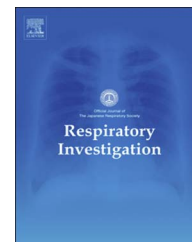




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Review

Role of microRNA in severe asthma

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ABSTRACT

The various roles of microRNAs (miRNAs) in the epigenetic regulation of human disease are gaining importance as areas of research, and a better understanding of these roles may identify targets for development of novel therapies for severe asthma. MiRNAs, a class of small non-coding RNAs that serve as post-transcriptional gene repressors, are recognized as critical components in regulating tissue homeostasis. Alteration in miRNA expression disrupts homeostasis and is an underlying mechanism for development of chronic respiratory diseases, including asthma. Differential profiles of miRNA expression are involved in inflammation and remodeling pathogenicity via activating airway structural cells and immune cells and inducing cytokine releases. miRNA action leads to asthma progression from mild to severe stages. Here, current knowledge of the heterogeneous roles of miRNAs in severe asthma, including biological mechanisms underlying Th2 and macrophage polarization, type 2 innate lymphoid cell (ILC2) biology regulation, steroid-resistant asthma phenotype, airway smooth muscle (ASM) dysfunction, and impaired anti-viral innate immune, are reviewed.

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Abbreviations: miRNA, microRNA; (ILC2), type 2 innate lymphoid cells; Th2 cells, type 2T helper cells

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1. Introduction

MicroRNAs (miRNAs) are a class of small non-coding RNAs that play a critical role in cell differentiation, proliferation and survival by binding to complementary target mRNAs, leading to RNA translational repression or degradation [1]. miRNAs primarily interact with their mRNA targets, via short sequence signatures, making them perfectly suited for combinatorial effects with other miRNAs or RNA-binding proteins (RBPs) that associate with the same mRNA. In mammals, miRNAs are estimated to control expression of 50% of all protein-coding genes. Functional studies demonstrate that miRNAs are involved in the regulation of almost every cellular process and that their dysfunctional expression is associated with many human pathologies [2].

Basic mechanisms of miRNA biogenesis and function have been extensively investigated during the past decade, and more recent studies demonstrate that miRNA themselves are subjected to tight control at levels of miRNA metabolism and function [3–7]. Numbers of individual miRNAs expressed in humans are comparable to numbers of transcription factors or RBPs, and many miRNA are expressed in tissue-specific or developmental stage-specific manners, thereby helping define cell-type-specific profiles of protein expression. With potential to target dozens or even hundreds of different mRNAs, individual miRNAs can coordinate or fine-tune protein expression. Strict and dynamic regulation of miRNA levels and activity is required, particularly during rapid developmental transitions or changes in cellular environments.

2. miRNA biogenesis and function

miRNA biogenesis cascades are catalyzed in tightly controlled pathways involving four key enzymes – Drosha, exportin 5, Dicer and argonaute 2 (AGO2) [8,9]. The first step of miRNA biogenesis is initiated by transcription of precursor molecules (called pri-miRNAs) that are either regulated by RNA polymerase II (RNA Pol II) from independent genes or as introns of protein-coding genes [2]. The majority of genes encoding miRNAs are located in intronic regions and contain their own promoter regions [8]. Transcription of miRNA genes is regulated in a similar manner to regulation of protein-encoding genes, and transcriptional control is primary means for regulating tissue-specific or development-specific expression of miRNAs. Pri-miRNAs fold into hairpins, which serve as substrates for two members of the RNase III family of enzymes, Drosha and Dicer. These enzymes operate in complexes with dsRNA-binding proteins (dsRBPs), for example as DGCR8 and transactivation-responsive (TAR) RNA-binding protein (TRBP) in mammals. Drosha catalyzes the first nuclear step of miRNA production. Following RNA Pol II-induced synthesis of pri-miRNAs transcripts, two enzymatic cleavage steps produce mature miRNAs. Drosha along with the cofactor protein DGCR8, binds to the primary miRNA (pri-miRNA). Two Drosha RNase domains

process pri-miRNAs into an ~70-nucleotide precursor hairpin species (pre-miRNA). Some pre-miRNAs are generated from very short introns (mirtrons) through a Drosha-DGCR8-independent process by splicing and debranching [2,8]. Subsequently, the exportin 5-RAN•GTP complex mediates the transport of pre-miRNAs from the nucleus into the cytoplasm. There, the RNase III Dicer and TRBP bind to the pre-miRNAs and cleave the terminal loop, resulting in a miRNA duplex [2,8]. Processing of the miRNA duplex is mediated by the argonaute (AGO) family of proteins, in conjunction with several cofactors such as PACT (also known as PRKRA, one strand of miRNA duplex – the guide strand, is preferentially incorporated into a miRNA-induced silencing complex (miRISC); the other strand (passenger) is released and degraded. Following unwinding and strand selection, mature miRNAs base-pair to complementary sites in target mRNA and induce translational repression or deadenylation and degradation. Efficient mRNA targeting requires continuous base-pairing of miRNA nucleotides 2 to 8 (the seed region) [3]. Mechanistic contexts of miRNA-mediated translational repression are not as well known as are details of mRNA deadenylation mediated by 182 kDa glycine-tryptophan protein (GW182) [5,6,10]. (Fig. 1).

3. Regulation of constituents of miRNA processing pathways

miRNA processing is regulated via changes in expression and activity of Drosha, Dicer, and their double-stranded RBP partners. Drosha and Dicer function primarily in complexes with these RBP partners (e.g. DGCR8 and TRBP). Both levels and activity of all of these proteins are subject to regulatory processes that induce accumulation of miRNA. For instance, DGCR8 mediates a stabilizing effect on Drosha by interacting with its middle domain, whereas Drosha regulates DGCR8 levels by cleaving hairpins present in the DGCR8 mRNA, thereby inducing its degradation [11,12]. The Drosha to DGCR8 ratio is likely important since a threefold excess of DGCR8 markedly suppresses Drosha processing activity *in vitro* [13]. Haem also facilitates pri-miRNA processing by binding to DGCR8, which promotes its dimerization [14].

Dicer, a large (~200 kDa) multi-domain protein, is involved in the cleavage of pre-miRNAs and also participates in loading miRNAs into miRISC. The amino-terminal helicase domain of Dicer may have an autoregulatory function since its deletion stimulates catalytic activity of human Dicer in a manner similar to stimulation by TRBP. Proteolysis may play a role in regulating Dicer activity or even in changing its specificity towards DNA. The accumulation of Dicer is regulated by its partner TRBP through TRBP-controlled Dicer stabilization and a decrease in TRBP results in pre-miRNA processing defects. Mechanisms of TRBP stabilization involve mitogen-activated protein kinase (MAPK) and extracellular regulated kinase (ERK)-mediated serine phosphorylation. TRBP phosphorylation enhances cell growth and survival, possibly resulting from

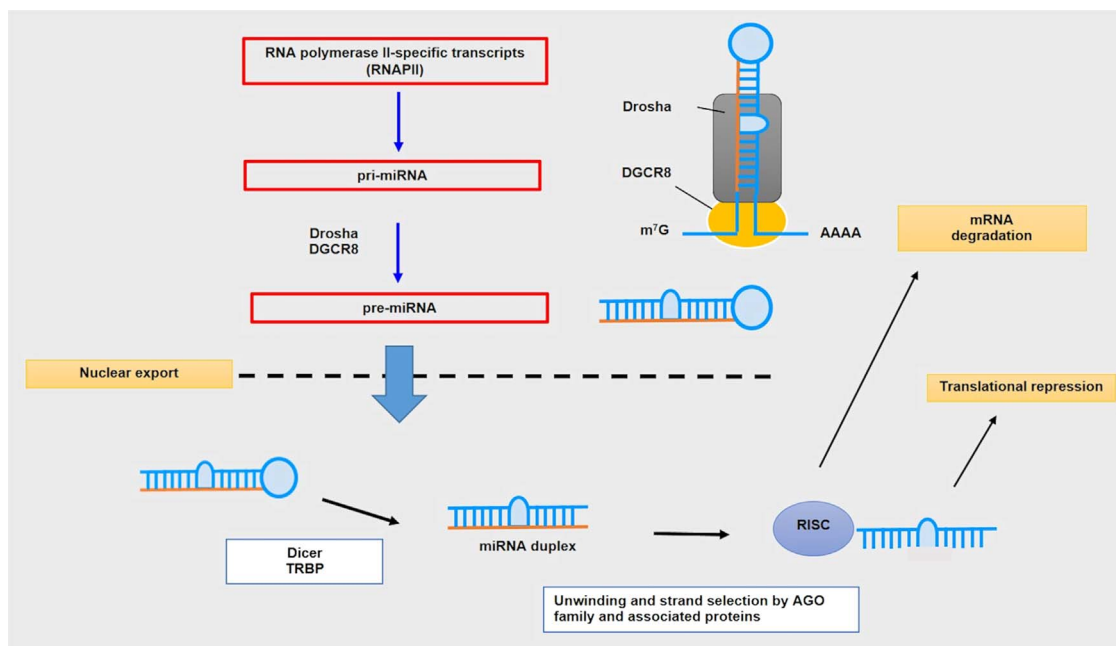


Fig. 1 – miRNA biogenesis and primary function. The processes of miRNA biogenesis begins with pri-miRNA synthesis and are tightly controlled. miRNA is produced in a step by step manner that relies on several key enzymes. miRNA act in the cytoplasm via mRNA degradation and translational repression.

upregulation of growth-stimulatory miRNAs in parallel with downregulation of let-7, a known suppressor of proliferation [15]. Further, let-7 could suppress Dicer mRNA, thereby forming a negative feedback loop with the potential to broadly influence miRNA biogenesis at both physiologic and pathologic levels, the latter being observed in cancers [2,16]. This mechanism underlies hormone-regulated Dicer expression [15,17]. For example, steroid hormones, including estradiol and progesterone, could stimulate or suppress miRNA expression in models of breast and uterine cancer through modulation of Dicer and exportin 5 expression. However, suppression of the primary process of miRNA biogenesis is outside the spectrum of corticosteroid functions [18,19].

4. Regulation of key elements of allergic and non-allergic asthma: Th2 polarization and type 2 innate lymphoid cells (ILC2)

Allergic asthma is the most common phenotype of asthma where chronic airway inflammation is dominated by a CD4⁺ T helper 2 (Th2) cells that produce GATA-3-regulated Th2 cytokines, particularly IL-4, IL-5 and IL-13 [20]. The immune response establishes self-enforcing loops, promoting Th2-driven cellular immunity and leaving the host unable to terminate inflammation. Persistent Th2 polarization is induced by inhaled allergens through mechanisms of dendritic cell (DC) sensitization involving DC cytokines-directed suppression of Th1 polarization. miRNAs, one post-transcriptional regulator, are critical for maintaining immune homeostasis and their dysfunction contributes significantly to human disease [21–23]. In multiple experimental asthma models, miR-21 has been shown to be upregulated [24]. Despite different methods of disease induction, each system

shares similar pathological phenotypes, including airway eosinophilia, Th2-associated inflammation, mucus production, and airway hyperresponsiveness [25–30]. Indeed, miR-21 is the most overexpressed miRNA in inflamed lung tissues, with the highest expression levels localized in macrophages and dendritic cells [24]. Because IL-12p35 3'UTR contains a highly evolutionarily conserved target sequence for miR-21 [24], miR-21 could potentially regulate Th polarization from Th1 toward Th2 phenotypes by downregulating the expression of IL-12 as a driver for Th1 polarization through IL-12p35 transcript degradation. Evidence that supports such regulation includes; miR-21 inhibition of IL-12p35 3'UTR in a dose-dependent manner, whereas genetic mutation of miR-21-binding sites in IL-12p35 3'UTR attenuates miR-21-mediated IL-12 repression [24]; and miR-21-deficient CD4⁺T cells exhibit increased IFN- γ but decreased IL-4 production and therefore enhanced delayed hypersensitivity response [31]. All data suggest that miR-21 potentiates Th2 polarization by suppressing Th1 differentiation. Upregulation of miR-21 has been demonstrated in multiple human Th2-mediated diseases, including eosinophilic esophagitis, atopic dermatitis, and ulcerative colitis. Silencing miR-21 expression with antagomiRs or anti-miRs could be a useful target for asthma treatment, as a recent report found that intranasal anti-miR-21 administration prior to allergen challenge in a HDM model of asthma had no significant effect on eosinophil recruitment or Th2 cytokine production [32]. In addition, Collison A et. al. showed that selective inhibition of miR-145 function but not that of miR-21 or let-7b with antagomiRs inhibited production IL-5 and IL-13 by TH2 cells, the recruitment of eosinophils to the airways, mucus hypersecretion, and the development of AHR. In contrast, administration of anti-miR-21 after the early phase of allergen sensitization at the time when an imbalance in Th polarization toward Th2 phenotype had

likely already been established could inhibit allergic airway inflammation. These findings indicate that miR-21 plays a proactive role in early stage sensitization. AntagomiRs might not reproduce the phenotype of miR-21 deficient mice because of either the type of antagomiR used or the mode and frequency of administration, as suggested by a lack of consistency among recent antagomiR and gene-deficient murine studies [33–36]. Results of these studies indicate that minimal alteration in miRNA levels can have profound effects on adaptive immune response. Recent research further enhances our understanding of roles of miRs in asthma in findings of the collective impact of five Th2-related miRs (miR27b, miR206, miR106b, miR203, and miR23b) on Th2 inflammation. Antagonism of these miRs led to a marked reduction of the Th2 phenotype [37].

Kinetics of miR-126 expression levels show that sustained upregulation of miRNAs may not be necessary for development of chronic persistent asthma. MiR-126 was upregulated in response to acute allergen challenge through myeloid differentiation primary response gene-88 (MyD88)-dependent TLR signaling, leading to the development of Th2-mediated allergic airway inflammation [38]. However, in a chronic model of asthma, upregulation of miR-126 expression in response to allergen exposure lasted a few weeks then declined to near basal levels [39]. In addition, silencing of miR-126 by antagomiRs in chronic asthma suppressed only recruitment of intraepithelial eosinophils in the airways and not mucus cell hyperplasia or subepithelial fibrosis [39].

ILC2s are a potent source of type 2 signature cytokines, IL-13 and IL-5, that drive allergic inflammation [40–42]. ILC2s could induce a steroid-resistant phenotype when activated by thymic stromal lymphopoietin (TSLP) through the signal transducer and activator of transcription 5 (STAT5) signaling pathway as demonstrated in severe asthma [43,44]. miRNAs are able to regulate lung ILC2 function in a mouse model of allergic airway inflammation and are implicated in the pathogenesis of severe asthma in humans [45]. ILC2 miRNAome shares features with Th2 cells, in which the miR-17~92 cluster is required for ILC2 expansion and promotion of IL-5 and IL-13 production by silencing suppression of cytokine signaling 1 (SOCS1) and A20 genes [45].

5. Epigenetic regulation of macrophage polarization

Macrophages are endowed with remarkable heterogeneity and plasticity allowing them to switch from one phenotype to another. This process is observed in lung tissues where intrinsic differentiation pathways in recruited macrophages interact with microenvironmental chemicals released from neighboring cells. Many macrophage polarization pathways involve complex interplay between microenvironmental signals and a hardwired differentiation program that directs macrophage identity and functional specialization. Macrophages undergo polarization into either classically activated (or M1) or alternatively activated (or M2) phenotypes which display different functions and transcriptional profiles. Regulation of macrophage polarization involves interactions among various cytokines, chemokines, transcriptional factors, and immune-

regulatory cells. Compelling evidence is available to show that epigenetic mechanisms have a significant role in controlling macrophage activation and polarization. This control is exerted when environmental stimuli induce epigenetic changes (microRNAs, DNA methylation, and histone modification), which contribute to alternations in cellular signaling and signature gene expression during M1 and M2 polarization [46,47]. Several transcription factors including peroxisome proliferator-activated receptors, signal transducers and activators of transcription, CCAAT-enhancer-binding proteins, interferon regulatory factors, Kruppel-like factors, GATA binding protein 3, nuclear transcription factor- κ B, and c-MYC, promote expression of specific genes that dictate the direction of macrophage polarization. miRNAs regulate macrophage polarization through these transcription factors and complement regulation of functional gene expression through translation or mRNA degradation in response to microenvironmental signals [48–50].

Macrophage polarization has been associated with development of asthma in disease initiation, progression, and severity [51–54]. Several miRNAs are involved in macrophage polarization in asthma, including miR-155, miR-146, Let-7c, miR-21, and miR-511-3p [55–62]. MiR-155 stimulates M1 macrophage while inhibiting M2 macrophage polarization [63]. miR-155-downregulation of M2 polarization is mediated by suppression of STAT-6 [46] and Smad2 [64], whereas upregulation of M1 polarization is driven by repression of SOCS1, SHIP1, and BCL6 [65–67]. Two isoforms of miR-146 (miR-146a and miR-146b) have opposing functions in regulating macrophage polarization. miR-146a promotes M2 macrophage polarization and suppresses proinflammatory cytokine production by targeting Notch1, PPAR γ , classical NF- κ B, IRAK, and TRAF6 [68–71], whereas miR-146b suppresses M1 macrophage signature genes by targeting IRF5 [72]. Similarly, Let-7c enhances the M2 phenotype by targeting p21-activated kinase 1 and C/EBP δ that are required the M1 phenotype [73,74]. miR-21 also promotes the M2 phenotype by enhancing CSF-1R signaling required for M1 suppression [75] and IL-10 production [76] while suppressing PTEN and PDCD4-related proinflammatory signals [77].

6. Modulation of airway smooth muscle cells

Airway smooth muscle (ASM) cells (ASMC) are the principal effectors of airway hyperresponsiveness and therefore play an essential role in asthma pathogenesis [78]. Airway smooth muscle contraction is regulated by intracellular mechanisms regulating calcium handling including cell surface molecules (i.e. CD38) and signal transductions (i.e. small GTPase RhoA) [79–84]. These two intracellular pathways are primary for calcium mobilization in ASM [83]. Ligand access to its cognate G protein-coupled receptor induces inositol 1, 4, 5 trisphosphate (IP3) that causes sarcoplasmic reticulum (SR) calcium release. Calcium-induced calcium release (CICR) results from activation of ryanodine receptor (RyR) channels by calcium and cyclic ADR-ribose (cADPR) whereas cADPR-associated calcium release is inhibited by 8-Bromo-cADPR (8-BrcADPR) [83]. Stimulation of G-protein coupled receptor could induce CD38 and cADPR production, though the mechanisms are unclear. CD38, a 45-kDa transmembrane glycoprotein

associated with the plasma membrane of ASMC, possess two enzymes, namely – ADP-ribosyl cyclase and cADPR hydrolase. ADP-ribosyl cyclase converts β -NAD to cADPR, which is then converted to ADPR by cADPR hydrolase [83]. Despite the lack of studies showing the direct correlation with severe asthma, CD38 expression in human ASMC is enhanced by a cytokine (e.g., TNF- α) enriched milieu that contributes to corticosteroid refractoriness. Its expression is post-transcriptionally regulated by miR-140-3p and miR-708 through direct binding to the 3'UTR of CD38 and through modulating activation of signaling mechanisms [79]. CD38-associated regulatory signaling modules that control ASM biology are PI3K/Akt, and phosphatase and tensin homolog (PTEN) signaling, that function as activator and inhibitor, respectively, for ASMC remodeling in asthmatic patients [85–87]. The PI3K/Akt axis induces CD38 expression that potentiates airway hyperresponsiveness (AHR) and ASMC proliferation whereas PTEN mediates the cell cycle arrest program in ASMC by upregulating p21 and down-regulating cyclin D1 [85–89]. In contrast, TNF- α could increase expression of miR-708, which induces upregulation of PTEN and MAPK phosphatase-1 (MKP-1), that subsequently inhibits PI3K/Akt, MAPK, and JNK, leading to downregulation of CD38 expression and reduced ASM hyperreactivity [90]. In addition, miR-708 directly suppresses CD38 expression [90].

Excessive ASM remodeling (smooth muscle hypertrophy and hyperplasia) within the airway wall is recognized as a dominant feature of severe asthma and causes chronic air-flow obstruction that may require treatment with bronchial thermoplasty [91,92]. Identification of key drivers of ASMC contractility and proliferation, particularly via epigenetic mechanisms, are critical for characterizing novel targets for limiting or reversing ASM remodeling. Overexpression of protein arginine methyltransferase 1 (PRMT1), regulated by ERK 1/2 MAPK associated STAT1 signaling in ASM, plays a causative role in ASM pathologies [93]. In normal ASMCs, posttranscriptional suppression of ERK by miR-19a limits

PRMT1 expression. This limitation does not emerge in asthmatic ASMCs because of miR-19a downregulation that leads to constitutive high expression of PRMT1 [94]. Some miRNAs, including miR-139-5p may prove beneficial for asthma treatment. miR-139-5p inhibits proliferation while promoting apoptosis of ASMCs by targeting Brg1, a chromatin-remodeling factor that is upregulated in ASMCs [95]. Stimulation of miR-139-5p is thus a possible therapeutic target for restoration of abnormal ASM remodeling.

Transforming growth factor (TGF)- β promotes aberrant ASM function by increasing ASMC proliferation and size, and by inducing ASM-mediated proinflammatory effects [96,97]. In severe asthma, this action of TGF- β is mediated through miR-221-downregulated cyclin-dependent kinase inhibitors p27^{kip1} and p21^{WAF1}. This down regulation leads to decreased ability of corticosteroids to suppress ASMC proliferation and IL-6 release, which is independent of miR-221 effects [98]. Recent studies have identified miRNA therapeutic targets for attenuation of airway remodeling in asthma. Epithelial miR-34/449 plays a role in autophagy-mediated airway remodeling by targeting insulin growth factor binding protein-3 (IGFBP-3). Normal expression of miR-34/449 attenuates airway remodeling by suppressing Nur77-mediated autophagy. In contrast, downregulation of miR-34/449 in asthmatic patients eliminates airway protection against IL-13-upregulated autophagy-related proteins, proinflammatory factors, and fibrosis-related proteins [99].

7. MicroRNA involved in the mechanistic link between airway epithelial cells (AECs), severe neutrophilic asthma, and posttranscriptional regulation of epithelial cell-derived cytokines

The critical role of airway epithelium in maintaining airway homeostasis by regulating the balance between tolerance and

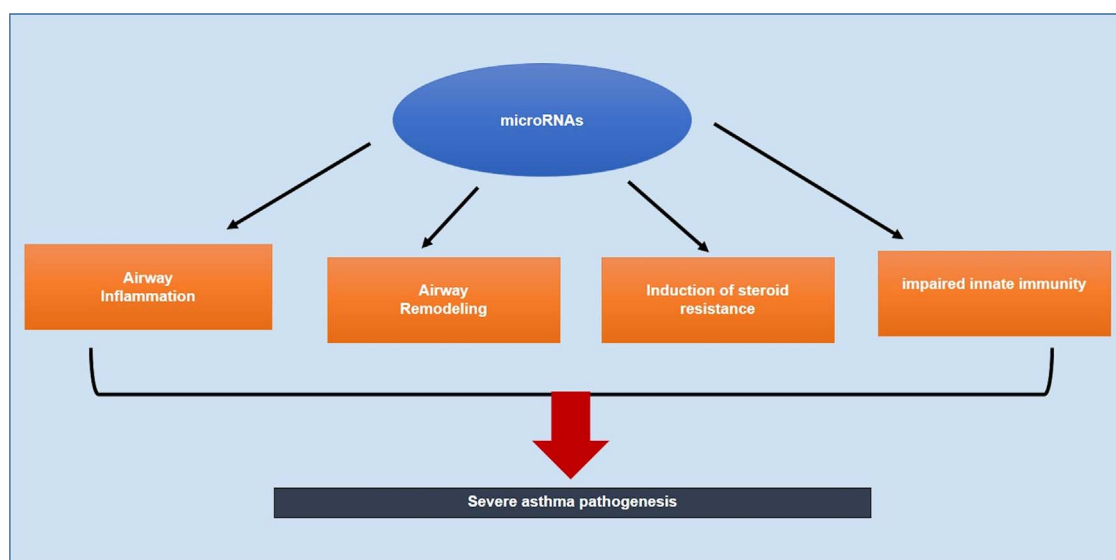


Fig. 2 – Mechanisms of miRNA action involved in severe asthma pathogenesis. Four processes are regulated by miRNA expressed in different relevant airway cell types to produce severe asthma phenotypes including, airway inflammation, airway remodeling, induction of steroid resistance, and impaired innate antiviral immune response. Regulation of airway target genes by miRNA could worsen or alleviate asthma pathology.

Table 1 – Selected miRNAs and their roles in pathology of severe asthma.

Pathology	MicroRNA	Cell type expressing microRNA	Target gene silencing	Effects	References
Airway inflammation	miR-21	Dendritic cells	IL-12p35 3' UTR	Enhances Th2 polarization by suppressing Th1 polarizing cytokine and allergic airway inflammation shown in ovalbumin-challenged mice	Lu et al. [31]
	miR-17~92	Mouse ILC2	SOCS1 and A20	Control of ILC2 homeostasis in a mouse model of allergic airway inflammation	Singh et al. [45]
		T cells	- PTEN, SOCS1, and deubiquitinase A20 for Th2 bias - Ikaros and PTEN for Th17 response	- Promotes IL-5 and IL-13 production - Enhances Th1, Th2, Th17 response demonstrated in airway CD4⁺ T cells from asthmatic patients - Suppresses Treg function and IL-10 production in mice	Kuo et al. [112]
	miR-126	Airway wall cells	POU domain class 2 associating factor 1 (OBF.1/ BOB.1) that primarily induces expression of PU.1, a major positive regulator of TLR4 and a negative regulator of GATA-3	- Modulates gene translation to bridge TLR4/ MyD88 signaling and Th2 response in BALB/c mouse model of HDM-induced allergic asthma - Modulates gene translation to bridge TLR4/ MyD88 signaling and Th2 response in BALB/c mouse model of HDM-induced allergic asthma - Inhibition of OBF.1/BOB.1 that leads to GATA-3 upregulation and TLR4 downregulation; the latter induces miR-126 expression	Mattes et al. [38]
	miR-145	Airway smooth muscles	Transcripts of negative regulators of inflammatory pathways	Eosinophilic inflammation, mucus hypersecretion, AHR, Th2 cytokine production in mice	Collison et al. [32]
	miR-629-3p	Airway epithelial cells of severe asthmatics	Target genes require further investigation	Induction of airway epithelial IL-8 and IL-6 expression demonstrated in sputum specimens	Maes et al. [102]
	miR-223-3p	Neutrophils, monocytes, macrophages of severe asthmatics	Possible target genes - FBXW7 (TLR4-mediated inflammatory gene suppressor via C/EBPδ - PARP1	- Induces airway epithelial IL-8 expression - Induces TGFBR1 (TGF-β receptor 1) expression - Upregulates of miR-142-3p	
	miR-142-3p	Neutrophils, monocytes, macrophages	Target genes required further investigation	Induces of TGFBR1 (TGF- β receptor 1) expression	
	miR-200b	Bronchoalveolar lavage cells from asthmatic patients compared to healthy control	IL-33	Suppresses IL-33 expression, however a reduction in these miRs that leads to severe asthma	Tang et al. [104]
	miR-200c miR-487b-3p	Alveolar epithelial cells	IL-33	Inhibits IL-33-mediated allergic airway inflammation in Mex 3b ^{-/-} mice. However, if expression of Mex-3B is active, it may upregulate IL-33 expression by inhibiting miR-487b-3p-mediated repression	Yamazumi et al. [105]

	miR-221-3p	Airway epithelial cells from symptomatic asthmatic patients	Chemokine (C-X-C motif) ligand (CXCL) 17 served as an anti-inflammatory chemokine by inhibiting CCL24 (eotaxin-2), CCL26 (eotaxin-3), periostin through p38 MAPK pathway	Exacerbates eosinophilic airway inflammation, however; reduces airway miR-221-3p	Zhang et al. [106]
Airway remodeling	miR-19a	Airway epithelial cells of severe asthmatics isolated from bronchial biopsied specimens Human airway smooth muscle cells (ASMCs) from severe asthmatics	- TGF-β receptor 2 (TGFB2) - ERK	- Enhances airway epithelial proliferation as result of loss of TGFB2-mediated SMAD3 phosphorylation - Enhances airway epithelial proliferation as result of loss of TGFB2-mediated SMAD3 phosphorylation	Haj-Salem et al. [103] Sun et al. [94]
	miR-145	Mice alveolar epithelial cells	EGFR	Alleviates airway remodeling involving goblet cell hyperplasia and mucus production	Cheng, et al. [108]
	miR-34/449	Lung epithelial cell line	IGFBP-3	- Suppresses IGFBP-3-mediated autophagy - Decreases airway remodeling in fibrosis components by downregulating fibrosis-related proteins	Yin et al. [99]
	miR-708-5p	Human ASMCs	- CD38 - NF-κB - AP-1	- Downregulates proinflammatory cytokines - Reduced TNF-α-induced airway hyperresponsive ness	Guedes et al. [79]
	miR-140-3p miR-221	Human ASMCs from severe asthmatics	Cyclin-dependent kinase inhibitors, p21^{WAF1} and p27^{kip1}	Enhances ASMC proliferation	Perry et al. [98]
Virus-induced asthma exacerbation	miR-150	Alveolar macrophages isolated from bronchoalveolar specimens from severe asthmatics	TLR7	Impairs innate antiviral immune response resulting from decreased TLR7-mediated IFN production	Rupani et al. [111]
	miR-152 miR-375				
Respiratory infection-induced steroid resistance	miR-21	Animal model of asthma	PTEN	Enhances PI3K/Akt-suppressed HDAC2 expression	Kim et al. [109]

specific immune response is a subject of increasing interest [100]. This balance is aberrantly dysregulated in asthma and airway epithelium becomes a central orchestrator of airway inflammation through production of cytokines and chemokines that induce both airway eosinophilia and neutrophilia. Suppression of the latter is outside the spectrum of corticosteroid action, neutrophil apoptosis is as at least in part steroid-resistant [101]. A recent report shows that upregulated miR-629-3p in airway epithelial cells from severe asthmatics enhances IL-8 release to recruit neutrophils into airways [102]. In addition, upregulation of miR-223-3p and miR-142-3p expression in recruited neutrophils, monocytes, and macrophages was significantly associated with the degree of airway obstruction [102]. Finally, *in silico* analysis and functional experiments on HBECs transfected with miRNA mimics suggested that identified miRNAs were modulating pathways related to inflammation and wound response [102]. These effects of miRNAs possibly lead to intense airway neutrophilia and remodeling and contribute to a severe asthma phenotype. Similarly, miR-19a enhances AEC proliferation through activation of TGF- β -mediated SMAD3 phosphorylation by suppressing TGF- β receptor 2 expression in severe asthmatics [103]. In contrast, miRNAs may negatively regulate cytokine production by airway epithelium. Reduced miR-200b and miR-200c enhances asthma severity through potentiating IL-33 release from lung epithelial cells. IL-33 production requires RNA binding protein Mex-3B for inhibition of miR-487b-3p-mediated repression of IL-33, suggesting the tight and complex regulation of IL-33 by miRNAs [104,105]. Similarly, although epithelial miR-221-3p primarily exacerbates eosinophilic airway inflammation through the induction of CCL24 (eotaxin-2) and CCL26 (eotaxin-3), its levels of expression are reduced in asthma, suggesting that the anti-inflammatory chemokine CXCL17 is a target of miR-221-3p that, if upregulated, could to suppress airway eosinophilia [106].

Mucus plugs are a plausible mechanism of chronic airflow limitation in severe asthma; plug formation is mediated by eosinophil peroxidase (EPO)-generated oxidants [107]. miRNAs suppress production of mucin 5AC (MUC5AC), a major airway mucin that is primarily expressed in goblet cells, and is highly upregulated in asthma by the induction of epidermal growth factor receptor (EGFR). Targeting EGFR by miR-145 caused downregulation of MUC5AC and attenuated airway remodeling [108].

8. miRNA-mediated mechanisms underlying respiratory infection-induced severe steroid-resistant asthma and compromised anti-viral innate immune responses

Respiratory tract infections caused by *Chlamydia*, *Haemophilus influenzae*, influenza, and respiratory syncytial virus induce miR-21-mediated severe steroid-insensitive asthma. After respiratory tract infection one of these pathogens in a mouse model, miR-21 levels increased in ovalbumin-induced allergic asthma. Overexpressed miR-21 directly inhibited PTEN while inducing PI3K/Akt activity that mediated HDAC2 suppression [109].

Heightened susceptibility to viral infection and virus-induced exacerbations in individual asthmatic patients results from an impairment in the innate anti-viral immune response of type I interferon (IFN) production [110]. Several defects in the immune axis related to type I IFN responses cause severely impaired functions in inflammatory cells. These defects occur in AEC, CD8⁺ T cells, plasmacytoid DCs, and recruited monocytic cells, in cooperation with potent suppressive effects of eosinophils and alternatively activated macrophages on type I IFN production [110]. In addition, a newly reported mechanism underlying defective type I IFN expression has recently been explored in alveolar macrophages. In healthy subjects, normal expression of TLR7 by alveolar macrophages keeps anti-viral type I IFN production in check [111]. Alveolar macrophages could not perform this function in severe asthmatic patients, since upregulation of a diverse network of miRNAs including miR-150, miR-152, and miR-375 caused reduced expression of TLR7 [111]. This upregulation resulted in high viral loads, abundant airway inflammation, and inescapably prolonged asthma exacerbations.

9. Conclusions

The concept that miRNAs are critical to pathogenesis of severe asthma via airway inflammation and hyperresponsiveness, is now widely accepted and is supported by a large amount of data. (Fig. 2 and Table 1) Although diverse, rather than single, miRNAs are key to biological dysfunction underlying severe asthma, prioritizing the importance of individual miRNAs is important in the context of severe human asthma, and the associated search for the best miRNA inhibitors as a novel therapeutic agents for severe asthma. A major area of research in the future, now made possible by advances in genomic technologies, will be testing the therapeutic efficacy of appropriate antagomiRs in silencing miRNA expression.

Conflicts of interest

The author has no conflicts of interest to declare.

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