRAK1 and TRAF6 protein expression [27], with earlier studies showing a similar effect on regulated and normal T cell expressed and secreted (RANTES) and IL-8 via IRAK1 regulation. In the past, miR-146 has also been shown to inhibit cytokine-induced apoptosis in human bronchial epithelial cells, suggesting that it has an important role in asthma in terms of both inflammation and airway remodeling.

Changes in miR-146a and miR-146b have been reported'in asthma. Feng *et al.* [28] observed that miR-146a and miR-146b (and miRNA-181a) are proinflammatory factors in asthma, and that downregulation of miRNA-146a may partially account for the anti-inflammatory effect of dexamethasone. How these findings tally with Tsitsiou *et al.* observations regarding reduced miR-146a in $CD4^+$ and $CD8^+$T cells *in vivo* in patients with severe asthma remains to be elucidated [14].

miR-106a

miR-106a is known to inhibit IL-10 in a post-transcriptional manner. Recently, knockdown of

miR-106a in an established murine allergic airway inflammation was shown to significantly alleviate many features of asthma including AHR, airway inflammation, increased Th2 response, goblet cell metaplasia, and subepithelial fibrosis along with increase in IL-10 levels in lung. The authors claim that this work represents the first in-vivo proof of a miRNA-mediated regulation of IL-10, with a potential to reverse an established asthmatic condition [29].

miR-221

It has been suggested that miR-221 plays a role in the pathogenesis of asthma from studies showing that its expression is increased in asthmatics. Furthermore, antagonism of miR-221 in a mouse OVA model reduces airway inflammation [30]. Mayoral *et al.*, [31] having carried out mouse studies using miR-221 lentiviral overexpression and depletion system, suggest that it has mast cell-specific effects and contributes to mast cell degranulation and cytokine production.

microRNA that impact on bronchial smooth muscle

A characteristic of the asthmatic lung is the altered smooth muscle response, whereby BSM cells become hyperresponsive to acetylcholine stimulation.

Hyperresponsiveness and miR-133

The first suggestion that miR-133 could impact asthma was from a study showing that miR-133 influenced cardiac muscle contraction and the hypothesis that miR-133 could have a similar effect on BSM [32]. This investigation elegantly demonstrated that miR-133 could directly downregulate RhoA protein expression, which is known to be a key protein in hypercontractive smooth muscles seen in asthma. It is also known that IL-13 can stimulate an increase in RhoA protein and promote hypercontractiveness via STAT6. Chiba *et al.* [32] demonstrated that IL-13 can decrease miR-133 expression directly in a STAT6-independent mechanism. Thus, in addition to its direct method of increasing RhoA protein, IL-13 can indirectly increase RhoA by lowering its regulator: miR-133 [33].

Phenotype and hypertrophy and miR-25

Although the hyperresponsiveness of smooth muscle is an obvious concern, the proliferation and change in phenotype of smooth muscle cells also contribute to the heightened response to stimulus and the production of inflammatory mediators. Kuhn *et al.* [34] determined how an inflammatory stimulus would affect miRNA expression and in turn how this change in miRNA levels would affect the smooth muscle cell phenotype. They first stimulated airway smooth muscle (ASM) cells with a mixture of IL-1β, TNF-α, and IFN-γ and identified several lowered miRNAs including miR-25. miRNA databases identified several genes targeted by this miRNA including vitronectin receptor integrin aV, myosin 1B, myocyte enhancer factor 2D, Kruppel-like factor 4 (KLF4), and suppressor of cytokine signaling 5. Stimulated ASM cells given antimiR-25 had a comparative decrease in many cytokines including RANTES, IL-18, and eotaxin, as well as myosin heavy chain (MYH11) that is an essential protein in muscle contraction. Nevertheless, no predicted binding sites exist for miR-25 in the MYH11 transcript, and the explanation that KLF4 is the mediator of these effects may be flawed in that both miR-25 and KLF4 are decreased upon cytokine stimulation. Therefore, although miR-25 could certainly be important in asthma, its role requires further elucidation.

microRNA and genetics

HLA-G had been discovered to be an 'asthma gene', that is, a gene in which single-nucleotide polymorphisms (SNPs) predispose an individual to have asthma. HLA-G is produced by fetal cells at the maternal–fetal boundary and helps to produce maternal tolerance of the fetus by enhancing T_H2 immunity. Maternal asthma is currently the greatest risk factor for asthma in children, and HLA-G would be a logical source of this predisposition as it is highly expressed when and where the fetus is in direct contact with the mother. In essence, it is during this time that the fetus experiences its first immune exposure. Tan *et al.* [35] focused on how SNPs in HLA-G could impact on miRNA targeting. They found that three miRNAs were predicted to target HLA-G: miR-148a, miR-148b, and miR-152. In the MRE for all of these miRNAs, there is a SNP associated with asthma: +3142C/G. Binding of these miRNAs to the HLA-G mRNA occurs with a G/G genotype, but with a C/C genotype, this binding is less stable. Thus, a loss of miRNA regulation because of an alteration of the MRE in the HLA-G gene and an overexpression of HLA-G protein could provide a mechanism for its association with asthma. This is further supported by HLA-G role in T_H2 immunity as asthma is defined by a T_H2 response.

Su *et al.* [36▪] conducted a study on SNPs in miRNA sequences. Of the four SNPs studied (chosen based on previous work), two appeared to be associated with a decreased risk of asthma: *rs2910164G/C*

and *rs2292832C/T*. What is interesting is that the *rs2910164* SNP exists in the premiRNA sequence of miR-146, the negative regulator of the TLR4 pathway that targets TRAF6 and IRAK1. This SNP is located in the stem loop of the premiR, potentially leading to a change or loss of premiR-146 processing or miR-146 function. Jimenez-Morales *et al.* [37] recently provided evidence that *rs2910164* may play a role in the susceptibility to childhood-onset asthma by demonstrating a difference between asthmatic and control women wherein the C allele was significantly associated with protection to asthma.

CONCLUSION

Although knowledge of the role of miRNAs in asthma is still in its infancy, several miRNAs have been identified that could promote or relegate the progression of asthma. PremiR medicines based on let-7 and miR-146 have the potential to correct the overexuberant IL-13 and proinflammatory cytokine expression, evident in the asthmatic lung, whereas those based on miR-133 could potentially repair the hyperresponsive defect in BSM cells. Conversely by inhibiting expression of various miRs (miR-21, miR-106a, miR-126, miR-145, miR-155, and miR-221) using antimiR approaches, aberrant cytokine expression and inflammation may be controlled. As with all miR-based medicines, the efficacy and specificity of the chosen miRs/antimiRs, their ability to be effectively delivered to the airways, and their chronicity once they reach their intended location are all aspects for development.

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Conflicts of interest

The authors declare no conflict of interest.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:

- ■ of special interest
- ■■ of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 000–000).

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