miR-487b mitigates allergic rhinitis through inhibition of the IL-33/ST2 signaling pathway

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Abstract. – OBJECTIVE: To investigate the potential effect of miR-487b/IL-33-ST2 axis on the pathology of allergic rhinitis (AR) and the relevant mechanism.

PATIENTS AND METHODS: The expression level of interleukin-33 (IL-33), a homolog of sulfotransferase (ST2), and miR-487b were detected in patients with or without allergic rhinitis. Luciferase assay was performed to evaluate the interaction between miR-487b and IL-33, and the effects of miR-487b/IL-33-ST2 axis on allergic rhinitis mice were determined by established allergic rhinitis model in mice by ovalbumin (OVA). The levels of OVA-specific immunoglobulin E (Ig-E), proinflammatory cytokines (IL-4, IL-5, and IL-13), and pathological alterations were detected.

RESULTS: The level of IL-33 and its specific ligand ST2 were found increased in allergic rhinitis patients while miR-487b expression level was markedly repressed. To confirm whether miR-487b has a regulation effect on IL-33, we checked it in three publicly available algorithms, TargetScan, miRDB, and microRNA. We found that IL-33 is a direct target of miR-487b, and Luciferase assays confirmed our hypothesis, the subsequent experiments showed that up-regulation of miR-487b could inhibit expression of IL-33 and ST2, resulting in the decrease of the immunoglobulin E (Ig-E), proinflammatory cytokines and mitigation of pathological alterations.

CONCLUSIONS: Our research discovered the suppressor function of miR-487b in allergic rhinitis and revealed that miR-487b/IL-33-ST2 axis may be a potential therapeutic target for the treatment of allergic rhinitis.

Key Words: Interleukin-33 (IL-33), Homolog of sulfotransferase (ST2), miR-487b, Allergic rhinitis.

Introduction

Allergic rhinitis (AR) is a common and frequent disease of the respiratory system, which not only seriously endangers people’s health, but also induces a variety of diseases easily. AR mainly refers to the inflammation of nasal mucosa due to the inhalation of allergens and the release of inflammatory mediators. At the same time, its pathogenesis is closely related to the physical, genetic, immune, and other factors. The main clinical manifestations of AR are stuffy nose, runny nose, nasal itching, sneezing, and olfaction disorders, etc. Also, it may cause or lead to diseases simultaneously, such as sinusitis, nasal polyps, pharyngitis, otitis media, trachitis and bronchitis, asthma and allergic conjunctivitis, seriously affecting people’s sleep, study, work, and life quality. Conventional drugs or surgical treatment have bottlenecks in the treatment of disease for a long time, it is urgently needed to find effective treatment methods. Therefore, deepening the understanding of the pathogenesis of AR and finding new diagnostic and therapeutic targets for allergic rhinitis are particularly important.

Interleukin (IL)-33 is a newly discovered cytokine in 2005 by Schmitz et al in 2005. It can be expressed by a variety of immune cells (such as mast cells, macrophages, and dendritic cells) and non-immune cells (such as endothelial cells, epithelial cells, smooth muscle cells, and fibroblasts). Scholars have found that IL-33 can promote Th2 cells, mast cells, basophils, eosinophils, and newly-found innate immune cells to produce Th2 cytokines, indicating that IL-33 may participate in the promotion of Th2 cytokine-mediated allergic reactions, such as asthma and AR. Moreover, homolog of sulfotransferase (ST2) was first identified as a member of the IL-1 receptor superfamily by Tominaga, previously known as “orphan receptor”. Latest studies have found that ST2 is a specific receptor for IL-33, and it binds to IL-33 to promote not only the accumulation of a variety of inflammatory cells in the nasal...
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mucosa, but also the release of IL-4, IL-5, IL-13, etc., through a series of signal transmission, thus contributing to the occurrence of AR. Therefore, the IL-33/ST2 signal transduction pathway is closely related to the occurrence of AR.

At present, several investigations have found that miRNA is involved in the occurrence and development of allergic diseases, including AR. MicroRNA (miRNA) is a newly-discovered short-chain non-coding regulatory RNA with an average length of 18-25 nucleotides. miRNA plays an important regulatory role in various physiological and pathological processes of the human body, such as embryonic development, organ formation, and tumorigenesis. Currently, a growing number of studies have found that miRNA is involved in the development of allergic disease, including allergic rhinitis. Scholars showed that miR-487b plays an important role in the regulation of inflammatory responses by targeting IL-33. It was suggested that Mex-3B could be a promising molecular target for the treatment of allergic asthma by regulating IL-33 expression.

The mouse model of allergic rhinitis was proposed in this work to investigate the potential effect of miR-487b/IL-33-ST2 axis on the pathology of allergic rhinitis.

**Patients and Methods**

**Human Tissue**

This study included 20 allergic rhinitis (AR) patients and 20 control patients at the Aviation General Hospital of China Medical University. The liquid nitrogen was used to freeze nasal mucosal tissues and were kept in -80°C refrigerator. After all, the Declaration of Helsinki should be mentioned and respected. This study was approved by the Ethics Committee of Aviation General Hospital of China Medical University. The signed informed consents were obtained from all participants before the study.

**Animals**

Male BALB/c mice aged 8-10 weeks old purchased from Beijing University of Chinese Medicine Animal Center were fed freely at room temperature for 1 week for testing. Then mice were randomly divided into 4 groups: Sham-operated group, (Sham group n=15), AR mice group (AR group n=15), AR mice treated with miR-487b lentivirus group (AR + miR-487b group, n=15), and AR mice treated with blank lentivirus (AR+ NC group, n=15). This study was approved by the Animal Ethics Committee of Beijing University of Chinese Medicine Animal Center Animal Center.

**AR Models**

AR model was induced according to previously reported protocol. Briefly, mice were sensitized twice. Firstly, 25 μg ovalbumin (OVA) and 2 mg of aluminum hydroxide (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in 200 μL of saline, the mice in AR groups were intraperitoneally injected with a mixed saline on days 0, 7, and 14 to set up primary sensitization. The secondary immunization was built one week after the primary sensitization. The mice in AR groups were suffered by 20 μL of saline dissolved 3% OVA intranasally from day 20th for one week. The Sham group was given normal saline instead of OVA and aluminum hydroxide. The lentivirus with miR-487b over-expression was intranasally administered to mice in AR + miR-487b group before 3 h of OVA challenge on days 28-34, and the blank lentivirus (GenePharma, Shanghai, China) was given to AR+NC group. Three mice chosen randomly from each group were sacrificed to detect the expression of miR-487b.

**Quantitative Reverse Transcriptase-Polymerase Chain Reaction (qRT-PCR) Analysis**

Total RNA was procured by TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's protocol. SYBR green qPCR assay was used to measure the level of IL-33 and ST2 expression, then endogenous controlled by glyceraldehyde 3-phosphate dehydrogenase (GAPDH). TaqMan miRNA assay (Applied Biosystems, Foster City, CA, USA) was used to measure the level of miR-487b expression normalized to miRNA U6.

**Luciferase Reporter Assays**

The human embryonic kidney cells (293T) purchased from the Chinese Academy of Sciences (Shanghai, China) were used in the current experiment link. In TargetScan, miRDB and microRNA websites, it was found that IL-33 is the target gene of miR-487b. The binding sequence of miR-487b at the 3’-end of IL-33 was mutated using a point mutation kit (Agilent Technologies, Santa Clara, CA, USA), and the mutated IL-33 (Mut-type) and non-mutant IL-33 (WT-type) were con-
nected with the pGL3-Basic luciferase reporter vector (Promega, Madison, WI, USA). PGL3-Basic vector with mutant IL-33 was transfected with lentivirus intervention on the 12-well plate. The same treatment was performed on the pGL3-Basic vector connected with the non-mutant IL-33 according to steps in the Luciferase Reporter Gene Assay Kit. Then, the luciferase activity was detected in a multi-function microplate reader.

**Western Blot Analysis**

The nasal mucosa tissues of mice were frozen and stored at −80 °C for protein analysis. Radioimmunoprecipitation assay (RIPA) tissue lysate (Beyotime, Shanghai, China) was added into the specimen, and the protein supernatant was extracted after homogenate and centrifugation. The protein level was determined using the bicinchoninic acid (BCA) method. According to the level, 20 μg protein was taken for loading, followed by 6% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). After blotting onto polyvinylidene difluoride (PVDF) membrane, samples were incubated with specific antibodies against IL-33, anti-ST2, and β-actin (1:1000) (Cell Signaling Technology, Danvers, MA, USA) at 4°C overnight. Then, the membrane was sealed with 5% skim milk powder at room temperature for 2 h; after washing for 3 times, the membrane was then incubated with electrochemiluminescence (ECL; Millipore, Billerica, MA, USA) for luminescence generation. The proteins were visualized and detected. The results were analyzed via Image-J software, and the grey level of each protein was normalized against the GAPDH.

**Histological analyses**

After the final intranasal sensitization, the mice were killed, and the Fresh nasal mucosa tissue was taken, fixed with formaldehyde, and dehydrated with gradient ethanol, followed by embedding, sectioning, then hematoxylin-eosin (HE) staining and Sirius red staining were performed. The distribution and morphology of the nasal mucosa epithelium were observed, and the number of eosinophils in the nasal mucosa was detected.

**Detection of OVA-Specific Immunoglobulin E (IgE)**

The blood samples were acquired when mice were killed. We get the serum through centrifuging the blood sample at 1000 × rpm for 15 mins. The OVA-specific Ig-E was measured with enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) as previously described. The samples were analyzed by measuring the optical density (OD) at 450 nm.

**Measurement of Cytokines**

After the sera were harvested, the IL-4, IL-5, and IL-13 levels were determined in the serum using the Cytometric Bead Array (CBA) Flex Set (BD Biosciences, San Diego, CA, USA) as previously described according to the manufacturer’s instructions. The samples were analyzed on a BD FACSVerse flow cytometer (BD Biosciences, San Diego, CA, USA).

**Statistical Analysis**

Statistical analysis was performed with a Student’s t-test or F-test. All p-values were two-sided and p<0.05 were considered statistically significant and analyzed by Prism 6.02 software (La Jolla, CA, USA).

**Results**

**The Expression of IL-33, ST2, and miR-487b in Patients**

To study the role of IL-33 and its specific ligand ST2 in AR development, the level of IL-33 and ST2 were detected in nasal mucosal tissues of AR patients or control patients by qRT-PCR. The results showed that both levels of IL-33 and ST2 were much higher in AR cases by comparing with the control cases (Figure 1A, 1B).

**IL-33 is a Direct Target of miR-487b**

To find the potential regulating miRNA of IL-33, we checked it in three publicly available algorithms, TargetScan, miRDB, and microRNA. It was found that IL-33 was a supposed target of miR-487b (Figure 2A). Therefore, the expression of miR-487b in the patients was detected. The result showed that the level of miR-487b expression was significantly declined in AR cases compared to the control cases (Figure 1C). Further, we established luciferase reporter vectors containing the wild or mutant-type miR-487b seed sequences of the IL-33 3’UTR. Upregulation of miR-487b with mimics resulted in the decrease of the luciferase activity of the wide-type IL-33 3’UTR reporter gene, while with no effect on mutant-type (Figure 2B), suggesting the expression of IL-33 could be regulated by miRNA 487b.
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and IL-33 might have some regulating effect in the progression of AR.

**Effect of MiR-487b on the Expression Level of IL-33 and ST2**

A mice model of AR induced by OVA was established to study further. In AR group, mice had allergic rhinitis symptoms such as frequent nose catching and sneezing. The symptom of the disease was improved after treatment of miR-487b lentivirus. The mice in AR + miR487b group that treated with miR-487b lentivirus could increase the expression of miR-487b, demonstrating that the lentivirus with miR-487b overexpression can up-regulate the expression of miR-487b (Figure 3A). Using Real Time-PCR and Western blot, we found that the expression level of IL-33 and ST2 was decreased by up-regulation of miR-487b (Figure 3B-3F). These data indicated that IL-33 and ST2 could be negatively regulated by miR-487b.

**Effects of miR-487b on the Pathological Alterations**

In HE images, nasal mucosa epithelium becomes hyperemia, edema, necrosis, and aberrant structure after AR groups. After intervened with lentivirus of miR-487b, all these pathological alterations were ameliorated (Figure 4). In Sirius red staining images, we measure the number of eosinophils cells which were stained red. The results

![Figure 1](image1.jpg)

**Figure 1.** The expressions of IL-33, ST2, and miR-487b in patients with or without Allergic rhinitis by RT-PCR in nasal mucosal tissues. (**p<0.0001 compared with Control cases).**

![Figure 2](image2.jpg)

**Figure 2.** IL-33 is a direct and functional target of miR-487b. A, Diagram of putative miR-487b binding sites of IL-33. B, Relative activities of luciferase reporters (**p<0.001).**
showed that miR-487b markedly reduced the number of eosinophils induced by AR (Figure 5A).

**Effect of MiR-487b on the Level of Ig-E**

The concentration of OVA-specific Ig-E in mice were measured by ELISA assay. The resulting histogram declared that the concentration of Ig-E was significantly increased in mice of AR groups; however, the concentration of Ig-E was reduced in mice treated with lentivirus of miR-487b (Figure 5B).

**Effect of MiR-487b on the Level of IL-4, IL-5, and IL-13**

Levels of proinflammatory cytokines, including IL-4, IL-5, and IL-13 were markedly elevated in serums of mice in AR groups. While the levels of IL-4, IL-5, and IL-13 were significantly diminished by intervening of miR-487b when comparing with AR + NC group (Figure 5C-5E).

**Discussion**

Allergic rhinitis (AR) is a kind of chronic inflammatory reaction disease of nasal mucosa involving a variety of immune cells, cytokines, and inflammatory mediators. Its pathogenesis is complicated. Clinically, the treatment of AR often cannot obtain satisfactory results. In recent years, the incidence rate of allergic diseases around the world shows an increasing trend, attracting more and more attention from the society. Therefore, searching for new diagnostic and therapeutic targets has become the main purpose of current research.

miRNA is a kind of endogenous small non-coding RNA with about 22 nucleotides in length, found in eukaryotes in recent years, which can recognize specific target mRNA and regulate the gene expression through promoting the degradation or inhibiting the translation of target mRNA at the post-transcriptional level. miRNA possesses important regulatory functions, which are pivotal for various activities of the body’s life. MiR-487b is located on chromosome 14q32.31, and there are few researches on miR-487b. Existing studies have shown that miR-487b is abnormally expressed in malignant tumors, such as neuroblastoma, and lung cancer, which is associated with the prognosis of the disease. Besides, it is highly expressed in the lung fibrous degeneration, pertussis, etc. Wang et al. have shown that miR-487b can inhibit the chronic heart failure through the IL-33/ST2 pathway.
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The IL-33/ST2 pathway is closely related to AR. Kamekura et al. found via ELISA using nasal mucosa of 11 AR patients and serum of 45 AR patients as samples that the serum IL-33 level in patients is significantly higher than that in normal group, and both IL-33 and ST2 receptor are highly expres-

**Figure 4.** miR-487b mitigate the pathological alterations in nasal mucosa of AR mice.

**Figure 5.** MiR-487b reduces the inflammatory response. 

A, the number of eosinophils in each microscopic field detected from Sirius red staining in nasal mucosa of AR mice. B, Expression level of total IgE by ELISA assay. C-E, Expression level of proinflammatory cytokines in serums of mice including IL-4, IL-5, and IL-13 by CBA. (**p<0.001 vs. Sham group; #p<0.05, ##p<0.01 vs. AR+NC group).
sed in nasal mucosa epithelial cells; after stimulation of IL-33 with allergen, mRNA in mast cells will also be up-regulated, and the expression level is increased. IL-33 can promote the production of cytokines/chemokines (such as IL-8 and granulocyte-colony stimulating factor) and directly act on the inflammatory reaction of nasal mucosa through ST2 in forms of autocrine and paracrine. The IL-33/ST2-mediated inflammatory responses are regulated by different signaling pathways in the nasal mucosa. The treatment with fluticasone propionate can reduce the expressions of IL-33 and ST2, thereby playing a therapeutic role.

In this work, the expression of IL-33 and ST2 in human tissue were analyzed, and it was found that the expression level of IL-33 and ST2 were significantly increased in AR patients. To study the molecular mechanism of abnormally high expression of IL-33 in AR, it was found first through bioinformatics that IL-33 was a regulatory target of miR-487b. Besides, the level of miR-487b observably reduced in AR patients. Then, a mice of AR model induced by OVA was performed to study further, and luciferase reporter gene assay showed that the lentivirus transfected with miR-487b significantly reduced the fluorescence expression of pGL3-Basic vector with WT-IL-33, but had no effect on the fluorescence expression of pGL3-Basic vector with Mut-Type, indicating that IL-33 is a target gene of miR-487b. As expected, the transfection with miR-487b resulted in the low expression of IL-33 and ST2 in both PCR and Western blot experiment. The s OVA-specific IgE together with proinflammatory cytokines: IL-4, IL-5, and IL-13 were markedly declined by intervened of miR-487b. Moreover, we found that miR-487b markedly the inhibited the pathological changes induced by OVA-induced AR. However, the experimental study at present is limited to experimental animals, and there is still a long way to go for clinical treatment.

**Conflict of Interest**

The Authors declare that they have no conflict of interest.

**References**


5. **Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Mosheri M, Qin J, Li X, Gorman DM, Bazin JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. Immunity 2005; 23: 479-490.**


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12) **Tomimaga S.** A putative protein of a growth specific cDNA from BALB/c-3T3 cells is highly similar to the extracellular portion of mouse interleukin 1 receptor. FEBS Lett 1989; 258: 301-304.


15) **Pecar-Petroski T, Didichenko SA, Kaeppner S, Piecz N, Dahinden CA.** Human basophils and eosinophils are the direct target leukocytes of the novel IL-1 family member IL-33. Blood 2009; 113: 1526-1534.


20) **Liu HJ, Zhang AF, Zhao N, Li XZ.** Role of miR-146a in enforcing effect of specific immunotherapy on allergic rhinitis. Immunol Invest 2016; 45: 1-10.


23) **Wang EW, Jin XS, Ruan CW, Ge ZR.** miR-487b mitigates chronic heart failure through inhibition of the IL-33/ST2 signaling pathway. Oncotarget 2017; 8: 51688-51702.


