



Role of microRNAs in allergic asthma: present and future

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Purpose of review

MicroRNAs (miRNAs) modulate gene transcription in response to environmental stressors and other stimuli. A role for miRNAs in inflammation and immunity has been demonstrated and further evidence suggests that miRNAs also play a role in allergic asthma.

Recent findings

Studies investigating the differential expression of miRNAs in biological fluids between asthma patients and controls have been published, as have their role in immune cell subsets. Further development of miRNAs in therapy has been addressed. miRNA-146a has been implicated in autoimmunity and allergic inflammation and miRNA-155 in the development of atopy. Targeting of miRNA-1 and miRNA-145 has been used to inhibit lung inflammation in mouse models of asthma. Although these recent findings need to be confirmed, miRNAs may prove to be useful as potential biomarkers of disease. However, their use as therapeutic targets in the lung remains unclear.

Summary

There may be a potential role for using circulating miRNAs as biomarkers of disease status or response to therapy. The use of miRNAs as asthma therapy remains to be determined.

Keywords

allergy, asthma, microRNAs, noncoding RNAs

INTRODUCTION

Asthma is a chronic inflammatory disorder of the airways that is characterized by airway hyperresponsiveness and airway inflammation usually described as eosinophilic, although other inflammatory subtypes are also described, and remodelling of the airways that includes airway smooth muscle (ASM) hyperplasia and subepithelial airway fibrosis. It is commonly associated with an allergic state usually observed with an increased level of circulating IgE with upregulation of T helper (Th)2 cytokines such as interleukin (IL)-4, IL-5 and IL-13. Cross-linking of IgE molecules on IgE receptors of basophils and mast cells leads to the release of chemical mediators such as histamine and leukotrienes that induce smooth muscle contraction and airway oedema.

Noncoding RNAs (ncRNAs) have emerged as novel molecules that may be important in lung disease [1[■]]. ncRNAs can be broadly subclassified into three groups: housekeeping RNAs (ribosomal, transfer and splicesomal), long noncoding (pseudogenes, intronic and intergenic), and the small ncRNAs [piwi-associated RNA, endogenous short

interfering RNA (siRNA) and microRNAs (miRNAs)]. Of these, the miRNAs are the most studied in lung disease, including asthma. miRNAs are small RNAs, consisting of 18–25 nucleotides that are highly conserved across species and act as regulators of both genes and gene networks [2]. They are able to induce messenger RNA (mRNA) degradation and/or inhibit mRNA translation, and as many as 60% of mRNAs may be targets for miRNAs [3]. miRNAs control the signalling pathways in most cell types, have a role in the development and phenotype of immune cells and regulate the inflammatory response in tissues. Thus, miRNAs have been proposed to have a pathophysiological role in

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KEY POINTS

- MicroRNA-146a and microRNA-155 are important in autoimmunity and inflammation and the development of atopy, respectively.
- MicroRNAs can be detected in biological fluids and used to differentiate between disease subtypes in asthma.
- Targeting of microRNA-1 and microRNA-145 can be used to inhibit lung inflammation in mouse models of asthma.
- Modifications to microRNA-1 may allow it to be used as a novel therapeutic in the treatment of human asthma.

asthma. Furthermore, because miRNAs have been found to be extremely stable in serum, they may also be used as biomarkers to differentiate subtypes of asthma. This review will focus on the association between miRNAs and allergic asthma by reviewing the current knowledge and recent publications in the last 12 months ([4[■],5[■],6[■]–15[■]], Table 1).

MICRORNAS AND THE REGULATION OF IMMUNE RESPONSES

An efficient immune system needs to be able to detect and remove harmful pathogens. On the contrary, repeated exposures and a disproportionate response to pathogens, antigens, damaged cells, toxins, allergens or irritants can result in a dysregulated inflammatory response, tissue damage, autoimmunity and allergic disease [16,17,18[■]]. miR-21, miR-146a and miR-155 have been the most frequently studied miRNAs in both innate and adaptive immune responses [19,20[■],21]. Recently, it has been reported that differences between normal and asthmatic patients can be detected via miRNA expression in exhaled breath condensates (EBCs) in a study in which miRNAs were isolated from EBCs with 50–100 molecules of miRNAs reproducibly quantified. Both miR-21 and miR-146a have been proposed as being airway epithelial biomarkers that differentiate between subtypes of asthma severity [22[■]], and these were also reported to be reduced in expression when measured in the exhaled condensates of asthmatic patients [4[■]]. Furthermore, an *in-silico* analysis of disease-specific Th2 cells between healthy and asthmatic individuals identified differential histone modification profiles, with a possible role for a number of miRNAs including miR-21, miR-146a and miR-155. These miRNAs may be useful

biomarkers for asthma susceptibility or may be targeted to provide novel therapies [23[■]].

A crucial role for miR-155 has been suggested in the development of postnatal immune regulation and atopy through the modulation of the growth and differentiation of forkhead box P3 regulatory T cells [5[■]]. The lack of circulating miR-155 found in infants fed with infant-formulated diet compared with those fed on natural milk may explain why infants fed on a diet of formula are more prone to developing atopy and obesity [5[■]]. miR-155 is now reported to be essential in Th2-mediated eosinophilic inflammation in ovalbumin (OVA) mouse models of asthma [6[■]], by targeting the transcription factor PU.1, a negative regulator of T(H)2 cytokine production, and by regulating interferon gamma (IFN- γ) production in human natural killer (NK) cells through the SH2-containing inositol phosphatase 1 [7[■]].

A number of other miRNAs, miR-10a, miR-17-92, miR-181a, miR-182 and miR-29a/b, have been proposed as regulators of the differentiation and plasticity of T cells, through as yet unknown mechanism(s) [8[■]]. miR-150, miR-371-5p, miR-718, miR-940, miR-1181, miR-1207-5p, miR-1915 and miR-3663-3p have been found to be differentially regulated in human primary ASM cells following mitogen stimulation and corticosteroid treatment, and their expression correlates with IL-6 release [9[■]]. miR-375 has been shown to regulate thymic stromal lymphopoietin (TSLP) in eosinophilic esophagitis (EoE) [10[■]].

MICRORNAS AND ASTHMA

Differential expression of miRNAs has been documented in various models of asthma. Wang *et al.* [11[■]] detected differential expression of miR-1229-3p, miR-145-5p, miR-338-3p, miR-3620-3p, miR-4485, miR-4707-3p and miR-636 in serum from asthmatic patients when compared with individuals with chronic obstructive pulmonary disease (COPD). Sinha *et al.* [24[■]] reported 11 miRNAs that were differentially expressed in EBC from asthmatic patients compared with healthy patients. These results were extended by Roff *et al.* [12[■]] who found lower levels of miR-570-3p in serum and EBC from asthmatic patients, and these were inversely correlated with lung function. miR-570-3p has a diverse effect on the expression of cytokines and chemokines in human airway epithelial cells with upregulation of chemokine (C-C motif) ligand (CCL)4, CCL5, tumour necrosis factor and IL-6 in response to tumour necrosis factor and suppression of tumour necrosis factor induced CCL2 and chemokine (C-X-C motif)8 [12[■]]. Finally, a significant difference

Table 1. Recent studies in microRNAs and allergy and asthma

miRNA	Disease	Function	Targets
miR-1248	Asthma	Exhaled condensates showed consistent reduction in miRNA expression in asthmatic patients [4 [■]]	?
let-7a			
miR-155			
miR-21			
miR-328			
miR-133a			
miR-155	Atopy	Involved in postnatal immune regulation and a protective factor for the development of atopy [5 [■]]	IL-2/STAT5
miR-155	Asthma	Required for Th2-mediated eosinophilic inflammation in the OVA-induced mouse model of asthma [6 [■]]	PU.1
miR-155	Asthma	Functions as a positive regulator of IFN- γ production in human NK cells [7 [■]]	SHIP1
miR-10a	Asthma	Differentiation and plasticity of T cells [8 [■]]	?
miR-17-92			
miR-181a			
miR-182			
miR-29a/b			
miR-150	Asthma	Differential miRNA expression in primary ASM cells following mitogen stimulation and treatment with corticosteroid [9 [■]]	CAP1, CDC25A, CFL2, MAPRE1, SLC7A1, SRSF3, TAGLN, TCF19 and VASP
miR-371-5p			
miR-718			
miR-940			
miR-1181			
miR-1207-5p			
miR-1915			
miR-3663-3p			
miR-375	EoE	Positive regulator of TSLP in human primary lung epithelial cells [10 [■]]	?
miR-1229-3p	Asthma	Different expression profile in serum when compared with COPD patients [11 [■]]	IP-10, TNFR, IGF2R, FGF3P3, GGT6 and serine proteases
miR-145-5p			
miR-338-3p			
miR-3620-3p			
miR-4485			
miR-4707-3p			
miR-636			
miR-570-3p	Asthma	Examined in both serum and EBC, and was found to be significantly lower in EBC of asthmatic patients and inversely correlated to lung function [12 [■]]	HuR, STAT1 α , CCL2, IL8, VDR, CCL8, tumour necrosis factor, IL6R, IL6, CCL4 and CCL5
miR-221	Asthma	miR-221 regulates the proliferation and IL-6 release of ASM cells from patients with severe asthma [13 [■]]	p21 ^{WAF1} and p27 ^{kip1}
miR-1	Asthma	Downregulated by VEGF in the lung endothelium, intranasal administration of miR-1 inhibited inflammatory responses to OVA, HDM and IL-13 [14 [■]]	Mpl
miR-150	ACD	Nanovesicles containing miR-150 induced antigen-specific tolerance in ACD model in mice [15 [■]]	?

?, unknown; ACD, allergic contact dermatitis; ASM, airway smooth muscle; CAP, cyclase-associated protein; CCL, chemokine (C-C motif) ligand; CDC25A, cell division cycle 25A; CFL, cofilin; EBC, exhaled breath condensate; EoE, eosinophilic esophagitis; FGF3P3, fibroblast growth factor binding protein 3; GGT6, gamma-glutamyltransferase 6; HDM, house dust mite; HuR, Human antigen R; IGF2R, insulin-like growth factor 2 receptor; MAPRE, microtubule-associated protein, RP/EB family; miRNA, microRNA; Mpl, myeloproliferative leukemia virus oncogene; OVA, ovalbumin; SHIP1, SH2-containing inositol phosphatase 1; SLC7A1, solute carrier family 7 (cationic amino acid transporter, γ -system), STAT, signal transducer and activator of transcription; SRSF3, serine/arginine-rich splicing factor 3; TAGLN, taglin; TCF, transcription factor; Th2, T helper 2; TNFR, tumor necrosis factor receptor; TSLP, thymic stromal lymphopoietin; VASP, vasodilator-stimulated phosphoprotein; VDR, vitamin D (1,25-dihydroxyvitamin D3) receptor; VEGF, vascular endothelial growth factor.

in bronchoalveolar lavage fluid exosomal miRNA profiles was detected in patients with mild asymptomatic asthma compared with healthy individuals [25[¶]].

The expression of miR-21 and miR-126 was found to be upregulated in airway epithelial cells from asthma patients and decreased in patients on inhaled corticosteroids, and IL-13 increased the expression of miR-21 and miR-126. These observations suggest that these miRNAs may be associated with the occurrence of asthma and may act as a biomarker of therapy [26[¶]].

A number of miRNAs, mRNAs and the little known long ncRNAs are differentially expressed in human primary ASM cells following mitogen stimulation and corticosteroid treatment [9[¶]]. We have demonstrated altered expression of miR-221 in ASMs from healthy individuals, nonsevere asthmatic patients and severe asthmatic patients, and that miR-221 regulates the aberrant proliferation and inflammatory state observed in these cells [13[¶]]. Recent results have confirmed the critical role of miR-146a in the regulation of inflammation in ASM cells from asthmatic patients and that miR-146a downregulation of cyclooxygenase-2 (COX-2) and IL-1 β is linked to reduced expression of the RNA binding protein human antigen R (HuR) [27[¶]]. miR-146a and miR-146b expression were inducible in ASM by proinflammatory cytokines, and miR-146a expression was greater in asthmatic cells. Only miR-146a was found to be an endogenous negative regulator in ASM. These results suggest that miR-146 mimics may be an attractive candidate as an anti-inflammatory treatment of asthma. HuR is also a direct target of miR-570-3p. The potential role of miR-155 in the regulation of COX-2 and prostaglandin E2 secretion from human ASM cells and its potential involvement in β 2-adrenoceptor hyperresponsiveness has also been reported [28[¶]]. miRNA-155 expression was positively correlated with COX-2 expression in the asthmatic ASM and could contribute to the elevated expression observed in these cells. Finally, miR-10a, one of the most abundant miRNAs expressed in primary human ASM cells [9[¶]], regulates mitogen-induced ASM proliferation by targeting the phosphoinositide 3-kinase (PI3K) pathway through suppression of the expression of the catalytic subunit of PI3K, PIK3CA [29^{¶¶}].

Asthma and allergic rhinitis commonly coexist and have been considered by some as manifestations of the same disease. Downregulation of miR-18a, miR-126, let-7e, miR-155 and miR-224 and upregulation of miR-498, miR-187, miR-874, miR-143 and miR-886-3p were observed in nasal biopsies of asthmatic patients in comparison with

controls irrespective of the presence of allergic rhinitis. These miRNA expression profiles did not allow differentiation of asthma based on levels of severity [30[¶]]. Because extracellular miRNAs have been reported as dynamic nonvesicular biomarkers of skeletal muscle turnover [31[¶]], miRNAs differentially expressed in ASM may also be good candidates when used as serum-based biomarkers.

Asthma can be exacerbated by many components of environmental pollutants including gaseous components such as ozone or the particulate fractions. Diesel exhaust particles and ambient particulate matter can upregulate TSLP, a cytokine that links innate and Th2-adaptive immune disorders and also the expression of miR-375 in human bronchial epithelial cells [10[¶]]. This upregulation of TSLP in human bronchial epithelial cells involves an interaction of miR-375 with complex regulatory effects on the aryl hydrocarbon mRNA. Ozone is an air pollutant that is associated with numerous adverse health effects that increased the expression levels of 10 miRNAs (miR-132, miR-143, miR-145, miR-199a*, miR-199b-5p, miR-222, miR-223, miR-25, miR-424 and miR-582-5p) in human bronchial airways *in vivo* at a concentration of 0.4 ppm for 2 h [32[¶]]. In addition, environmental dust pollution that can cause dyspnoea has been linked to changes in miRNA expression in serum, bronchoalveolar lavage fluid and urine in military personnel serving in Iraq and Afghanistan [33^{¶¶}]. In particular, miR-371-5p was differentially overexpressed in all three fluids studied [33^{¶¶}]. The expression of several of these miRNAs correlated with changes in forced vital capacity and gas exchange efficiency.

Airway remodelling in chronic allergic asthma is characterized by an increase in ASM mass and sub-epithelial fibrosis. House dust mite (HDM) exposure in sensitized mice and in primary lung epithelial cells is associated with a reduction in the expression of let-7g miRNA in a c-jun N-terminal kinase (JNK)-1 dependent manner and overexpression of let-7g in lung epithelial cells reversed HDM-induced α -smooth muscle actin (SMA) expression. These results indicate that let-7g may be associated with airway remodelling but not with airway hyperresponsiveness and inflammation [34^{¶¶}]. These findings also demonstrate an important requirement for JNK-1 in promoting HDM-induced fibrotic airway remodelling.

Response to drug treatment may also be associated with miRNA expression and genetic background. The mir-152 family (148a, 148b and 152) suppressed major histocompatibility complex, class I, G (HLA-G) expression and a common single nucleotide polymorphism (SNP) in the HLA-G 3'UTR region (rs1063320) modulated miRNA

binding. Individuals who carried the G minor allele of rs1063320 had reduced asthma-related exacerbations compared with noncarriers after treatment with statins [35[¶]].

MIRNA-BASED THERAPEUTICS

Although currently available therapies for allergic asthma are usually effective, when used for treating severe asthma, they are less effective [18[¶],36[¶]]. New approaches are needed for treating severe asthma as well as eventually curing the disease. miRNA-based therapeutics may be used to develop new treatments for preventing exacerbations in patients with severe asthma or reversing the allergic state or treating the viruses that can cause severe exacerbations of asthma. In addition, the remodelling process for which we have no method of treatment may be targeted through the use of miRNA targeting as this process is likely to be the basis of chronic airflow obstruction. Targeting miRNAs in either a cell or tissue-specific manner is an attractive avenue for novel therapeutics as they can be both inhibited [i.e. locked nucleic acids (LNAs)] or overexpressed (i.e. plasmids), and are less likely to result in side-effects when compared with other approaches [37].

At present, there are only two miRNAs in clinical trials unrelated to allergic asthma. A lipid-formulated miR-34 mimic (MRX34) inhibits tumour growth and increases overall survival in mouse models, and is currently in phase I testing in patients with primary or metastatic liver cancer [38[¶]]. Antagomirs of miR-122 are in human clinical trials for hepatitis C [39^{¶¶}]. Targeting miRNAs has been limited to animal models of allergic asthma. Takyar *et al.* [14[¶]] have shown that intranasal administration of miR-1 inhibits inflammatory responses to OVA and HDM in mouse models of asthma. There are common problems when utilizing miRNAs as a therapy that need to be overcome. For example Kakiuchi-Kiyota *et al.* [40[¶]] note that different LNAs seem to affect distinct mechanistic pathways, which suggests that the toxicity issues may vary dramatically with different sequences. However, attaching N,N diethyl 4-(4 nitronaphthalen 1 ylazo)-phenylamine (ZEN) to both ends of an anti-miR considerably enhances its binding affinity, results in more potent miRNA inhibition and has low toxicity in cell culture [41[¶]]. Furthermore, even when miRNAs are efficiently delivered into target tissues at therapeutically meaningful doses, another limiting factor is determining how to reach a sufficient dose within the cells in order to achieve efficient miRNA inhibition. The efficiency of the internalization and subsequent release of miRNAs is extremely low.

An imaging-based analytical method using fluorescence and electron microscopy to track the intracellular transport and release of siRNAs demonstrated that lipid nanoparticles enter cells through clathrin-mediated endocytosis and macropinocytosis, and that less than 2% of the miRNAs escaped from the endosomes [42^{¶¶}]. However, it may be possible to use nanovesicles to overcome this problem [43[¶]]. Nanovesicles containing miR-150 are capable of entering effector T cells, suppressing allergic contact dermatitis and promoting antigen-specific tolerance in mice [15[¶]]. However, human application remains unknown.

Finally, the mechanism of action of miRNAs may not be as simple as first thought. Evidence suggests that miRNAs may have a range of functions including regulation of transcription through epigenetic mechanisms, modulation of translation by acting as decoys and by their effects upon long ncRNAs [9[¶],44[¶]]. Therefore, using miRNAs as a potential therapy may produce numerous 'off-target effects' that we are, as yet, unaware of in primary human cells. However, improved techniques for targeting miRNAs, and increasing knowledge of their biological function in primary cells could lead to the production of specific gene expression modulators for allergic asthma in the future.

CONCLUSION

Recent studies indicate that miRNAs may be important in controlling the immune response, both on a cell-based and system-wide manner. Whether a single miRNA or a collection of them acting coordinately is most important is unclear. However, miRNAs appear to be important in all areas of asthma including diagnosis (as possible biomarkers), disease severity and treatment response. There is a potential for miRNA research to uncover as yet unknown mechanisms in the pathogenesis of allergic asthma as well as being developed into novel therapies.

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Conflicts of interest

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