

Chapter 17

microRNA and Allergy

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Abstract Allergy is a common hypersensitivity disorder of the immune system, which, along with other factors, is also subjected to regulation by microRNAs. The most common allergic diseases are allergic rhinitis, asthma, atopic dermatitis, and food allergy, which all are multifactorial and very heterogeneous conditions, highlighting the need for more individualized treatment techniques. More particular key questions in relation to allergic diseases are how microRNAs influence the differentiation, polarization, plasticity and functions of T helper and other immune cells, as well as the development of immune tolerance. In addition, microRNAs can affect allergic inflammation and tissue remodeling through their functions in epithelial and other tissue cells. Among immune system-related microRNAs, miR-21, miR-146a, and miR-155 are the most intensively studied and have convincingly been demonstrated to regulate immune responses and tissue inflammation in allergic diseases. Further characterization of microRNA functions is important, as similar to other conditions, the modulation of microRNA expression could potentially be used for therapeutic purposes in allergic diseases in the future. In addition, miRNAs could be implemented as biomarkers for endotyping complex allergic conditions.

Keywords Noncoding RNA • Allergy • Asthma • Rhinitis • Dermatitis • Food allergy

Introduction

Immune responses in health and disease are controlled by the dynamic and multifactorial regulation of gene expression in each cell type involved. A healthy immune system detects and eliminates pathogens very efficiently. However, excessive and inappropriate responses to self-antigens, pathogens, tissue damage, toxins, irritants, and allergens may cause unreasonably strong inflammation, leading to autoimmune

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or allergic diseases [1–3]. The most common allergic diseases are allergic rhinitis, asthma, and atopic dermatitis (AD), which can occur either alone or in combination [4, 5]. A predisposition toward developing certain allergic reactions is also known as atopy. Immunologically, atopy is characterized by the expansion of the Th2 cell subset and IgE-expressing memory/effector B cells and plasma cells producing IgE antibodies specific for common environmental allergens. Because allergic diseases mainly affect tissues that interface with the environment, the role of epithelial cell responses is important. In addition, various nonpathogenic and pathogenic microbes and viruses affecting the same tissues enhance the complexity of the molecular events that lead to or protect against the development of allergic inflammation [6]. For instance, human rhinoviruses (HRVs), which typically only cause the common cold, may also cause long-lasting infection and acute asthma exacerbations in asthmatics [7, 8].

Atopic diseases possess a strong genetic component, which is well characterized for AD. Numerous genetic studies of AD have identified candidate genes linked either to the epidermal barrier, such as filaggrin (*FLG*) [9], or to immune processes, such as *IL4/IL13* receptor [10, 11], *IL13* [12] and the α -chain of the high affinity receptor for IgE, *FCER1A* [13–15]. Distinct functional *IL13* variants have been associated with asthma susceptibility as well [16]. A recent meta-analysis of genome-wide association studies confirms previous findings regarding the involvement of both the epidermal barrier function and immune dysregulation in AD pathogenesis [17].

Many studies have addressed the domination of Th2 cells in atopic diseases. As one possibility, high IFN- γ -secreting Th1 cells in peripheral blood selectively undergo activation-induced cell death and skew the immune response toward surviving Th2 cells in AD patients [18]. Albeit Th2 cells are dominant in the periphery of atopic patients, Th1 type responses, such as an enhanced level of IFN- γ , in fact appear to have a stronger influence on epithelial cells in the chronic phase of inflammation [14, 19–23].

Allergic diseases are very heterogeneous [24]. For example, both asthma and AD were initially divided into either extrinsic or allergic or intrinsic, i.e., nonatopic, disease. Recently, it has become clear that asthma and AD are far more complex diseases composed of a number of variants with different underlying pathophysiologies. Accordingly, it has been proposed that asthma can be divided into distinct disease entities called asthma endotypes, which link both the clinical characteristics and underlying biology [25]. However, the currently used diagnostic techniques and biomarkers do not allow for precise asthma endotyping.

The existing therapies for atopic diseases aim to control the symptoms. Despite extensive research, there is no established method to prevent asthma and AD, and the severe forms of these diseases are not well controlled [3]. Therefore, further characterization of the molecular mechanisms contributing to the pathogenesis of allergic diseases is important and ultimately could help in the development of more personalized therapeutic approaches for these diseases.

Although studies on the functions of microRNAs in allergic diseases have emerged during the last 5 years, the roles of miRNAs in the regulation and pathogenesis of these diseases remain largely uncharacterized [26, 27]. Here, we provide an overview of the microRNAs that influence the development of allergic

diseases or impact the strength of inflammation in affected tissues. In addition, we discuss the potential of microRNAs to serve as biomarkers and targets of gene therapy in allergic diseases.

Three Guardians of the Immune System

Three microRNAs, miR-21, miR-146a, and miR-155, are most likely the most intensively studied immune system-related miRNAs and have also been shown to play important roles in the development of allergic diseases and inflammation in several studies. The functions and best characterized targets of miR-21, miR-146a, and miR-155 are presented in Fig. 17.1.

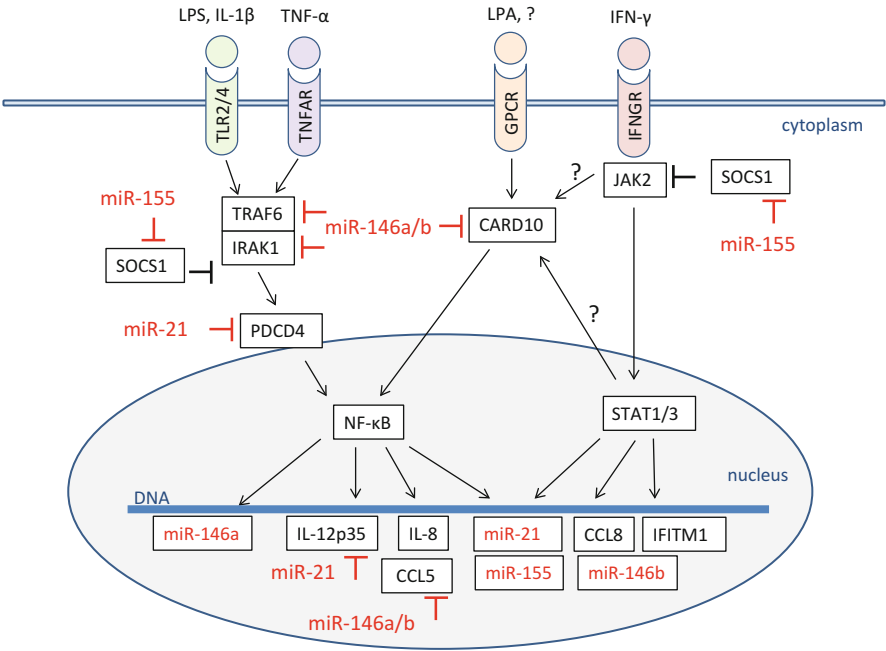


Fig. 17.1 The expression regulation and functions of miR-146a/b, miR-21, and miR-155. The expression of miR-146a and miR-146b can be stimulated by activation of the NF-κB and JAK-STAT signaling, respectively, while the expression of miR-21 and miR-155 can be upregulated by both these pathways. miR-21 inhibits inflammatory responses by targeting tumor suppressors and a pro-inflammatory protein (PDCD4) that promotes the activation of [30] and contributes to Th2 polarization by targeting IL-12p35 [53]. miR-146a/b inhibit most probably the same set of target genes. miR-146a/b directly downregulate IL-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6) [31], which can be activated by TLR ligands, TNF-α and IL-1β, as well as caspase recruitment domain-containing protein 10 (CARD10), which can be activated through G protein-coupled receptors (GPCRs) by lysophosphatidic acid (LPA) or other unidentified factors, as well as by IFN-γ in unknown manner [33, 34]. miR-155 promotes type I IFN signaling and contributes to the activation of NF-κB pathway by targeting suppressor of cytokine signaling 1, SOCS1 [41]

miR-21 is mainly studied in connection with cancer, but it has been shown to regulate several immunological processes as well [28]. miR-21 is highly expressed in many cell types and can be further upregulated by signal transducers and activators of transcription 3 (STAT3) and nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B) [29]. In mouse macrophages, miR-21 has been shown to inhibit inflammatory responses by targeting tumor suppressors and a pro-inflammatory protein (PDCD4) that promotes the activation of NF- κ B and controls the production of immune-suppressor cytokine IL-10 [30].

Similarly, miR-146a is an anti-inflammatory miRNA known to inhibit the activation of the NF- κ B pathway. miR-146a directly downregulates IL-1 receptor-associated kinase 1 (IRAK1), TNF receptor-associated factor 6 (TRAF6) [31], Relb, a member of the non-canonical NF- κ B/Rel family [32], and caspase recruitment domain-containing protein 10 (CARD10, also known as CARD recruited membrane-associated protein 3, CARMA3) [33, 34]. miR-146a^{-/-} mice develop autoimmunity at approximately 6 months of age, which is caused by the overactivation of STAT1 in Treg cells, leading to increased IFN- γ production and an inability to suppress immune responses mediated by Th1 cells [35–37]. Recent studies demonstrate that in addition to miR-146a, miR-146b is expressed and plays important roles in several different cell types [38, 39]. Mature miR-146a and miR-146b differ from each other by two nucleotides, and they target the same or a very similar set of genes because of their identical seed sequence [31, 40]. Although the function of miR-146a has been studied intensively in immune system and in numerous diseases, the function and regulation of miR-146b is less well described. In monocytes, miR-146b is upregulated via an IL-10-mediated STAT3-dependent loop upon LPS stimulation and has been shown to directly target TLR4 [38]. In normal breast epithelial cells, the expression of miR-146b is induced by IL-6 and IFN- γ through activation of the STAT3 and STAT1 pathways, respectively [39].

In contrast to miR-21 and miR-146a/b, miR-155 acts primarily as a pro-inflammatory factor. miR-155 expression is induced upon vesicular stomatitis virus infection in macrophages, which promotes type I IFN signaling by targeting suppressor of cytokine signaling 1, SOCS1 [41]. The suppression of SH2 domain-containing inositol-5-phosphatase (SHIP1) by miR-155 results in activation of Akt kinase and the upregulation of IFN response genes during the cellular response to LPS [42]. In addition, miR-155 directly represses BCL6, a transcription factor that attenuates NF- κ B signaling, and this promotes atherosclerosis in mice [43]. miR-155 also functions in the development and activation of adaptive immune cells, including effector T cell subsets [44]. In addition, miR-155-deficient mice are defective in production of Th1 and Th17 cells and, therefore, are highly resistant to experimental autoimmune encephalomyelitis [45].

Numerous other miRNAs control innate and adaptive immune responses. For example, miR-301a, miR-9, miR-147b, and miR-125a have been shown to influence the activation of the NF- κ B pathway [46], and miR-10a, miR-17-92, miR-181a, miR-182, and miR-29a/b impact the differentiation and plasticity of T cells [47]. In addition, miR-17-92, miR-34a, miR-150, miR-181b, miR-125b, and miR-217 affect the development and functions of B cells [48]. Within the context of

allergic diseases, it is particularly interesting to study how miRNAs influence Th2 polarization, Treg cell suppression capacity, and Ig class switching in B cells.

miRNAs in the Regulation of Effector T Cell Responses in Allergic Diseases

Naïve T cells can differentiate into Th1, Th2, or Th17 cells, all of which can promote different types of inflammatory responses. Moreover, the established subsets of effector T cells are rather heterogeneous cell populations, which often express only one or two of the lineage specific cytokines and under certain conditions can coproduce different amounts of the cytokines characteristic of other lineages, thus leading to different functional outcomes [49–51]. Allergic diseases are characterized by the expansion of Th2 cells and IgE-expressing memory B cells and plasma cells that produce IgE antibodies specific to common environmental allergens [3].

The characteristic cytokines for Th2 cells are IL-5, IL-4, and IL-13; however, the Th1/Th2 balance is also influenced by Th1 cytokines, such as IFN- γ and IL-12, and by innate immune responses of antigen presenting cells (APCs), dendritic cells (DCs), and tissue macrophages, and responses of epithelial cells [52]. For example, epithelial cells produce thymic stromal lymphopoietin (TSLP) in response to various stimuli and that contributes to activation of Th2 type responses. Coordinately, all miRNAs regulating the balance of Th1/Th2 type cytokines and innate immune responses in APCs and epithelial cells influence the pathogenesis of allergic diseases. For example, in a mouse model of ovalbumin (OVA)-induced allergic airway inflammation, miR-21 was the most highly induced miRNA in transgenic mice overexpressing IL-13 in the lungs. Because miR-21 directly targets IL-12p35, it was suggested that miR-21 might contribute to Th2 polarization [53]. Consistent with this, OVA-challenged miR-21-deficient mice exhibited reduced lung eosinophilia and increased levels of IFN- γ when compared to wild-type mice [54]. miRNA-21 also has been shown to inhibit innate immune responses in TLR2 agonist-induced lung inflammation in mice [55] (Table 17.1).

In line with research in mouse models of airway inflammation, a correlation between reduced levels of miR-21 in mononuclear leukocytes from human umbilical cord blood samples and elevated antenatal IgE production in allergic rhinitis patients have been observed [56]. Additionally, the upregulation of miR-21 was observed in esophageal biopsies from patients with eosinophilic esophagitis (EoE) [57] (Table 17.2). EoE is characterized by very high IL-13 overproduction, very strong Th2 type inflammation and eosinophilia in the esophagus and can therefore be regarded as a model for Th2-type diseases [58]. miR-21 is also upregulated in lesional skin from subjects with allergic responses to diphenylcyclopropenone (DPCP) in humans and in a dinitrofluorobenzene (DNFB)-induced contact dermatitis mouse model, conditions in which Th17 and Th1 responses are dominant [59].

Ubiquitously expressed miRNA let-7 has been shown to influence the expression of IL-13 in lung epithelial cell-line A549. Indeed, intranasal administration of let-7

Table 17.1 The functions of miRNAs in asthma

miRNA	Function	Targets
miR-126	Inhibition reduced allergic inflammation in an HDM-induced mouse model of asthma [78] and eosinophilia in a mouse model of chronic airway inflammation [79]	?
miR-221	Upregulated in OVA-induced mouse models of asthma; silencing reduced allergic inflammation [82]	Spred-2
miR-145	Silencing reduced allergic inflammation in an HDM-induced mouse model of asthma [80]	?
miR-106a	Silencing reduced allergic inflammation in an HDM-induced mouse model of asthma [81]	IL-10
let-7a	Overexpression reduced allergic inflammation in an OVA-induced mouse model of asthma [60] Intravenous administration of LNA inhibitors impeded AHR in a mouse model of asthma [61]	IL-13
miR-21	Overexpressed in lung-specific IL-13-expressing transgenic mice with induced allergic airway inflammation [53] Overexpression reduced TLR2 agonist-induced lung inflammation in mice [55] Increased Th1 cytokines and reduced eosinophilia in an OVA-induced mouse model of asthma in miR-21-deficient mice [54]	IL-12p35
miR-146a	Overexpressed in splenic CD4+ T lymphocytes [76] Reduced expression in CD8+ and CD4+ cells in patients under oral corticosteroid treatment [119] Reduced cytokine induced apoptosis in human bronchial epithelial cells [94] Increased in human asthmatic SMCs [93]	IRAK1, TRAF6, CARD10, indirectly inhibits COX-2 and IL-1 β
miR-375	A positive regulator of TSLP in human primary lung epithelial cells [65]	?
miR-133a	Downregulated in mouse bronchial smooth muscle cells; augments airway contraction and hyperresponsiveness [89]	RhoA
miR-1	Downregulated by VEGF in lung endothelium; intranasal administration inhibited inflammatory responses to OVA, HDM, and IL-13 overexpression Mpl [88]	Mpl
miR-155	Required for Th2-mediated eosinophilic inflammation in an OVA-induced mouse model of asthma [77] miR-155-deficient mice spontaneously developed asthma-like inflammation in the lung and showed increased levels of IL-4 and IL-5 in T cells [44] Increased in asthmatic SMCs upon stimulation [95]	PU.1 c-Maf
miR-17~92 cluster	Downregulated in pulmonary hypertension, SMCs; specific depletion augments hypoxia-induced pulmonary hypertension in mice [91]	PDLIM5
miR-708	Inhibits airway SMC contractility [92]	CD38

Table 17.2 The functions of miRNAs in allergic diseases other than asthma

miRNA	Disease	Function	Targets
miR-21	Allergic rhinitis, ACD, EoE	Antenatal IgE production and development of allergic rhinitis [56]	IL-12p35
		Upregulated in contact dermatitis lesional skin [59]	
		Upregulated in EoE esophageal tissue [57]	
		Inhibits Th1-associated delayed-type hypersensitivity cutaneous responses in miR-21-deficient mice [54]	
miR-146a	AD	Upregulated in the skin and keratinocytes of AD patients; alleviates chronic inflammation in a mouse model of AD [34]	IRAK1, CARD10, CCL5
miR-375	EoE	Expression is modulated by IL-13 [58]	?
		Downregulated in EoE esophageal tissue [57]	
miR-155	AD	Influences development of AD by downregulating CTLA-4 negative regulator of T cell activation [102]	CTLA-4
miR-125b	CRS with nasal polyps	Upregulated in CRS with nasal polyps; influences IFN- α/β production [103]	4E-BP1
miR-150	ACD	Nanovesicles containing miR-150 induce antigen-specific tolerance in a mouse ACD model [110]	

family miRNAs led to reduced IL-13 and AHR as well as the resolution of allergic inflammation in an OVA-induced mouse model of asthma [60]. However, in another study, instead of exacerbating the disease, let-7 miRNA inhibition using intravenously administered locked nucleic acid (LNA) inhibitors hampered AHR in a mouse model of asthma [61].

miR-155 can impact the Th1/Th2 balance and the development of allergic disease through its function in macrophages, in which miR-155 targets IL-13RA, SOCS1, and SHIP1. When miR-155 levels are reduced in differentiating macrophages, STAT6 becomes more activated, leading to the establishment of an alternative M2 pro-Th2 phenotype [62]. A very interesting recent study using miR-155(−/−) or miR-146a(−/−) T cells identified that T cell-intrinsic miR-155 is required for type-2 immunity and that T cell-intrinsic miR-146a is required to prevent overt Th1/Th17 skewing [63]. Thus, if there is no miR-155 in macrophages, they tend to stimulate Th2 responses; however, if there is no miR-155 in T cells, there is less differentiation toward Th2 type direction suggesting that tightly regulated miR-155 in each participating cell type is needed for proper functioning of the immune system.

miR-375 is yet another miRNA that contributes to the differentiation of Th2 cells. miR-375 has been shown to be induced by IL-13 in gut mucosal epithelial cells and it positively regulates the expression of key Th2 cytokines, TSLP, and resistin-like beta (RELM β) [64]. In line with these results, diesel exhaust particles, which are known to aggravate asthma, induce TSLP mRNA and miR-375 in primary human

bronchial epithelial cells [65]. In contrast to gut mucosal epithelial cells, miR-375 is downregulated by IL-13 in human esophageal squamous and bronchial columnar epithelial cells, suggesting that the regulation of miR-375 is complicated and cell type specific [57].

In addition to the Th2 cells, recent studies propose an important role for Th22 cells in the chronic phase of the allergic tissue inflammation. Influence of miRNAs on development of Th22 cells, as well as on production of IL-22 has not been studied. This will be interesting because IL-22 might be also produced by Th17 cells; however, when IL-17 augments inflammation, IL-22 is known to play a tissue-protective role [66].

miRNAs and Regulatory T and B Cell Responses

T cells with immunoregulatory capacity can be classified into two groups: thymus-derived naturally occurring CD4⁺CD25⁺FOXP3⁺ Treg cells and inducible Treg cells (iTreg) generated outside the thymus after antigenic stimulation. Importantly, the balance between allergen-specific Treg cells and Th2 cells is decisive in the development of allergy. Studies on the mechanisms of immune responses to allergens have demonstrated that iTreg cells are dominant in healthy individuals [67]. Therefore, miRNAs that influence the development and functions of iTregs most likely play important roles in allergic diseases. For example, miR-10a has been shown to limit the phenotypic conversion of iTreg cells into follicular helper T cells and hinder the differentiation of the Th17 cell subset [68]. An interesting large-scale overexpression screen with T cell-expressed miRNAs in naïve mouse CD4⁺ T cells undergoing Treg differentiation identified 29 miRNAs with a negative effect and 10 miRNAs with a positive effect. miR-100, miR-99a, and miR-10b all specifically promoted Treg differentiation and inhibited the Th17 program without impacting viability, proliferation, and activation [69]. In addition to autoimmunity, Th17 cells play a dominant role in neutrophilic asthma, which is also associated with asthma exacerbations in response to rhinoviruses [70], indicating that these miRNAs potentially influence the development of neutrophilic asthma and asthma exacerbation.

iTreg cells not only suppress Th1 or Th2 cell responses but also have a direct influence on B cells. Both natural Tregs and iTregs reduce the IgE-secreting plasma cell frequency and simultaneously augment IgG4-secreting plasma cells. Recently, it has also been shown that regulatory IL-10-producing B cells induce Treg cells and alleviate symptoms in a murine model of allergic asthma [71, 72]. However, the roles of miRNAs in immunoregulatory B cells are almost fully unexplored. Interestingly, miR-21 was recently shown to stimulate B cells to produce IL-10 in a study exploring the role of miR-21 in nasopharyngeal cancer. Thus, in addition to its role in triggering Th2 type responses, miR-21 is required for the regulatory role of B cells, which suggests that similarly to miR-155, well-controlled miR-21 levels in every cell is needed for the proper functioning of the immune system.

The development of regulatory and effector T cells strongly depends on innate immune responses of APCs [73]. Several miRNAs may play important roles in the modulation of DC capacity to induce regulatory T cells. For example, miR-23b transfection into mouse or human *in vitro* differentiated DCs leads to increased IL-10 and decreased IL-12 production, inactivation of the Notch1 and NF- κ B signaling pathways, and increased capacity of DCs to promote FoxP3+ CD4+ T cell proliferation [74]. Interestingly, miR-146a has been shown to be differentially expressed by myeloid DC subsets and it desensitizes DCs to TLR2-dependent activation, which suggests that high constitutive miR-146a levels in the epidermis render Langerhans cells (LCs) in the epithelium less susceptible to inappropriate activation by commensal bacterial through TLR2 [75]. Thus, both miR-146a and miR-23b might essentially contribute to protection against the development of allergic responses through their action in APCs, the first line of the cells activated by allergens. As the modulation of microRNA expression in APCs can be considered easier compared with T and B lymphocytes, further studies about the functions of microRNAs in APCs has high potential also in light of few of the development of therapeutic approaches for allergic diseases.

miRNAs in Asthma

The first study to demonstrate the importance of miRNAs in airway diseases was published in 2007 by Rodriguez et al., who characterized miR-155-deficient mice and noted that approximately 50 % of aging miR-155-deficient mice spontaneously develop asthma-like lung inflammation with increased Th2-type cytokines and greater numbers of lymphocytes and macrophages but similar counts of eosinophils compared to wild-type mice [44]. In ensuing studies, the expression of several miRNAs, including miR-155, was found to be dysregulated in the airways or lymphocytes of patients with asthma, as well as in different mouse models of airway inflammation [76]. Interestingly, the same miR-155-deficient mice were recently demonstrated as developing an altered inflammatory response with diminished eosinophilic inflammation, reduced eotaxin-2/CCL24 and periostin levels, and reduced Th2 cell numbers in an experimental mouse model of allergic airway inflammation [77]. Concordantly, miR-155 was shown to be required for Th2-type responses [63]. Thus, it appears that in the case of miR-155 deficiency, a spontaneous development of airway inflammation occurs; however, in induced mouse models, miR-155 rather contributes to airway inflammation.

In addition to miR-155, several other miRNAs have been shown to modulate asthma-like lung inflammation in different mouse models (Table 17.1). The expression of miR-126 was found to be increased in the airways of mice upon exposure to house dust mite (HDM), and the inhibition of miR-126 using intranasal administration of miR-126 antagomir reduced allergic inflammation in the same mouse model of asthma [78] and also decreased eosinophilia in a mouse model of chronic airway

inflammation [79]. Similarly, the inhibition of miR-145 [80] and miR-106a [81] had anti-inflammatory effects in HDM-induced allergic airways disease in mice. Similar to miR-126, enhanced expression of miRNA-221 was detected in the lung biopsies of mice subjected to an OVA-induced mouse model of asthma, and the inhibition of miR-221 reduced inflammation in the airways [82]. Interestingly, miR-221 is also known to regulate the cell cycle of mast cells and is upregulated by stimulation with IgE-allergen complexes in these cells [83, 84].

Current therapies for asthma aim to control symptoms and mainly include fast-acting beta2-adrenoceptor agonists and corticosteroids for long-term control, yet these therapies are inefficient in controlling severe forms of asthma and allergic diseases [3]. Thus, one important question in research concerning asthma is how miRNAs impact the development of corticosteroid-resistant and severe asthma. One of the initial studies in a mouse model of corticosteroid-resistant asthma demonstrated a rapid change in miRNA levels in mouse lungs following LPS-induced inflammation; however, no influence of glucocorticoids was detected [33]. Another study showed that the expression of miR-146a is reduced in CD8+ and CD4+ T cells from severe asthma patients under continuous oral corticosteroid treatment [34]. In agreement with this observation, the expression of miR-146a was shown to be suppressed by dexamethasone in splenic CD4+ T lymphocytes from mice subjected to an OVA-induced model of asthma [53].

Together, these studies show that miRNAs can either enhance or reduce airway inflammation and suggest that a complex network of miRNAs impacts immune responses in asthma. Because asthma is a complex syndrome with heterogeneous endotypes or phenotypes [25, 85], miRNAs as fine-tuners of gene expression also may contribute to the development of different forms of the disease.

miRNAs in Bronchial Epithelium and Airway Smooth Muscle Cells

Disease-related changes in lung alveolar epithelial cells, endothelial cells, fibroblasts, and bronchial smooth muscle cells (SMCs) have strong impact on the pathogenesis of asthma, and miRNAs are clearly part of the regulatory network involved in tissue responses to asthma. An initial study demonstrated that miRNAs have different expression profiles in airway SMCs, bronchial epithelial cell-lines A549 and Beas2B, bronchial fibroblasts, and alveolar macrophages [86]. In epithelial cells from asthmatic patients, 66 miRNAs were detected as differentially expressed, and pathway analysis indicated that the putative targets of these miRNAs encode disease- or inflammation-related proteins such as TNF- α , IL-8, Cox2, IL-6, and AQP4 [87].

Several recent studies describe the functions of miRNAs in lung endothelial cells and SMCs. The lung-specific overexpression of vascular endothelial growth factor (VEGF) was shown to decrease miR-1 expression in the lung endothelium,

whereas intranasally delivered miR-1 inhibited inflammatory responses to HDM, OVA, and IL-13. In the same study, VEGF blockade and the knockdown of a miR-1 target, myeloproliferative leukemia virus oncogene (MPL), inhibited Th2-mediated lung inflammation, suggesting that VEGF controls lung Th2 inflammation via miR-1 and MPL [88]. Suppression of miR-133a leads to increased expression of the small GTPase RhoA, causing augmented contraction and hyperresponsiveness of bronchial SMCs in a mouse model of allergic asthma [89]. miR-140-3p is another microRNA that possibly has a strong impact on SMCs, as it is capable of activating p38 MAPK and NF- κ B in these cells [90]. Patients with pulmonary hypertension were found to express decreased levels of the miR-17~92 cluster, transforming growth factor beta (TGF- β), and SMC markers. Concordantly, SMC-specific knockout of miR-17-92 attenuated hypoxia-induced pulmonary hypertension (PH) in mice, suggesting a direct link between the downregulation of the miR-17-92 cluster and hypoxia-induced PH in these mice [91]. The cell-surface protein CD38, which mediates airway SMC contractility, was recently shown to be both directly and indirectly regulated by miR-708 through JNK MAPK and PI3K/AKT signaling pathways [92].

The expression of anti-inflammatory miR-146a was also shown to be enhanced in human asthmatic SMCs. Both miR-146a/b were found to negatively regulate pro-inflammatory cyclooxygenase-2 (COX-2) and IL-1 β in human SMCs [93]. miR-146a might also contribute to tissue remodeling in the lungs because it has been shown to reduce cytokine-induced apoptosis in human bronchial epithelial cells [94]. Consistent with the findings already described in this chapter, the expression of pro-inflammatory miR-155 has been shown to be increased in asthmatic SMCs upon stimulation, and its expression positively correlates with COX-2 expression in human asthmatic SMCs [95]. Together, these studies demonstrate that the increased activation of pro-inflammatory pathways, altered expression of tight junction proteins and other changes observed in epithelial cells, SMCs and endothelium of patients with asthma may also be partially attributed to underlying aberrant miRNA expression.

microRNAs in the Regulation of Virus-Induced Asthma Exacerbation

One major cause of morbidity among asthma patients is virus-induced recurrent exacerbations, among which HRVs are the most common triggering factors [7, 96]. As asthma exacerbations are not well controlled in the severe form of the disease, studies on the molecular mechanisms of asthma exacerbation are of great importance [3]. The first implications of miRNAs in the regulation of airway responses to respiratory viruses have been published very recently. The replication of human rhinovirus HRV-1B was increased after DICER knockdown in human bronchial epithelial cells, and the transfection of specific anti-miRs for miR-128 and miR-155

increased viral replication of HRV-1B [97]. microRNAs -18a, -27a, -128, and -155 were found to be downregulated in asthmatic bronchial epithelial cells; however, manipulation of the levels of these individual microRNAs in bronchial epithelial cells did not have an effect on any pathways potentially regulating responses to HRVs, and only simultaneous knockdown of the network of microRNAs led to a significant increase in IL-8 and IL-6 expression [98].

In an early study, IL-1 β -induced miR-146a was shown to reduce the levels of pro-inflammatory chemokines IL-8 and CCL5 (also known as RANTES) in human alveolar A549 epithelial cells [99], which is consistent with its strong inhibitory effect on NF- κ B in other cell types [31–34]. When infecting epithelial cells, HRVs activate both NF- κ B and interferon responses, whereas it has been proposed that deficient interferon induction and increased activation of NF- κ B in asthmatic bronchial epithelial cells leads to aggravated inflammation and possibly to the exacerbation of asthma [100, 101]. These findings suggest an important function for microRNAs targeting NF- κ B and stimulating interferon responses in protecting against exacerbation and long-lasting viral infections in patients with asthma.

miRNAs in Atopic Dermatitis, Allergic Contact Dermatitis, and Chronic Rhinosinusitis

The functions of microRNAs in atopic dermatitis (AD), allergic contact dermatitis (ACD), chronic rhinosinusitis (CRS), and allergic rhinitis (AR) have been described only in a small number of studies. miR-155 was determined to be overexpressed in skin from AD patients most likely due to infiltrating immune cells. The same study suggested that miR-155 influences the development of AD through the downregulation of cytotoxic T lymphocyte-associated antigen (CTLA)-4, a negative regulator of general T cell activation that also plays an important role in allergen tolerance [67, 102]. microRNA profiling of human skin challenged with DPCP and skin from a mouse model of contact dermatitis revealed that the same set of microRNAs, miR-21, miR-142-3p, miR-142-5p, and miR-223, are upregulated both in human and in mice [59]. Similar to asthmatic SMC cells, miR-146a expression was found to be increased in keratinocytes and chronic lesional skin of AD patients.

Intriguingly, the effect of miR-146a in the suppression of NF- κ B pathway keratinocytes appear to largely occur due to the suppression of CARD10. In line with experiments in keratinocytes, miR-146a-deficient mice developed stronger inflammation, characterized by increased expression of genes typical in the chronic skin inflammation of AD [34]. Consistent with its binding site in IL-12p35 and inhibitory function in Th2-type airway inflammation, the third microRNA among “the three guardians,” miR-21, has been shown to suppress Th1-associated delayed-type hypersensitivity cutaneous responses in mice [54].

miR-125b has been shown to be upregulated in eosinophilic CRS with nasal polyps. miR-125b overexpression influenced IFN- α/β production, most likely through

the suppression of eukaryotic translation initiation factor 4E (EIF4E)-binding protein 4E-BP1 in airway epithelial cells [103]. miR-135a, which is reduced in a mouse model of allergic rhinitis, has been shown to correct the Th1/Th2 imbalance in the same mouse model when applied intranasally [104]. miR-143 has been shown to decrease the mRNA and protein expression levels of eotaxin and mucin 5AC (MUC5AC) in IL-13-stimulated nasal epithelial cells through direct suppression of IL-13 receptor α 1 chain (IL13R α 1) [105].

An interesting aspect of microRNAs is their relative stability in the extracellular space when compared with mRNA. Moreover, it appears that miRNAs can be actively secreted via cell-derived membrane vesicles and by protein-miRNA complexes and can also be picked up by neighboring cells [106–109]. Indeed, a recent study demonstrated that CD8+ suppressive cells are capable of producing antigen-specific antibody-coated nanovesicles containing miR-150. These nanovesicles entered into effector T cells and suppressed induced ACD and promoted antigen-specific tolerance in mice; the nanovesicles from miR-150-deficient mice did not have a suppressive effect [110]. Despite the limited number, these studies clearly demonstrate that miRNAs are involved in the regulation of allergic inflammation in the skin and sinonasal epithelial cells and strongly suggest that microRNAs represent additional means of communication between different types of cells participating in the regulation of allergic responses and the development of tolerance.

Extracellular miRNAs as Biomarkers in Allergic Diseases

Asthma is a complex disorder based on underlying cellular and molecular mechanisms and can be subcategorized into endotypes [24, 25, 85]. For better management of allergic diseases, especially severe forms of asthma, the development of endotype-specific treatment modalities is important and depends on reliable biomarkers. The microRNA expression pattern varies in different cell types and disease conditions. microRNAs are stable and can be detected in different body fluids, such as serum, urine, and saliva, and recent studies suggest that similarly to other diseases, extracellular miRNAs could be potentially used as biomarkers in airway and allergic diseases as well. In serum from patients with chronic obstructive pulmonary disease (COPD), four miRNAs (miR-20a, miR-28-3p, miR-34c-5p, and miR-100) were significantly downregulated and one (miR-7) upregulated [111].

Of ten differentially expressed miRNAs in esophageal tissue from EoE biopsies, the differential expression of miR-146a/b and miR-223 was also detected in plasma from EoE patients [57]. Bronchoalveolar lavage fluid (BALF) exosomal miRNA profiles have been found to be different in patients with mild asymptomatic asthma and healthy subjects [112]. miRNAs have also been detected in exhaled breath condensate, and some differences have been reported between asthmatic patients and control subjects [113]. miRNA expression profiles of serum and urine from 30 children with AD and 28 healthy children revealed significant upregulation of miR-203

and miR-483-5p in serum from children with AD. At the same time, miR-203 was markedly decreased in urine from children with AD compared with healthy children, and the downregulated miR-203 in urine was significantly associated with the levels of serum IgE in the AD patients [114]. Clearly, future studies are needed to determine whether extracellular miRNAs can be used as reliable biomarkers to distinguish certain endotypes of asthma and other allergic diseases.

Potential of miRNA-Based Therapeutics in Allergic Diseases

The current therapies for asthma and allergic diseases are still inefficient in controlling severe forms of these diseases, and approximately 5–10 % of all people with these disorders have inadequately controlled, severe persistent asthma or severe atopic dermatitis [3]. These asthma and allergy patients use a large proportion of public health resources, and new, more effective therapies are urgently required. One direction in the development of novel therapeutics for allergic diseases is the biological modification of immune responses for better and more individualized control [3, 115]. Indeed, there is obvious potential for miRNAs as novel target molecules for the development of biological treatment modalities. Because the functions of miRNAs are shaped by evolution, miRNA-based therapeutics would have a low likelihood of inducing toxicity, suggesting that miRNA-based therapeutics would have few side effects [116]. However, in the field of miRNA research in allergic diseases, only a limited number of people are investigating the functions of microRNAs, which is most likely due to the complexity and specificity of this research direction. Regardless, we propose that improved techniques for microRNA overexpression and inhibition, combined with increased knowledge about the biological functions of microRNAs, will lead to the development of very specific gene expression modulators that could be implemented as personalized therapeutics for allergic diseases in the future.

Based on the current knowledge of the molecular mechanisms and functions of microRNAs in allergic diseases and inflammation in general, we outline here a few ideas, whereby the targeting or overexpression of different miRNAs could possibly be utilized therapeutically in allergic tissue inflammation (Fig. 17.2). Allergic tissue inflammation in the skin, sinus, or lungs is induced by the development of a strong allergen-induced Th2-type immune response, which is strongly supported by the production of TSLP by epithelial cells [117]. As miR-375 promotes the production of TSLP [64], the inhibition of miR-375 may reduce the development of Th2-type inflammation. Suppression of the positive regulators of Th2 cell development, miR-155 [63] and miR-21 [54], in CD4⁺ T cells would similarly reduce the skewing of immune responses toward the Th2 direction. During the chronic phase of tissue inflammation, secondary infections and innate immune response activation in epithelial cells occurs [118], which could be suppressed with the overexpression of miR-146a/b. In the case of asthma, overexpression of the miR-17-92 cluster (87) may reduce pulmonary hypertension.

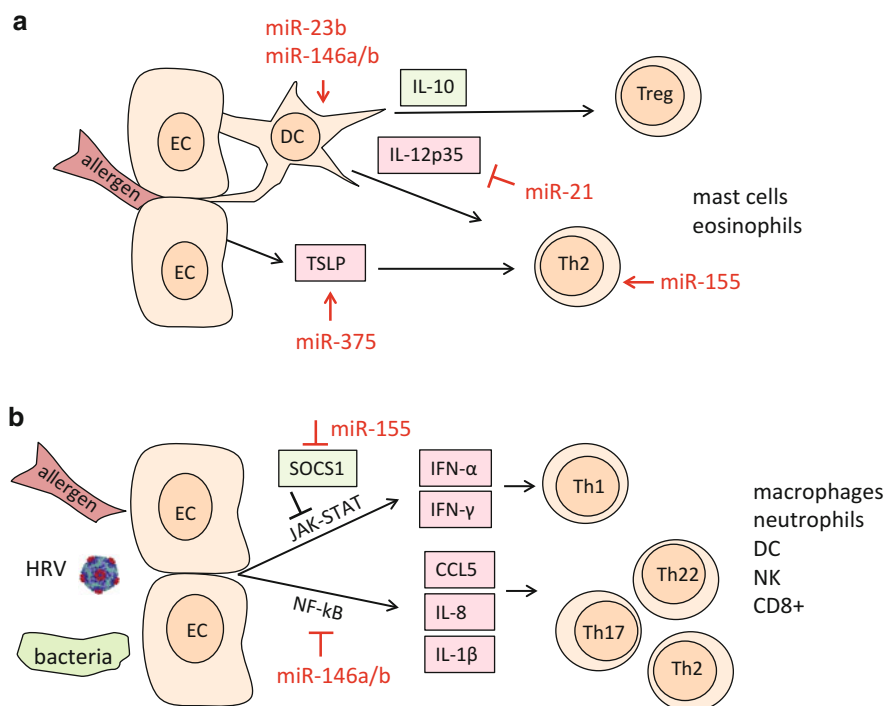


Fig. 17.2 microRNA as potential therapeutic targets in allergic inflammation. **(a)** Allergic tissue inflammation is induced by allergen-induced Th2-type immune response supported by the production of thymic stromal lymphopoietin (TSLP) by epithelial cells [117]. As miR-375 promotes the production of TSLP [64], the inhibition of miR-375 could reduce Th2 type inflammation. Suppression of the positive regulators of Th2 cell development, miR-155 [63], and miR-21, which targets IL-12p35, a positive regulator of Th1 cells [54], would also reduce Th2 type responses. Overexpression of miR-23b and miR-146a in DCs would render DCs less susceptible to the activation and might contribute to the development of Treg cells [74, 75]. **(b)** During the chronic phase of tissue inflammation, secondary infections and activation of innate immune responses in epithelial cells occurs [118], which could be suppressed by the overexpression of miR-146a/b. In HRV-induced airway inflammation or asthma exacerbation, the overexpression of miR-146a would possibly help to reduce the activation of the NF-κB pathway and accompanying inflammation, whereas the simultaneous overexpression of miR-155 would increase the interferon response and virus clearance

In HRV-induced long-term airway inflammation or asthma exacerbation, the overexpression of miR-146a would help to reduce the activation of the NF-κB pathway and accompanying inflammation, whereas the simultaneous overexpression of miR-155 would increase the interferon response and virus clearance. Overexpression of miR-23b and miR-146a in DCs would render DCs less susceptible to the activation and might contribute to the development of regulatory T cells [74, 75]. In addition, inhibition of miR-126, miR-221, miR-145, and miR-106a (Table 17.1) could possibly be used to reduce allergic inflammation in patients with asthma.

In conclusion, the roles of miRNAs in relation to allergic diseases are just beginning to be explored. Further studies are needed to reveal the full impact of miRNAs in the development of allergic diseases as well as to reveal their potential as therapeutic targets and non-invasive biomarkers in allergic conditions.

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