MicroRNAs and Asthma Regulation

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ABSTRACT

MicroRNAs participate in the regulation of asthma, the goal of this study is to summarize recent researches on the roles of microRNAs in the pathogenesis of asthma.

A review of the English medical literatures was conducted by searching PubMed for studies concerning asthma and microRNAs.

The results of the present study indicate that microRNAs play important roles in regulating asthma immune responses. MicroRNAs not only participate in determining DCs phenotype and then naive T lymphocyte differentiation, but also participate in the regulation of airway inflammation and airway remodeling in asthma. Furthermore, microRNAs are also shown to be targets for asthma therapy in the future.

Keywords: Airway Remodeling; Asthma; Inflammation; MicroRNAs; Therapeutics

INTRODUCTION

Asthma is a worldwide problem, the prevalence of asthma ranged from 1% to 18% of the population in different countries, with an estimated 300 million individuals suffering from asthma around the world. Annual worldwide deaths from asthma have been estimated about 250,000 and mortality does not appear to correlate well with prevalence. Although more and more progressions have been made about the pathogenesis of asthma in recent years, the increasing incidence and mortality validate further researches. As an airway inflammatory disease closely correlates with immune regulation, more and more evidences suggest that asthma is intensively regulated by a variety of microRNAs (miRNAs). Understanding the roles of miRNAs in asthma pathogenesis may also aid to explore new therapeutic targets.

MiRNAs Biology

MiRNAs are approximately 19- to 25-nucleotide single-stranded, noncoding RNAs that exist in both animals and plants and regulate gene/protein expression through direct complementarity between their 5' region and the 3' untranslated region of target mRNAs. Direct binding of miRNAs to a target mRNA may result in either mRNA degradation or inhibition of protein translation.[1] More than 2000 miRNAs have been discovered in humans. Each miRNA regulates as many as 200 predicted target genes in vertebrates.[2] MiRNAs are proposed to regulate up to one-third of all human genes at the post-transcriptional level by degrading or repressing target messenger RNA.[3] And it has been

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speculated that miRNAs may be associated with the regulation of almost every aspect of cell physiology.[4] A variety of biological processes including immune cell lineage commitment, differentiation, maturation, and maintenance of immune homeostasis and normal function have been recognized to be regulated by miRNAs.

MiRNAs Directing Dendritic Cells (DCs) Differentiation

DCs have a pivotal role in the regulation of naïve T cell differentiation. Lung DCs can also prevent harmful immune responses to innocuous inhaled antigens via induction of regulatory T cells or T helper (Th)1 cells.[5] Meanwhile, the function and immunity of DCs are closely regulated by miRNAs. Zhang et al demonstrated that microRNA let-7i was upregulated during lipopolysacchride (LPS) -induced DC maturation, while downregulation of let-7i significantly impeded DC maturation as evidenced by reduced CD80 and CD86 expression.[6] DC maturation and activation by TLR3, TLR4, and TLR9 agonists upregulate miR-148/152 expression, meanwhile downregulate CaMKIIα expression. By targeting CaMKIIα, miR-148 and miR-152 negatively regulate the innate response and Ag-presenting capacity of DCs, confirming that miR-148 and miR-152 contribute to immune homeostasis and immune regulation.[7] By silencing the transcription factor c-Fos, miR155 is required for efficient DC maturation and is critical for the ability of DCs to promote antigen-specific T-cell activation.[8]

MiRNAs Directing T Lymphocyte Differentiation

T lymphocytes are central effector cells in asthma regulation and their differentiation are closely modulated by miRNAs. In patients with atopic dermatitis, miR-155 was significantly overexpressed and contributed to skin inflammation by suppressing CTLA-4 expression, and thus promoting Th cell proliferative responses.[9] Mice deficient in miR-155 did not develop collagen-induced arthritis for lacking of pathogenic autoreactive B and T cells, since anticollagen antibodies and the expression levels of antigen-specific T cells were strongly reduced in miR-155(-/-) mice.[10] Higher level of miR-146a is expressed in human memory T cells compared to naïve T cells and is induced in human primary T lymphocytes upon T-cell receptor stimulation. Furthermore, miR-146a enforced expression impairs both activator protein 1

activity and interleukin (IL)-2 production induced by TCR engagement, thus suggesting a role of miR-146a in the modulation of adaptive immunity.[11] Ablation of miR-21 in mice led to increased levels of the Th1 cytokine IFN-γ but reduced lung eosinophilia after allergen challenge. Furthermore, the authors proved that miR-21 deficiency made DCs produce more IL-12 after LPS stimulation and OVA-challenged CD4(+) T lymphocytes to produce increased IFN-γ and decreased IL-4, identifying miR-21 as a major regulator of Th1 versus Th2 differentiation.[12]

Roles of miRNAs in Airway Inflammation

Asthma is a chronic airway inflammatory disease with genetic predisposition, involving multiple cells, cytokines, mediators and signals and closely related to immune regulation. More and more evidences demonstrated that miRNAs participate in airway inflammatory regulation in asthma.

Garbacki N demonstrated that in the mice models sensitized and challenged with ovalbumin mimicking acute, intermediate and chronic human asthma, 58, 66 and 75 miRNAs were found to be significantly modulated, respectively. Several signaling pathways such as matrix metalloproteinases, inflammatory responses and transforming growth factor β (TGF-β) signaling, and biological processes, including apoptosis and inflammation were identified to be linked with miRNA regulation.[13] Bronchial epithelial miRNA expression patterns in patients with asthma were demonstrated to be quite different from that of control subjects, with 217 miRNAs differentially expressed in steroid-naive subjects and 200 in steroid-using subjects with asthma, suggesting that alterations of airway epithelial cell miRNA levels are a common feature of asthma.[14] Jardim MJ et al also demonstrated that the expression of 66 miRNAs in bronchial epithelial cells were significantly different between mild asthma group and healthy control group, meanwhile, the expression of IL-8, Cox2, and tumor necrosis factor- α (TNF-α) were up-regulated in asthmatic cells, whereas the expression of IL-6 was lower compared with that in healthy control subjects.[15]

Upregulation of miR-221 and miR-485-3p was proved to downregulate Spred-2 level in murine asthma models,[16] which may regulate the pathogenesis of asthma. MiR-1248 was proved to serve as a positive regulator to increase IL-5 expression,[17] while IL-5 is a key Th2 cytokine and play central roles in

eosinophil recruitment, maturation and survival. Sensitized and chronically challenged mice expressed more miR-126 than naïve mice in the airway wall tissue, inhibition of miR-126 significantly reduced recruitment of intraepithelial eosinophils.[18] In severe asthmatic patients, circulating CD8+ T cells were activated but not CD4+ T cells, and this change is correlated with the downregulation of miR-146a/b and miR-28-5p, as well as changes in the expression of multiple species of lncRNA that might regulate CD8+ T-cell function.[19]

Studies also show that some of the miRNA expression in asthma with a dynamic way. Feng MJ et al demonstrated that the expression levels of microRNA-181a, -150, -146a and -146b were higher in the asthma group compared with the control group in the beginning of the disease, and after 5 days dropped to control group levels because there was no new airway challenge. Moreover, miR-181a, miR-146a and miR-146b had a positive linear correlation with

the number of inflammatory cells, and the miR-146a expression was down-regulated by treatment with dexamethasone.[20] The expression level of miR-192 in pre-challenged asthmatic patients was lower than that in healthy control subjects, and post-challenged asthmatic patients was significantly lower than that in pre-challenged patients, indicating that change in miR-192 levels may be implicated in asthma pathogenesis.[21]

Roles of miRNAs in Airway Remodeling

Airway remodeling is another main character of asthma and is closely correlated with recurrent asthma episodes. MiRNAs has also been demonstrated to be involved in airway remodeling in asthmatic mice model. Chiba Y et al demonstrated that RhoA expression is negatively regulated by miR-133a in bronchial smooth muscle cells (BSMCs) and that the miR-133a downregulation caused an upregulation of RhoA, resulting in an augmentation of BSMCs

Table 1. Roles of miRNAs in airway inflammation and remodeling

miRNA	Roles in asthma
miRNA-221	Mast cell adhesion, migration, cytokine production and degranulation; Downregulate Spred-2
miRNA-485-3p	Downregulate Spred-2
MiR-1248	Increase IL-5 expression
miR-126	Recruit eosinophil
miR-146a/b, miR-28-5p	Downregulate miR-146a/b and miR-28-5p expression and activate CD8+T cells
miRNA-181a, miRNA-146a, miRNA-146b	Increase the number of inflammatory cells in the airway
miR-133a	Negatively regulate RhoA expression and reduce BSM contraction
miR-26a	Induce human airway smooth muscle cells (HASMCs) hypertrophy
miR-10a	Reduce mitogen-induced HASMCs proliferation

Table 2. Targeting miRNAs in asthma therapy

miRNA	miRNAs Targeting in asthma therapy
miR-155	MiR-155 Knockout diminishes airway inflammation, mucus hypersecretion, Th2 cell numbers and airway Th2 cytokine levels
miR-145	MiR-145 blockage inhibits eosinophilic inflammation, mucus hypersecretion, Th2 cytokine production, and airway hyperresponsiveness
miR-106a	MiR-106a knockdown alleviates airway hyperresponsiveness, inflammation, increased Th2 response, goblet cell metaplasia, and subepithelial fibrosis
miRNA-126	MiRNA-126 blockade suppresses airway inflammation, diminishes Th2 responses, inflammation, airways hyperresponsiveness, eosinophil recruitment, and mucus hypersecretion
miRNA-221	MiRNA-221 blockade suppresses airway inflammation
let-7	Let-7 miRNA inhibition decreases IL-13 levels, resolutes airway inflammation, reduces airway hyperresponsiveness, and attenuates mucus metaplasia and subepithelial fibrosis
miR-133a	Negatively regulates RhoA expression, reduces BSMCs contraction

contraction.[22] MiR-26a is a hypertrophic gene, enforced expression of miR-26a induced human airway smooth muscle cell hypertrophy by attenuating the endogenous glycogen synthase kinase-3β protein expression, and miR-26 knockdown reversed this effect.[23] In another study, miR-10a was proved to be potent regulator for human airway smooth muscle (HASM) cells proliferation, overexpression of miR-10a reduced mitogen-induced HASM proliferation by ~50%, whereas inhibition of miR-10a increased HASM proliferation by -40%, in which 52 genes were down-regulated by miR-10a, including the catalytic subunit α of PI3K.[24] The roles of miRNAs in airway inflammation and remodeling in asthma were summarized in table 1.

MiRNAs and Asthma Therapeutics

MiRNAs are not only regulators in asthma pathogenesis, but are also targets for asthma theraputics which is shown in table 2. Malmhäll C et al demonstrated that MiR-155 knockout resulted in diminished eosinophilic inflammation and mucus hypersecretion in the lungs of asthmatic mice compared with wild type control animals and the phenomenon was accompanied by a reduction in Th2 cell numbers and airway Th2 cytokine levels and complete abrogation of allergen-induced airway eotaxin-2/CCL24 and periostin levels in miR-155 knock out mice.[25] Another study demonstrated that blocking of miR-145 inhibited eosinophilic inflammation, mucus hypersecretion, Th2 cytokine production, and airway hyperresponsiveness in house dust mite sensitized and challenged mice, and the anti-inflammatory effects of miR-145 antagonism were comparable to that of steroid treatment.[26]

According to the fact that miR-106a inhibits IL-10, an anti-inflammatory cytokine in a post-transcriptional manner, Sharma A et al demonstrated that knockdown of mmu-miR-106a in an established allergic airway inflammation significantly alleviated most of the features of asthma such as airway hyperresponsiveness, airway inflammation, increased Th2 response, goblet cell metaplasia, and subepithelial fibrosis along with increase in IL-10 levels in lung, representing mmu-miR-106a as a potential target for reversing an established asthmatic condition.[27] Selective blockade of miRNA-126 suppressed house dust mite induced allergic airway inflammation,

leading to diminished Th2 responses, inflammation, airways hyperresponsiveness, eosinophil recruitment and mucus hypersecretion.[28] MiR-221 blockade also resulted in a reduction of airway inflammation in the OVA-induced murine asthma model.[29]

Soibam B et al confirmed that in vitro IL-13 is regulated by mmu-let-7a, meanwhile, inhibition of let-7 miRNAs in vivo using a locked nucleic acid significantly inhibited the production of allergic cytokines and the disease phenotype.[30] Kumar M et al demonstrated that in an IL-13-dependent murine model of allergic airway inflammation, airway inflammation was associated with a reduction in most of the members of the let-7 family. Exogenous administration of let-7 mimic to lungs of mice with allergic inflammation resulted in a decrease in IL-13 levels, resolution of airway inflammation, reduction in airway hyperresponsiveness, and attenuation of mucus metaplasia and subepithelial fibrosis.[31] MiRNAs also involved in bronchial smooth muscle contraction by regulating RhoA expression, Chiba Y et al demonstrated that miR-133a negatively regulated RhoA expression in bronchial smooth muscle of challenged mice, inhibiting the function of endogenous miR-133a by its antagomir upregulate RhoA expression.[32] Thus targeting specific miRNAs may be potential theraputics not only for airway inflammation, but also for airway remodeling.

CONCLUSION

MiRNAs are small, endogenous RNAs, approximately 20-25 nucleotides long which regulate gene expression at a post-transcriptional level. MiRNAs are involved in diverse biological processes, including development, stress response, cancer, and cardiac hypertrophy, implicating them in normal and pathological processes. Asthma is chronic airway inflammatory disease involving a large number of cytokines, inflammatory cells and inflammatory mediators. Allergic airway inflammation may be particularly sensitive to miRNA regulation because it is characterized by marked changes in gene and protein expression in the lung. Importantly, miRNAs direct the differentiation of DCs, which play critical roles in innate and adoptive immunity. As recognition deepened about their roles in pathophysiology of asthma, new intervention method and theraputics will

be discovered in the future.

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