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Forum Minireview

MicroRNAs and Their Therapeutic Potential for Human Diseases: MiR-133a and Bronchial Smooth Muscle Hyperresponsiveness in Asthma

Yoshihiko Chiba[1,*] and Miwa Misawa[1]

[1]*Department of Pharmacology, School of Pharmacy, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan*

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Abstract. MicroRNAs (miRNAs) play important roles in normal and diseased cell functions. The small-GTPase RhoA is one of the key proteins of bronchial smooth muscle (BSM) contraction, and an upregulation of RhoA has been demonstrated in BSMs of experimental asthma. Although the mechanism of RhoA upregulation in the diseased BSMs is not fully understood, recent observations suggest that RhoA translation is controlled by a miRNA, miR-133a, in cardiomyocytes. Similarly, in human BSM cells (hBSMCs), our recent studies revealed that an upregulation of RhoA was induced when the function of endogenous miR-133a was inhibited by its antagomir. Treatment of hBSMCs with interleukin-13 (IL-13) caused an upregulation of RhoA and a downregulation of miR-133a. In a mouse model of allergic bronchial asthma, increased expression of IL-13 and RhoA and the BSM hyperresponsiveness were observed. The level of miR-133a was significantly decreased in BSMs of the diseased animals. These findings suggest that RhoA expression is negatively regulated by miR-133a in BSMs and that the miR-133a downregulation causes an upregulation of RhoA, resulting in an augmentation of the contraction. MiR-133a might be a key regulator of BSM hyperresponsiveness and provide us with new insight into the treatment of airway hyperresponsiveness in asthmatics.

Keywords: microRNA (miRNA), miR-133a, RhoA, bronchial smooth muscle, airway hyperresponsiveness, allergic bronchial asthma

1. Introduction

MicroRNAs (miRNAs) are a recently discovered class of small, noncoding, single-stranded RNAs that control expression of complementary target messenger RNAs (mRNAs) (1, 2). The mature miRNAs comprise about 22 nucleotides, derived from long transcripts primary miRNAs (pri-miRNAs) and precursor miRNAs (pre-miRNAs). There is increasing evidence that the mature miRNAs negatively modulate gene expression primarily through base pairing to the 3′ untranslated region (UTR) of target mRNAs, resulting in mRNA cleavage and/or translation repression (1 – 3). Evidence also suggests a role for miRNAs in a variety of basic biological functions, including normal immune function (4) and lung development (5). In addition, an implication of miRNAs has been suggested in diverse diseases including airway diseases such as chronic obstructive pulmonary disease (COPD) (6) and asthma (4, 7 – 10). It is thus possible that miRNAs are attractive new drug targets for the treatment of airway diseases.

In the current brief review, we will discuss the role of miR-133a in the development of bronchial smooth muscle (BSM) hyperresponsiveness, one of the characteristic features of allergic bronchial asthma.

2. Airway smooth muscle in asthma

The dramatic increase in the number of asthma cases over the last decades is of great concern for public health world-wide (11). Increased airway narrowing in response to nonspecific stimuli is a characteristic feature of human

*Corresponding author. chiba@hoshi.ac.jp
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obstructive pulmonary diseases, including bronchial asthma. This abnormality is an important sign of the disease, although the pathophysiological variations leading to the hyperresponsiveness remain unclear. It has been suggested that one of the factors that contribute to the exaggerated airway narrowing in asthmatics is an abnormality in the properties of airway smooth muscle (12). Rapid relief from airway limitation in asthmatic patients by β-stimulant inhalation may also suggest an involvement of augmented airway smooth muscle contraction in the airway obstruction. Thus, it may be important for development of asthma therapy to understand changes in the contractile signaling of airway smooth muscle cells associated with the disease.

Asthmatic patients have an increased contractility of airway smooth muscle (13). Asthmatic animal models also have hyperresponsiveness of airway smooth muscles (14, 15). Similarly, an increased responsiveness of bronchial smooth muscle has been demonstrated in a rat model of airway hyperresponsiveness induced by repeated antigen inhalation (16 – 19). In this animal model of airway hyperresponsiveness, the bronchial smooth muscle contraction induced by receptor agonists such as acetylcholine (ACh), but not by high K^+ depolarization, is markedly augmented (16 – 18). Similar results were also obtained in a mouse model of allergic bronchial asthma (20, 21). Moreover, it has also been demonstrated that muscarinic receptor density and antagonist affinity of airway smooth muscle are at normal levels (17). Thus, it is possible that the mechanisms responsible for the airway hyperresponsiveness exist, at least in part, in the downstream pathway of muscarinic receptor signaling, including agonist-mediated Ca^{2+} sensitization.

3. RhoA and BSM hyperresponsiveness

The Ca^{2+} sensitization of airway smooth muscle has been reported in canine (22), porcine (23) and rabbit tracheae (24), and human bronchus (24, 25). Likewise, we have also demonstrated that the Ca^{2+} sensitization is inherent in BSMs of rats (26) and mice (20), as determined by using permeabilized muscle strips. Since the Ca^{2+} sensitization induced by ACh is sensitive to C3 exoenzyme (20, 26) and Y-27632 (27), the RhoA/Rho-kinase pathway is involved in the signaling. RhoA and Rho-kinases are also expressed in BSMs of these animals (20, 26, 27). Activation of RhoA by ACh stimulation has also been demonstrated in the bronchial smooth muscles of rats (28) and mice (20). When the RhoA/Rho-kinase system is activated by contractile agonists, the activity of myosin light chain (MLC) phosphatase is reduced and the level of phosphorylated MLC is then increased, resulting in an augmentation of contraction. Interestingly, recent studies demonstrated that the agonist-induced, RhoA/Rho-kinase–mediated Ca^{2+} sensitization of BSM contraction is augmented in animal models of allergic bronchial asthma (20, 26). An importance of the RhoA/Rho-kinase system has also been demonstrated in human BSM (24), and the signaling of RhoA and its downstream Rho-kinases are now considered as a therapeutic target for the treatment of airway hyperresponsiveness in asthma (29).

As described above, the RhoA-mediated Ca^{2+} sensitization of BSM contraction is augmented in experimental asthma models (20, 26). An upregulation of RhoA has also been demonstrated in BSMs of these animal models of allergic bronchial asthma (20, 26). However, the mechanism(s) responsible for the upregulation of RhoA has yet to be elucidated.

4. Negative regulation of RhoA expression by miR-133a in BSM

The miR-133 family of miRNAs is the most highly expressed miRNAs in cardiac myocytes (30). Mature miR-133a is generated by processing of its pre-miRNAs, miR-133a-1, and miR-133a-2, that are transcripts of different genes. Reportedly, miR-133a-1 and miR-133a-2 are specifically expressed in skeletal muscle and cardiac myocytes (31). Although its expression in BSM was not determined by these investigators, our previous study using real-time PCR analyses revealed an expression of mature miR-133a in hBSMCs and mouse BSM tissues (32). The mature sequences of miR-133a and miR-133b referred from the database, miRBase (http://microrna.sanger.ac.uk/) (33), are shown in Table 1. There is no species difference between human and mouse in the mature sequence of miR-133a or miR-133b, but the expression of mature miR-133a*, a minor miRNA derived from precursors of miR-133a-1 and miR-133a-2, is not suggested in human (33).

To date, the transcriptional/translational mechanism of RhoA is not well understood. However, a negative regulation of RhoA expression by miR-133 has recently been suggested in cardiomyocytes (31). The RNA-hybrid analyses (http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/) (34) of human and mouse mRNAs for RhoA revealed putative binding sites of miR-133a in their 3′-UTRs (32). Based on these information, we have recently tested the hypothesis that downregulation of miR-133 may be a cause of the upregulation of RhoA in BSM.

As a result (32), an upregulation of RhoA protein was observed when the function of endogenous miR-133a was inhibited by its inhibitor, called antagomir-133a, in hBSMCs. Conversely, the hBSMCs transfected with precursor miR-133a (pre-miR-133a) showed a down-

regulation of RhoA protein. On the other hand, neither antagomir-133b nor pre-miR-133b had significant effect on RhoA protein expression. These findings indicate that miR-133a, but not miR-133b, is an endogenous modulator of RhoA protein expression, and that RhoA protein expression is negatively regulated by endogenous miR-133a in hBSMCs.

Our previous studies revealed that interleukin-13 (IL-13), one of the major cytokines upregulated in the airways of asthmatics (35, 36), is capable of induction of RhoA protein upregulation in hBSMCs (37, 38) and of induction of airway hyperresponsiveness in naive mice (37). IL-13 also caused an upregulation of RhoA mRNA and a downregulation of miR-133a in hBSMCs (32). The IL-13–induced upregulation of RhoA mRNA was abolished by coincubation with a STAT6 inhibitor, whereas, interestingly, this treatment had no effect on the downregulation of miR-133a induced by IL-13 (32). These observations suggest that IL-13 is capable of induction of RhoA protein upregulation both by increasing RhoA mRNA via STAT6-dependent mechanism and by increasing RhoA translation via the STAT6-independent downregulation of miR-133a in hBSMCs.

Previous evidence that IL-13 is a key factor for induction of BSM hyperresponsiveness and upregulation of RhoA protein in mouse model of allergic bronchial asthma (37) reminds us that miR-133a might also be downregulated in BSMs of the diseased animals. To test the idea, we also determined the expression level of miR-133a in BSM tissues of the repeatedly antigen-challenged mice. As a result, a downregulation of miR-133a was observed in BSM tissues of the antigen-challenged mice (32). Taken together, the findings suggested that miR-133a might also negatively modulate the expression of RhoA gene in the mouse BSM, and the downregulation of miR-133a might be a cause of upregulation of RhoA protein in the mouse model of allergic bronchial asthma. It is thus possible that introduction of synthetic miR-133a and/or its mimics specifically into the BSMs of asthmatics might cause a downregulation of RhoA protein and

Table 1. Mature sequence of miRNAs

ID	Accession	Sequence
hsa-miR-133a	MIMAT0000427	5′-UUUGGUCCCCUUCAACCAGCUG-3′
mmu-miR-133a	MIMAT0000145	5′-UUUGGUCCCCUUCAACCAGCUG-3′
mmu-miR-133a* (†)	MIMAT0003473	5′-GCUGGUAAAAUGGAACCAAAU-3′
hsa-miR-133b	MIMAT0000770	5′-UUUGGUCCCCUUCAACCAGCUA-3′
mmu-miR-133b	MIMAT0000769	5′-UUUGGUCCCCUUCAACCAGCUA-3′

The sequences are from the miRBase, Release 12.0 (http://microrna.sanger.ac.uk/) (33). hsa: *Homo sapiens*, mmu: *Mus musculus*. (†)Expression of mature miR-133a* (a minor miRNA) is not suggested in humans (33).

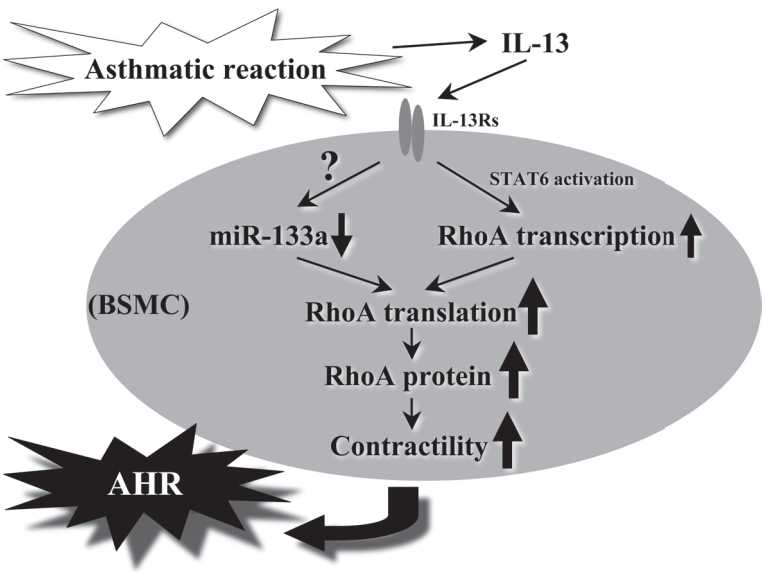

Fig. 1. A schematic representation of development of bronchial smooth muscle hypercontractility in allergic bronchial asthma. The asthmatic reaction causes an increase in Th2 cytokines such as interleukin-13 (IL-13) in the airways. The released IL-13 activates its receptors (IL-13Rs) on bronchial smooth muscle cells (BSMCs) and then increases RhoA transcription STAT6-dependently and decreases a miRNA, miR-133a, STAT6-independently. Both the upregulation of RhoA mRNA and the downregulation of miR-133a cause an upregulation of RhoA protein, resulting in an augmentation of the contraction, one of the causes of airway hyperresponsiveness (AHR) in asthmatics. See text for details.

an amelioration of BSM hyperresponsiveness, resulting in a suppression of airway hyperresponsiveness.

In addition to the miR-133a, recent studies also suggest that RhoA protein expression is negatively regulated by miR-155 (39) and miR-31 (40, 41) in cancer cells. Although their expression and function in smooth muscle cells have not been determined to date, whether these miRNAs also downregulate RhoA protein in the BSMCs is of great interest.

5. Conclusion

In conclusion, it has been suggested that RhoA protein expression is negatively regulated by miR-133a in BSMs. IL-13 is capable of reducing the miR-133a expression in BSMs. In BSMs of the repeatedly antigen-challenged mice, the miR-133a downregulation causes an upregulation of RhoA, presumably resulting in an augmentation of the contraction (Fig. 1). An importance of RhoA and its downstream Rho-kinases was also demonstrated in contraction of human BSM (24). The RhoA/Rho-kinase pathway has now been proposed as a new target for the treatment of airway hyperresponsiveness in asthma (29, 42 – 44). Thus, the findings might provide us with new insight into the treatment of airway hyperresponsiveness.

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