

MicroRNA in Chronic Rhinosinusitis and Allergic Rhinitis

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Abstract Inflammatory upper airway diseases, particularly chronic rhinosinusitis (CRS) and allergic rhinitis (AR), have a high worldwide prevalence. CRS and AR involve sustained and exaggerated inflammation that is associated with marked changes in gene and protein expression under tight regulation. A novel group of gene expression regulators is a class of short single-stranded RNA molecules termed microRNAs (miRNAs). miRNAs can cause gene silencing through degradation of target mRNAs or inhibition of translation. Dysregulated expression of miRNAs has been shown in various human diseases, such as cancer, inflammatory skin and bowel diseases, rheumatoid arthritis, and asthma. Although studies of miRNAs in inflammatory upper airway diseases are relatively new and few, emerging evidence implicates an involvement of miRNAs in shaping the inflammation pattern in upper airways. The purpose of this review is to provide an overview on our current understanding of miRNA expression and function in CRS and AR, and to underscore the potential for clinical usage of miRNAs in CRS and AR.

Keywords Allergic rhinitis · Chronic rhinosinusitis · Eosinophil · MicroRNA · Inflammation · Innate immunity · Biogenesis

Introduction

MicroRNAs (miRNAs) are recently discovered small non-coding RNA molecules that are only 18–22 nucleotides in length and are highly conserved throughout evolution [1]. miRNAs represent one of the fundamental epigenetic regulatory mechanisms used by cells that can mediate posttranscriptional gene silencing of target genes [2]. In mammals, the 5' end of mature miRNAs (the seed sequence) can bind to the 3'-untranslated region (3'-UTR) of their target mRNAs through at least 6- to 8-nucleotide-long complementary sequences [2]. This interaction is able to suppress target gene expression by degradation of mRNA or inhibition of translation initiation, or both, depending on the level of complementarity [3]. More than 1,000 miRNAs are encoded in the human genome (<http://www.mirbase.org/>). The expression of miRNAs is often tissue-specific and developmentally controlled [4]. Given the very short complementarity to the target mRNA, a single miRNA can target hundreds of genes [5]. An approximately 30 % of genes are thought to be subject to miRNA regulation [6]. On the other hand, individual genes may be targeted by multiple miRNAs, adding complexity to the regulatory network between miRNAs and target genes [7].

As fine tuning regulators of gene expression, miRNAs are involved in diverse biologic processes, including cell proliferation, apoptosis, and differentiation, organ development, metabolism, stress responses, and signal transduction [8, 9]. It is now apparent that abnormal miRNA expression is a common feature of various diseases. Aberrantly expressed miRNAs are primarily shown to participate in the development of multiple cancers [10, 11]. More recently, the important role of miRNAs in inflammation is highlighted by studies in which dysregulation of miRNAs has been demonstrated to accompany diseases associated with excessive inflammation and aberrant immune responses, such as psoriasis, rheumatoid arthritis, pulmonary fibrosis, and allergic asthma [12–16].

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Chronic rhinosinusitis (CRS) and allergic rhinitis (AR) are two widely prevalent inflammatory diseases in upper airways that severely impact the quality of life of affected individuals and are strongly associated with inflammatory lower airway diseases [17, 18]. Exaggerated and sustained airway inflammation is the hallmark of inflammatory upper airway diseases that require the interplay of different immune and resident cells accompanied by marked changes in gene and protein expression. [19–21]. Therefore, a better understanding of the complex regulatory mechanisms that are involved in inflammatory gene expression in CRS and AR will be critical to developing new approaches to treatment. Principal among these is the miRNA network. Although the evaluation of miRNA expression and function in patients with CRS or AR has received relatively little attention, a growing number of miRNAs have been implicated in tuning of inflammation and immune responses in CRS and AR in recent years. In this review, we outline the current knowledge of miRNA expression and function in CRS and AR, and underscore their potential as novel targets for the treatment and biomarkers for the diagnosis of CRS and AR.

MiRNA Biogenesis and Regulation

As illustrated in Fig. 1, human miRNAs genes are present in the non-protein-coding regions in genomic DNA and introns of protein-coding genes [8]. The generation and function of miRNAs require a set of proteins collectively referred to as the miRNA machinery [22]. Mature miRNA species can be generated from two sources [23]. One is that the mature miRNAs are derived from primary miRNAs (pri-miRNAs) transcribed from specific miRNA encoding regions of the genome [23]. Long pri-miRNA transcripts (generally >1 kb long) are initially transcribed by RNA polymerase II or III and then the RNase III endonuclease, Drosha, and its cofactor DiGeorge syndrome critical region gene 8 process pri-miRNAs into 60–70-nucleotide hairpin miRNA precursors (pre-miRNAs) in the nucleus [23]. The other source is from intronic sequences of protein-coding genes (mirtrons) that do not require Drosha processing. These miRNAs are co-transcribed with protein-coding genes and then spliced and debranched by lariat debranching enzyme to generate pre-miRNAs [24]. No matter what source that the pre-miRNAs originate from, the pre-miRNAs are exported into the cytoplasm and another RNase III enzyme, Dicer, cleaves these pre-miRNAs into mature miRNA [24]. One strand of this mature miRNA duplex is incorporated into the RNA-induced silencing complex (RISC) and guides the RISC complex to its targets [25]. Assembled into the RISC, the miRNA regulate gene expression by base pairing of the seed sequence to the 3'-UTR of target mRNA. Depending on the level of complementarity between miRNA and its target site, target mRNA degradation, translational

repression, or both occur, with imperfect base pairing favoring translational repression [7]. Recently, it has been estimated that about 84 % of miRNA-mediated gene repression is resulted from reduced mRNA levels in mammals, which exceeds previous estimates [25]. Human immunodeficiency virus transactivating response RNA-binding protein (TRBP) and protein activator of the interferon-induced protein kinase (PACT) interact with each other and associate with Dicer to promote the cleavage of pre-miRNA [26]. Depletion of TRBP affects the pre-miRNA processing and decreases the level of mature miRNA [27, 28, 29••]. Although PACT is not indispensable for the pre-miRNA cleavage reaction step, suppressing PACT strongly affects the accumulation of mature miRNA in vivo and moderately reduces the efficiency of miRNA-induced RNA interference [29••, 30]. Argonaute 2/eukaryotic translation initiation factor 2C,2 (EIF2C2) has been recognized as the catalytic center of RISC that is important for cleavage of the target mRNA [29••, 31].

The expression and function of miRNAs are under stringent regulation by multiple mechanisms at multiple levels. The miRNA expression can be affected by chromosomal abnormalities, gene mutations, gene single nucleotide polymorphisms and methylation status, dysregulation of transcriptional regulatory factors, and defect in miRNA machinery and transportation of pre-miRNAs out of the nucleus [32]. Polymorphisms within the 3'-UTR region of the target mRNA may compromise miRNA binding, thus influencing the target gene expression [33, 34].

MiRNAs in CRS

Chronic rhinosinusitis (CRS) is a heterogeneous group of sinus diseases [35, 36]. Based on the absence or presence of nasal polyps, CRS is currently divided into two types: CRS without nasal polyps (CRSsNP) and CRS with nasal polyps (CRSwNP) with distinctive expression of inflammatory and remodeling mediators [37–40]. CRSsNP is characterized by a Th1 milieu, whereas CRSwNP is featured by a Th2-biased eosinophilic inflammation [37–39]. Eosinophilic inflammation has been considered as a major pathologic hallmark of CRSwNP. It has been found in 65–90 and 50 % of total CRSwNP cases in white and Chinese individuals, respectively [35–37]. The etiology of CRS remains a matter of vigorous debate. A few emerging studies in miRNAs in CRS implicate the involvement of miRNA in shaping the inflammation pattern in CRS.

MiRNA Expression in CRS

Using miRNA arrays, we were the first to identify the miRNA expression profiles in CRSsNP and eosinophilic CRSwNP and found distinct expression profiles between CRSsNP and

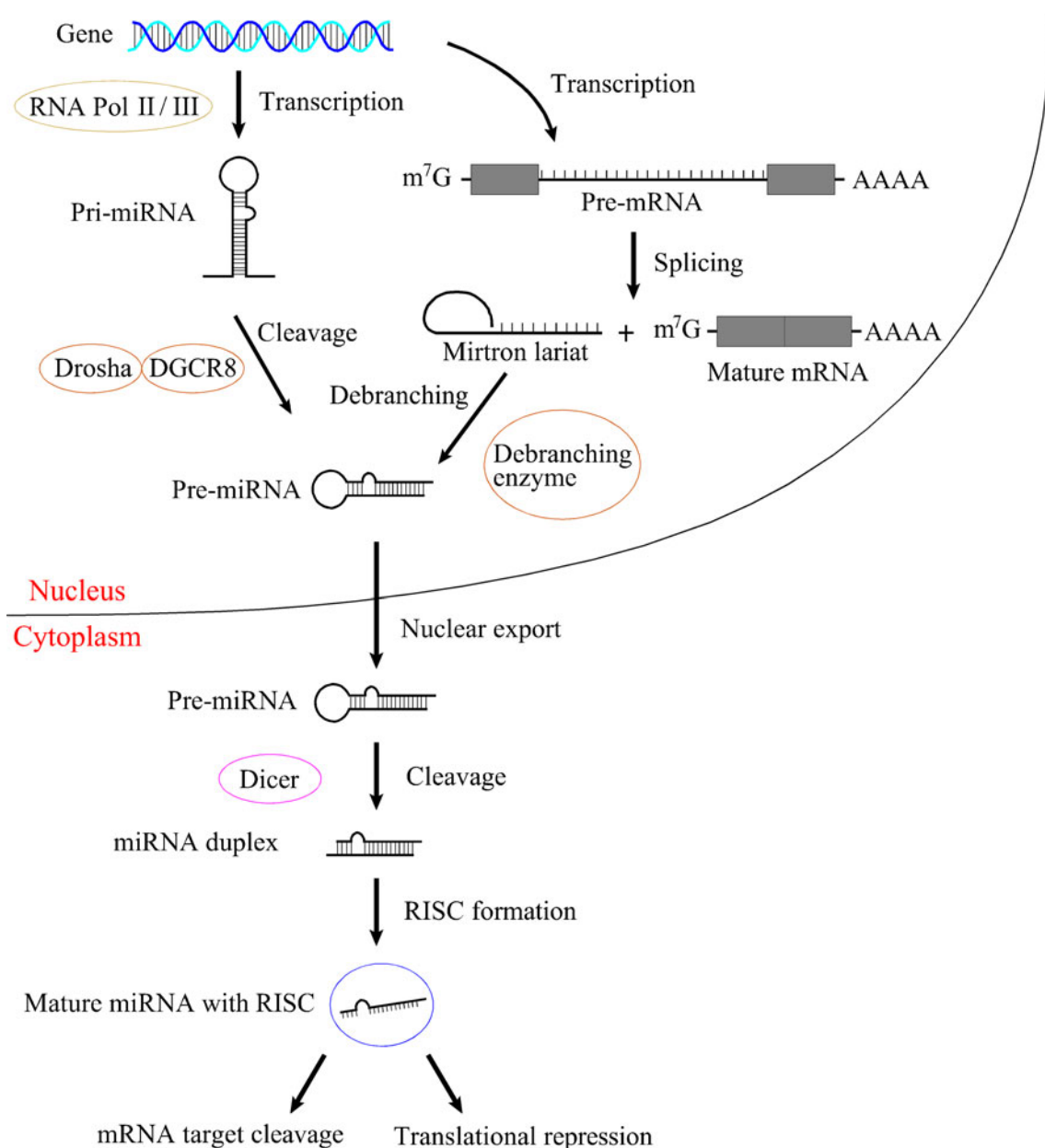


Fig. 1 Biogenesis and mechanism of action of miRNAs. Mature miRNA species can be generated from two sources. One is that the mature miRNAs are derived from primary miRNA (pri-miRNAs), which are transcribed by RNA polymerase II or III from specific miRNA encoding regions of the genome. And then the RNase III endonuclease, Drosha, and its cofactor DiGeorge syndrome critical region gene 8 (DGCR8) cleave pri-miRNAs into 60- to 70-nucleotide hairpin miRNA precursors (pre-miRNAs) in the nucleus. The other source is derived from intronic sequences of protein-coding genes (mirtrons), which are generated from

pre-mRNA and then spliced and debranched by lariat debranching enzyme to generate pre-miRNAs without requiring Drosha processing. No matter what source that the pre-miRNAs originate from, the resulting pre-miRNAs are exported into the cytoplasm and another RNase III enzyme, Dicer, cleaves these pre-miRNAs into mature miRNA. One strand of this mature miRNA duplex is incorporated into the RNA-induced silencing complex (RISC). Assembled into the RISC, the miRNA regulate gene expression by base pairing of the seed sequence to the 3'-UTR of target mRNA, followed by target mRNA degradation or translational repression

eosinophilic CRSwNP [41••]. Compared with control subjects, 31 differentially expressed miRNAs (30 down-regulated and 1 up-regulated miRNA) in eosinophilic CRSwNP and 4 differentially expressed miRNAs (2 down-regulated and 2 up-regulated miRNAs) in CRSsNP were found [41••]. Except for miR-26a, which was down-regulated in both CRSsNP and eosinophilic CRSwNP, there

were no other overlapping miRNAs in CRSsNP and eosinophilic CRSwNP [41••]. The distinct expression patterns of miRNAs in different subtypes of CRS indicate different endotypes for this heterogeneous disease. The only up-regulated miRNA in eosinophilic CRSwNP as revealed by microarray, miR-125b, was selected for the mechanistic study. Quantitative RT-PCR and in situ hybridization showed that

miR-125b was up-regulated in eosinophilic CRSwNP as compared with control, CRSsNP, and non-eosinophilic CRSwNP, suggesting an association between miR-125b and eosinophilic inflammation [41•]. It was also found that miR-125b was mainly produced by epithelial cells of sinonasal mucosa [41•]. In silico analysis identified eukaryotic translation initiation factor 4E binding protein 1 (4E-BP1) as one of the potential targets of miR-125b, which is an important downstream element of phosphatidylinositol-3 kinases/serine/threonine kinase Akt/mammalian target of rapamycin pathway and a negative regulator of type I interferon (INF) production by translation repression of interferon regulatory factor (IRF-7) [42]. Transfection and reporter assays verified 4E-BP1 as an understream target of miR-125b with miR-125b having the ability to suppress 4E-BP1 protein expression [41•]. Combining both in vivo and in vitro experiments, we showed that miR-125b induced the production of type I IFN via targeting 4E-BP1 in airway epithelial cells [41•]. Type I IFN expresses various biologic activities besides its antiviral function and has been implicated in both Th1 and Th2 responses. Consistent with previous study [43], we found that type-I IFN was able to induce B-cell activating factor (BAFF) production [41•], which is an important regulator of immunoglobulin class-switch recombination and production in B lymphocytes [43, 44]. Increased BAFF has been found in patients with CRSwNP and may contribute to the local activation of eosinophils through inducing IgA production [44]. We found that the expression of type I INF correlated with eosinophil infiltration in sinonasal mucosa, supporting a role of type I INF in eosinophilic inflammation in CRS [41•]. Type I INF is mainly induced by viral infection. The role of respiratory virus infection in CRS remains debated [45]. We did not find a significant difference in common respiratory virus infection in our cohort of CRS patients compared with controls [41•, 45]; however, the overexpression of miR-125b may lead to enhanced expression of type I INF, which may contribute to subsequent eosinophilic inflammation in eosinophilic CRSwNP [41•]. In our study, we revealed that the expression of miR-125b in nasal epithelial cells could be up-regulated by dsRNA, LPS, and IL-10 [41•]. Our study discovered a novel and important role for miR-125b in innate antiviral immunity and in shaping inflammation pattern in airways (Fig. 2). However, the cause–effect relationship between viral infection and miR-125b–4E-BP1–type I IFN pathway and eosinophilic CRSwNP needs to be tested in vivo, which is currently hampered by the lack of suitable animal models of CRS [41•]. Our findings indicate an attracting role of miRNAs in the development of CRS that need further researches.

MiRNA Machinery Component Expression in CRS

miRNA machinery protein expression is critical for the metabolism and homogenesis of the miRNAs under normal and

pathological conditions [26–28, 29•]. Disturbance of miRNA machinery proteins can lead to the abnormalities of the expression and function of miRNAs and subsequent dysfunction of cells. miRNA machinery abnormalities have been implicated in the carcinogenesis and regulation of immune cell function. Dicer and PACT, and fragile X mental retardation protein 1 (FXR1) levels have been found to be up-regulated in various cancer [22, 29•, 46]. Drosha and Dicer have been implicated in immune regulation and prevention of lethal inflammatory diseases [29•, 47]. Treg cell-mediated tolerance has been suggested to be critically dependent on the Dicer-controlled miRNA metabolism [29•, 48]. We explored the expression of the major miRNA machinery components in sinonasal mucosa from normal controls, CRSsNP, eosinophilic CRSwNP, and non-eosinophilic CRSwNP patients, which include Drosha, Dicer, PACT, TRBP, FXR1, and EIF2C2 [29•]. We discovered no significant difference in mRNA expression levels of miRNA machinery components except PACT among different study groups [29•]. The immunohistochemistry study confirmed the increased protein expression of PACT in CRSwNP as compared with controls and CRSsNP. A further significant increase in PACT protein expression was found in eosinophilic CRSwNP as compared with non-eosinophilic CRSwNP [29•]. PACT was found mainly expressed in CD138+ plasma cells [29•]. The up-regulated local production of immunoglobulins has been shown in CRSwNP, and certain miRNAs, such as miRNA-155, have been associated with the regulation of the generation of immunoglobulin class-switched plasma cells [49]. However, whether the cell-specific expression pattern of PACT can affect miRNA generation and immunoglobulin production in plasma cells needs further studies. Currently, our study, at least, underscores an association between miRNA machinery protein and inflammatory reaction in CRSwNP.

MiRNAs in AR

There are three reports about miRNA expression profiles in AR. Two of them have examined miRNA profiles in nasal biopsy and one investigated miRNA expression in cord blood (CB) cells obtained from AR patients [50•, 51, 52•]. Yu S et al. compared the miRNA expression profiles in nasal mucosa from AR patients and nonallergic controls by means of microarray [50•]. They identified 9 differentially expressed miRNAs with more than a 2-fold change including 2 up-regulated miRNAs and 7 down-regulated miRNAs. Further quantitative RT-PCR assay confirmed the down-regulation of hsa-miR-224, hsa-miR-187, and hsa-miR-143 in AR [50•]. Employing quantitative RT-PCR, Soujalehto H et al. measured the expression levels of selected 35 miRNAs that were formerly identified as differentially expressed miRNAs in AR in Yu's study or considered to relate to inflammation or

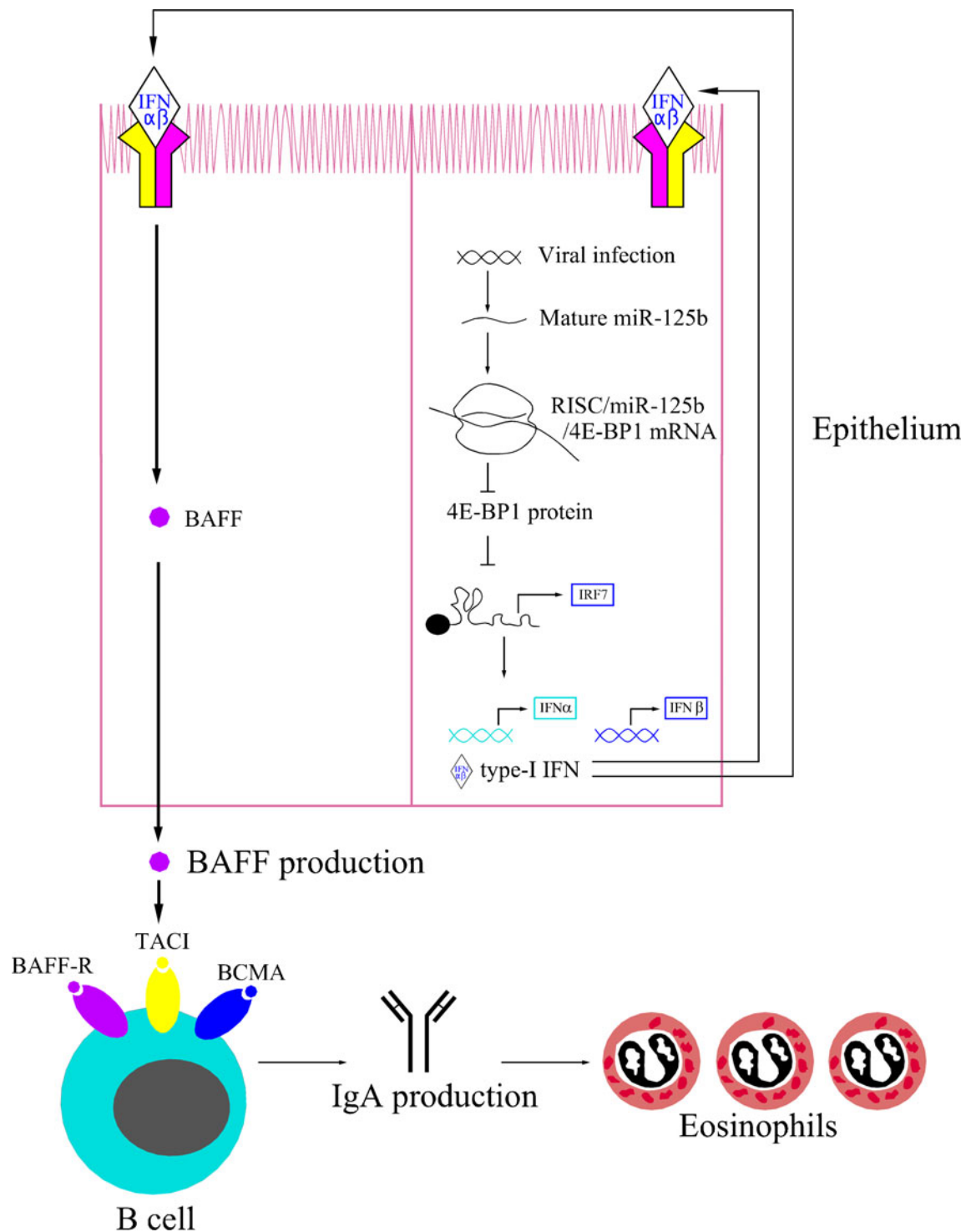


Fig. 2 Summary of the the role of miR-125b in innate antiviral immunity in airways. Viral infection up-regulates miR-125b expression in epithelial cells, which leads to eukaryotic translation initiation factor 4E binding protein 1(4E-BP1) translational silence and rescues interferon regulatory factor (IRF)-7 translational repression. The increased IRF-7 expression can up-regulate type I interferon (IFN) gene transcription. Type-I IFN is able to induce B cell-activating factor (BAFF) secretion from epithelial

cells, which binds to the 3 receptors that are selectively expressed on B cells and plasma cells, including BAFF receptor (BAFF-R), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI), and B-cell maturation antigen (BCMA). The interaction between BAFF and its receptors can promote the IgA production from B cells and plasma cells and then contributes to the local activation of eosinophils. RISC, RNA-induced silencing complex

immunological responses by earlier studies [51]. They found that subjects with current AR symptoms had increased levels

of miR-155, miR-205, and miR-498, but reduced levels of let-7e in nasal mucosa. They also found that patients with positive

skin prick test showed increased miR-155 and miR-205 and decreased let-7a expression as compared to subjects with negative skin prick test [51]. Therefore, whether the expression of these miRNAs is intrinsically related to AR or atopy awaits further investigations. It is confusing that we can see more discrepancies than consistence in miRNA profiles in AR between Yu's and Soujalehto's study. The underlying reasons are unclear, but may be related to the heterogeneity of the tissue. Although studying the miRNA expression profiles in tissue can provide us with an overview of the gene regulation in diseased mucosa, there may be a significant degree of cellular heterogeneity across individual biopsies. The other limitation of their studies is that the functions of aberrantly expressed miRNAs in AR have not been investigated. Chen RF et al. screened for the expression of a panel of 157 miRNAs in mononuclear leucocytes from human umbilical CB samples and analyzed the association of miRNAs expression and development of AR [52••]. They found that miR-21 and miR-126 levels were significantly lower in CB with elevated IgE levels and in monocytes from AR children [52••]. Transfection of miR-21 precursor into monocytes suppressed transforming growth factor beta receptor 2 (TGFBR2) expression, suggesting a role of miR-21 to regulate TGFBR2 [52••]. TGFBR2 is critical for the TGF- β function. A significant increase in TGFBR2 expression in nasal epithelium from AR patients has been reported previously [53]. Chen showed that TGFBR2 expression on monocytes was significantly up-regulated in CB with elevated IgE levels and in AR patients. These results indicated the regulatory association between miR-21 and TGFBR2 in AR, and that miR-21 could potentially be an early predictor of AR. However, the association of miR-21 down-regulation and the development of atopy and AR is contrast to its known function in suppressing IL-12 expression [54]. Therefore, further studies on the tissue- and cell-specific effects of miR-21 in AR patients are needed.

Clinical perspective

Compared with mRNAs and even proteins, miRNAs are in a relatively stable form, which make miRNAs a potential candidate as noninvasive biomarkers. The preclinical studies on cancer offer an exciting possibility of miRNAs acting as biomarkers for disease diagnosis, stratification, and monitoring [11, 55, 56]. The finding that miR-21 has the potential to facilitate the diagnosis of AR in newborn also highlights the possibility of miRNAs as biomarkers for diagnosis and prognosis of inflammatory upper airways diseases [52••]. Suppression of overexpressed miRNAs or restoration of down-regulated miRNAs in different disease conditions may be of therapeutic profit. Intravenous or local delivery of synthetic miRNAs and their inhibitors in mice has been shown little evidence of toxicity [57]. Currently, there is a lack of in vivo

study regarding the therapeutic effect of miRNAs in inflammatory upper airway diseases. However, therapeutic benefits of miRNAs have been demonstrated in other diseases. Silencing of miR-122 has been shown to repress hepatitis C virus replication in primates, and miR-122-targeting treatment in patients with chronic hepatitis C has already reached the clinical trial [58]. In asthma, a potential therapeutic application of miRNA has been demonstrated in animal models. For example, administration of anti-miR-145 decreased airway hyperreactivity and mucus production but had no effect on eosinophil infiltration in an experimental allergic asthma model [59]. Inhibition of miR-106a by antagomiRs attenuated experimental asthma severity after the asthma phenotype had been firmly established [60]. There is no doubt that the translational researches on the clinical usage of miRNAs in inflammatory upper airway diseases will finally change our approach toward the diagnosis, prognosis, and treatment of AR and CRS. Nevertheless, despite the promising efficacy and safety of miRNAs, the efficacy and specificity of the chosen miRNAs/anti-miRNAs, their ability to be effectively delivered to the disease site, and their chronicity once they reach their intended location, are all aspects for intense development in future.

Conclusions

Although a number of miRNAs have been associated with CRS and AR, our knowledge of the role of miRNAs in inflammatory upper airway diseases are still in its infancy. The results from different studies are inconsistent, the number of patient population in these studies is relatively small, the function pathways of most miRNAs have not been established, and therapeutic effect of these miRNAs in CRS and AR is unknown. Therefore, it is critical to address two important challenges in future studies: (1) identification of miRNAs that are consistently dysregulated in CRS and AR; and (2) to identify the key targets of miRNAs and the importance of the miRNA/mRNA network in the pathogenesis of CRS and AR. The identification of regulatory role for miRNAs in inflammatory upper airway diseases may not only decipher new elements of the pathogenesis of inflammatory upper airway diseases but also provide new insights into the diagnosis and development of new therapeutic strategies.

Compliance with Ethics Guidelines

Conflict of Interest This study was supported by National Natural Science Foundation of China (NSFC) grant 81325006 and 81020108018 to Z.L., and a grant from Ministry of Health of China (201202005).

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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