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LncRNA MIAT Promotes Allergic Inflammation and Symptoms by Targeting MiR-10b-5p in Allergic Rhinitis Mice

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Abstract

Background: Allergic rhinitis (AR) is one of the most common noninfectious respiratory diseases caused by immunoglobulin E (IgE) response.

Objective: The study sought to explore the relationship between lncRNA MIAT and miR-10b-5p and their interaction in the regulation of allergic phenotypes in allergic rhinitis (AR) mice.

Methods: A mice model of AR was constructed using ovalbumin (OVA) sensitization. AR mice were treated with miR-10b-5p agomiR and LNA mediated lncRNA MIAT. The targeting relationship between MIAT and miR-10b-5p was analyzed by the ENCORI website and dual-luciferase reporter assay. The numbers of rubbing and sneezing of mice were counted. Hematoxylin-eosin (HE) staining visualized the eosinophils infiltration in nasal mucosa tissues of mice. The percentage of Th17 cells was quantitated by flow cytometry analysis. ELISA was used to detect the levels of serum OVA-specific IgE, the Th12 cytokine IL-4, and inflammatory cytokines (IL-6, IL-17).

Results: MIAT was up-regulated in the nasal mucosa of AR mice, while miR-10b-5p was down-regulated. MIAT directly suppressed miR-10b-5p expression in AR mice. The numbers of rubbing and sneezing, the percentage of Th17 cells, and the levels of OVA-specific IgE, IL-4, IL-6, and IL-17 in AR mice were decreased by miR-10b-5p overexpression, which was reversed by MIAT overexpression. The eosinophils infiltration in AR mice was inhibited by miR-10b-5p overexpression, which was also reversed by MIAT overexpression.

Conclusion: The present study demonstrates that MIAT overexpression Promotes allergic inflammation and symptoms by activating Th17 immune response via miR-10b-5p inhibition.

Keywords

allergic rhinitis, inflammation, LncRNA MIAT, miR-10b-5p, Th17 cells

Introduction

Allergic rhinitis (AR) is one of the most common noninfectious respiratory diseases caused by immunoglobulin E (IgE) response.[1] AR is characterized by symptoms such as nasal sneezing, runny nose, nasal congestion and nasal itching when people suffering from allergic diseases contact specific allergens.[2] AR can affect up to 30% of the global population and its prevalence has been reported to be on the increase.[3] AR does not threaten people's lives, but it seriously impacts one's quality of life and work efficiency, which causes great social and economic burdens.[4] Furthermore, 10–40% of AR patients are accompanied by allergic asthma.[5] Despite the rapid progress in the research on AR, the outcomes of the current treatments, such as immunotherapy and antihistamines, remain unsatisfactory.[6] Thus, it is essential to further investigate the pathogenesis of AR to

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support fundamental research on the prevention and treatment of AR, which will hopefully help improve the quality of life of AR patients and the effectiveness of treatment.

The actions of Th2 cells and some functions of Th1 cells are involved in AR development.[4] IL-17, a key cytokine produced by Th17 cells, induces allergen-specific Th2 cell activation, eosinophil and neutrophil accumulation, and serum IgE production in asthma; all of these features may play important roles in AR.[7] Th17 mediated immunity may be involved in the airway disease of children with allergic asthma and allergic rhinitis.[8] Upon allergen sensitization, Th17 cells are activated and promote inflammation.[7]

MicroRNAs (miRNAs) are small, single-stranded, highly conserved, non-coding RNAs with a length of about 21–25 nucleotides.[9] MiRNAs can bind to partial complementary sites of targeted mRNA 3′-UTR to regulate gene expression according to the principle of complementary base pairing.[10] MiRNAs are implicated in a broad variety of physiological and pathological processes.[11] More recently, growing evidence has revealed that the modulation of post-transcriptional regulation by miRNAs is crucial to AR pathogenesis.[12–14] Chen et al. demonstrated that miR-10b-5p is a novel Th17 regulator and its overexpression inhibits the production of IL-17A in both total CD4 and differentiating Th17 cells.[15] Formaldehyde-induced inflammatory signals originating in the nose were associated with decreased miR-10b levels.[16] This study identified miR-10b-5p as a miRNA of interest in AR pathogenesis.

Long noncoding RNAs (lncRNAs) are non-protein-coding RNA molecules longer than 200 nucleotides and have been reported to be critical players in biological processes.[17] An increasing number of studies indicated that lncRNAs might be involved in the pathogenesis of AR.[18,19] LncRNA MIAT, a highly conserved mammalian lncRNA, is thought of as a critical role in tumor, atherosclerosis and nephropathy,[20–22] and thereby may have the potential to affect immune-related functions. It has been demonstrated that some lncRNAs may harbor miRNA-binding sites and can serve as targets of miRNA molecules.[23]

In the current study, an AR mouse model was constructed using ovalbumin (OVA). We then explored the mutual regulation between MIAT and miR-10b-5p and their interaction on Th17 cell immune response and pathophysiological characteristics in AR mice.

Materials and Methods

Ethics Statement

All animal experiments were performed in Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine in compliance with the guidelines of the China Council on Animal Care and Use. This research was recommended by the Committee of Experimental Animals of Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine (AH + 20,190,722,002). Every effort was made to minimize animal discomfort and suffering.

Mice Treatment

Forty five-week-old male BALB/c mice (20–22 g) obtained from the Vital River Laboratory Animal Technology Co., Ltd., (Beijing, China) were raised and maintained under specific pathogen-free conditions. As displayed in Figure 1, the mice were divided into four groups as follows: control, OVA, MC and M groups, with 10 mice in each group. After 3 days of adaptive feeding, the experiment was conducted.

Control group: mice were only intraperitoneally injected with PBS.

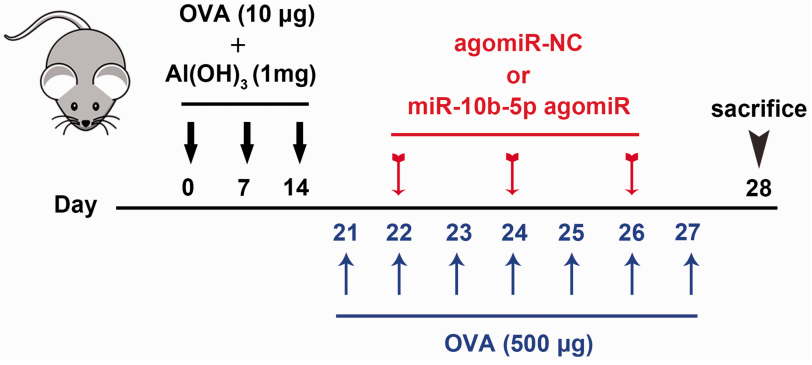


Figure 1. The experimental protocol of the present study. Mice in the MC, M, MIAT-NC, MIAT and MIAT+M groups were sensitized with ovalbumin (OVA) on days 0, 7 and 14, while control group mice were given PBS only. All groups received intranasal injection of OVA between days 21 and 27, except for the control group. Selected groups of mice were treated with miR-10b-5p agomiR or agomiR-NC via intraperitoneal injection 2 h before intranasal OVA challenge on days 22, 24 and 26.

OVA group: on days 0, 7 and 14, mice were sensitized by intraperitoneal injection of 500 μL of PBS containing 10 μg of ovalbumin (OVA; A5503, grade V; Sigma, USA) and 1 mg of aluminum hydroxide (A110525, Aladdin, Shanghai, China). Between day 21 and day 27, the AR mice were sensitized by intranasal injection of 20 μL of PBS containing 500 μg of OVA.

MC group: on days 0, 7, and 14, mice received the same treatment as that in the OVA group. Prior to OVA induction, namely on days 22, 24 and 26, mice were injected intraperitoneally with agomiR NC (miR4N0000001-4-5, RiBoBio), and then subjected to induction of AR.

M group: on days 0, 7, and 14, mice received the same treatment as that in the OVA group. Prior to OVA induction, namely on days 22, 24 and 26, mice were injected intraperitoneally with miR-10b-5p agomiR (miR40000208-4-5, RiBoBio), and then subjected to induction of AR.

To further explore the potential mechanism of rhinallergosis, mice were divided into six groups as following: OVA, MC, M, MIAT-NC, MIAT and MIAT+M groups, with 10 mice in each group. Mice in the OVA, MC and M groups were treated as described above.

MIAT-NC group: on days 0, 7, and 14, mice received the same treatment as that in the OVA group. Prior to OVA induction, namely on days 22, 24 and 26, mice were injected intraperitoneally with LNA-control (500 μg/100 g body/day, Exiqon, Vadbaek, Denmark) and then subjected to induction of AR.

MIAT group: on days 0, 7, and 14, mice received the same treatment as that in the OVA group. Prior to OVA induction, namely on days 22, 24 and 26, mice were injected intraperitoneally with LNA mediated lncRNA MIAT (500 μg/100 g body/day, Exiqon, Vadbaek, Denmark), and then subjected to induction of AR.

MIAT+M group: on days 0, 7, and 14, mice received the same treatment as that in the OVA group. Prior to OVA induction, namely on days 22, 24 and 26, mice were injected intraperitoneally with LNA mediated lncRNA MIAT and miR-10b-5p agomiR, and then subjected to induction of AR.

Symptom Score

In our work, 30 min after the final OVA injection on day 27, AR mice received a behavioral test as documented in previous reports.[24,25] The frequencies of rubbing and sneezing of mice in each group were blindly observed and recorded.

Haematoxylin-Eosin Staining

Histopathological examination was performed by haematoxylin-eosin (HE) staining as previously described.[1] In short, 24 h after the final intranasal sensitization, mice were sacrificed under ketamine (50 mg/kg) and xylazine (5 mg/kg) anesthesia. The heads of the mice were removed and promptly fixed in fresh 4% paraformaldehyde for 24 h at 4 °C. The tissue samples were dehydrated with increasing ethanol series and embedded in paraffin. Next, the nasal mucosa tissues were prepared into 4 μm-thick sections and dyed using an H&E staining kit (G1120, Solorbio, China). The eosinophils infiltration was visualized using a Sirius red kit (G1470, Solarbio). Images were captured by a 200 × inverted microscope (Ts2r-FL, Nikon, Japan).

Elisa

To evaluate the allergic response, OVA-specific IgE, Th12 cytokine IL-4, and inflammatory cytokines (IL-6 and IL-17) in serum were assayed using ELISA kits. Mouse IL-4 (SEKM-0005) and IL-6 ELISA kits (SEKM-0007) were purchased from Beijing Solarbio Science & Technology Co., Ltd. Mouse OVA-specific IgE (EIA06159m) and IL-17 ELISA kits (EIA05898m) were purchased from Wuhan Xinqidi Biological Technology Co., Ltd. (http://www.qidibio.com/). Procedures were performed based on the manufacturers' protocols.

Flow Cytometry Analysis of Th17 Differentiation

Th17 differentiation in $CD4^+$ T cells was evaluated by determining IL-17 A content using flow cytometry analysis.[26] Heparinized blood (10 mL) was harvested from each group, and peripheral blood mononuclear cells were separated from the heparinized blood using Ficoll-Hypaque density-gradient centrifugation. $CD4^+$ T cells were isolated from the peripheral blood mononuclear cells using human CD4 microbeads (130-049-201, Miltenyi Biotec, USA). Next, the $CD4^+$ T cells were pretreated with 1 μg/mL phorbol myristate acetate (P1585, Sigma-Aldrich, USA) and 1 μg/mL ionomycin (I9657, Sigma-Aldrich) in the presence of 1 μg/mL monensin (554,715 BD, USA) for 8 h.[26] The activated cells were washed and treated with an FITC conjugated anti-mouse CD4 monoclonal antibody (11-0041-82, eBioscience, USA). The cells were then washed, fixed, and permeabilized using a BD Cytofix/Cytoperm™ Fixation/Permeabilization Solution Kit (554,714 BD, USA). The cells were treated with a PE-conjugated anti-human IL-17 A monoclonal antibody (12-7182-82, eBioscience, USA). A rat IgG2a kappa Isotype Control was used as an isotype control (12-4321-80, eBioscience). Data were analyzed using a FACScan flow cytometer (BD, USA) equipped with FlowJo software.

Dual-Luciferase Reporter Assay

The possible binding sites between lncRNA MIAT and miR-10b-5p were predicted by ENCORI. HEK293T cells (CRL-11268, American Type Culture Collection (ATCC)) were used for dual-luciferase activity assay. Wild type (WT) lncRNA MIAT-3'-UTR containing the putative miR-10b-5p binding site and mutant (MUT) lncRNA MIAT-3'-UTR (with the mutated miR-10b-5p binding site) were synthesized by GenePharma Co., Ltd. and cloned into luciferase reporter plasmids (E1330, Promega, Madison, WI, USA), and the resulting luciferase reporter plasmids were named as MIA-WT and MIA-MUT, respectively. The WT or MUT reporter plasmid was co-transfected with miR-10b-5p mimic (M, miR10000254-1-5, GenePharma) or mimic control (MC, miR1N0000001-1-5, GenePharma) into cells using Lipofectamine 2000 (11,668 ThermoFisher, USA). After incubation for 48 h, the luciferase activity in the transfected cells was evaluated using the Dual Luciferase Reporter Assay Kit (E1910, Promega). The activity of firefly luciferase was normalized to that of Renilla luciferase.

Quantitative Reverse Transcriptase Polymerase Chain Reaction

Total RNA was separated from nasal mucosa using TRIzol® (15596-026, Invitrogen). Complementary DNA was synthesized from the total RNA sample using PrimeScript™ RT Master Mix (RR036A, TaKaRa). Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using a SYBR Premix Ex Taq II Kit (RR820A, Takara) on the ABI 7500 real-time PCR system (Applied Biosystems, Foster City, CA). Total miRNAs were extracted from nasal mucosa using a miRNA Extraction Kit (B1802, HaiGene). qRT-PCR was performed using a miRNA cDNA Synthesis Kit (D1802, HaiGene) and a miRNA qPCR Kit (TAP02259, HaiGene) for detection of miR-10b-5p, based on the manufacturers' protocols. Primers used for qRT-PCR were as follows: MIAT forward: 5′-TTCCAGACACGTTCATGTG-3′, reverse: 5′- TTCT GTCTCCTCTGTCCCA -3′; GAPDH forward: 5′-AG GTCGGTGTGAACGGATTTG-3′, reverse: 5′-GGGG TCGTTGATGGCAACA-3′. U6 forward: 5′-CTCGC TTCGGCAGCACA-3′, reverse: 5′-AACGCTTCACG AATTTGCGT-3′. The nucleotide sequence of the forward primer for mouse miR-10b-5p was TACCCT GUAGAACCGAATTTGTG, and a universal reverse primer was provided in the kit. GAPDH and U6 were used as endogenous controls of MIAT and miR-10b-5, respectively. The relative gene levels were calculated using the $2-\Delta\Delta Ct$ method.[27]

Statistical Analysis

A Student's *t*-test and one-way ANOVA analysis followed by Bonferroni's post hoc test were performed using SPSS Statistics version 21 (SPSS 21.0; SPSS Inc) to determine the statistical significance of observed differences. Data are shown as the mean ± standard deviation (SD). $P < 0.05$ was considered to indicate statistical significance.

Results

Overexpression of miR-10b-5p Alleviated Allergic Symptoms and Infiltration of Eosinophils in AR Mice

The numbers of nose-scratching and sneezing were increased significantly in AR mice compared to control mice (Figure 2(A) and (B), $p < 0.001$), but they were decreased in miR-10b-5p agomiR-treated AR mice compared to AR mice (Figure 2(A) and (B), $p < 0.001$). Increased infltration of eosinophils is a predominant diagnostic index for AR. As shown in Figure 2(C), there was a striking infiltration of eosinophils in the nasal mucosa of AR mice compared to control mice. By contrast, intraperitoneal injection of miR-10b-5p agomiR markedly inhibited the infiltration of eosinophils in the nasal mucosa of AR mice (Figure 2(C)).

Overexpression of miR-10b-5p Reduced the Levels of OVA-Specific IgE and IL-4 in AR Mice

OVA-specific IgE level was remarkably increased in AR mice compared to the control mice (Figure 2(D), $p < 0.001$). By contrast, miR-10b-5p agomiR reduced OVA-specific IgE level in AR mice (Figure 2(D), $p < 0.001$). Besides, the level of IL-4 in serum was strikingly elevated in AR mice (Figure 2(E), $p < 0.001$). However, miR-10b-5p agomiR decreased IL-4 level in AR mice (Figure 2(E), $p < 0.001$).

Overexpression of miR-10b-5p Suppressed Th17 Immune Response in AR Mice

The percentage of allergen-reactive Th17 cells and the levels of IL-6 and IL-17 in serum were increased in AR mice compared to the control mice (Figure 2(F), $p < 0.001$). However, intraperitoneal administration of miR-10b-5p agomiR reduced the percentage of Th17 cells and the levels of IL-6 and IL-17 in AR mice (Figure 2(E), (G), and (H), $p < 0.001$).

MIAT Directly Targeted miR-10b-5p

As described in Figure 3(A), ENCORI showed that MIAT possessed binding sites for miR-10b-5p. The result from dual-luciferase reporter assay showed that

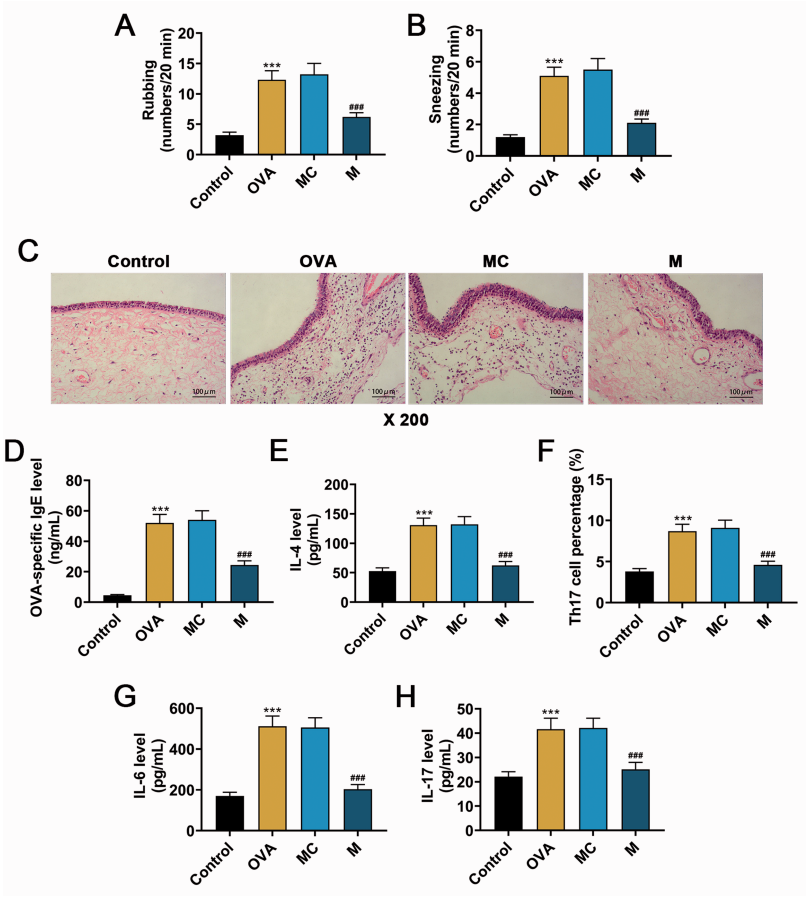


Figure 2. Overexpression of miR-10b-5p alleviated allergic symptoms and inflammatory response in allergic rhinitis (AR) mice. (A and B) Overexpression of miR-10b-5p reduced the numbers of rubbing (A) and sneezing (B) in AR mice. (C) Hematoxylin and eosin (H&E) staining was used to evaluate the effect of miR-10b-5p overexpression on the infiltration of eosinophils in the nasal mucosa of AR mice. (D and E) ELISA was used to measure the levels of serum ovalbumin (OVA)-specific IgE (D) and IL-4(E) in AR mice. (F) The percentage of Th17 cells was quantitated by flow cytometry analysis. (G and H) ELISA was used to evaluate the levels of IL-6 (G) and Th17 cell differentiation (IL-17) (H) in serum of AR mice. M, miR-10b-5p agomiR; MC, agomiR NC. $^{***}p < 0.001$ vs. Control; $^{\#\#\#}p < 0.001$ vs. MC. Data are shown as the mean ± standard deviation (SD).

luciferase activity was significantly decreased in the MIAT-WT+M group compared to the MC group (Figure 3(B), $p < 0.001$), while it remained stable in the MIAT-MUT+M group compared with the MIAT-MUT+MC group (Figure 3(B)).

MIAT Promoted Th17 Immune Response in AR Mice Through miR-10b-5p

MIAT level was significantly increased in AR mice compared to control mice (Figure 4(A), $p < 0.001$); intraperitoneal injection of MIAT further increased MIAT level in AR mice compared to AR mice (Figure 4(A), $p < 0.001$). MiR-10b-5p level was significantly decreased in AR mice compared to control mice (Figure 4(B), $p < 0.001$), which was partially reversed by miR-10b-5p agomiR (Figure 4 (B), $p < 0.001$); intraperitoneal injection of MIAT further decreased miR-10b-5p level in AR mice compared to AR mice (Figure 4(B), $p < 0.001$), which was partially reversed by miR-10b-5p agomiR (Figure 4(A), $p < 0.001$). The data suggested that MIAT can negatively regulate miR-10b-5p expression in AR mice. The percentage of Th17 cells and the levels of IL-6 and IL-17 in serum were increased in AR mice compared to the control mice (Figure 4(C)–(E), $p < 0.001$), which was partially reversed by miR-10b-5p agomiR (Figure 4(C)–(E), $p < 0.01$); intraperitoneal administration of MIAT further increased the percentage of Th17 cells and the levels of IL-6 and IL-17 in AR mice compared to AR mice without MIAT treatment (Figure 4 (C)–(E), $p < 0.001$), which was partially reversed by miR-10b-5p agomiR (Figure 4(C)–(E), $p < 0.001$).

MIAT Promoted Allergic Phenotypes and Eosinophils Infiltration in AR Mice Through miR-10b-5p

The numbers of nose-scratching and sneezing were increased in AR mice compared to the control mice (Figure 4(F) and (G), $p < 0.001$), which was partially

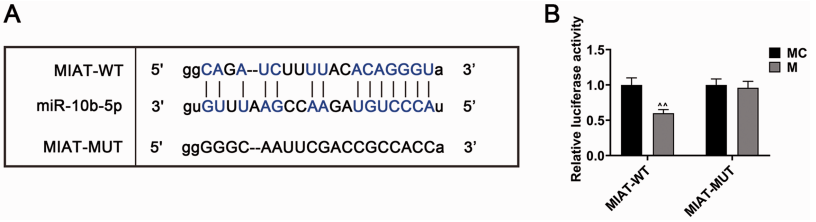


Figure 3. MIAT directly targeted miR-10b-5p. (A) ENCOR predicted a miR-10b-5p binding site in the 3'UTR of MIAT. (B) The cells were transfected with miR-10b-5p mimics (M) or its corresponding negative control (mimics negative control (MC)), together with wild-type (WT) or mutant MIAT 3'UTR. Dual luciferase reporter assay verified that miR-10b-5p was the target gene of MIAT. M, miR-10b-5p mimic; MC, mimic control. $^{\wedge\wedge}p < 0.01$ vs. MC. Data are shown as the mean ± standard deviation (SD).

reversed by miR-10b-5p agomiR (Figure 4(F) and (G), $p < 0.001$); the numbers of nose-scratching and sneezing were further increased in AR mice treated with MIAT compared to AR mice without MIAT treatment (Figure 4(F) and (G), $p < 0.001$), which was partially reversed by miR-10b-5p agomiR (Figure 4(F) and (G), $p < 0.001$). As shown in Figure 4(H), there was a significant infiltration of eosinophils in the nasal mucosa of AR mice compared to control mice; miR-10b-5p agomiR treatment suppressed the infiltration of eosinophils in the nasal mucosa of AR mice compared to AR mice without miR-10b-5p agomiR treatment, which was partially reversed by MIAT treatment. The levels of serum OVA-specific IgE and IL-4 were significantly elevated in AR mice compared to control mice (Figure 4(I) and (J), $p < 0.001$); however, miR-10b-5p agomiR decreased the levels of serum OVA-specific IgE and IL-4 in AR mice compared to AR mice without miR-10b-5p agomiR treatment (Figure 4(I) and (J), $p < 0.001$), which was partially reversed by MIAT treatment (Figure 4(I) and (J), $p < 0.001$).

Discussion

AR is primarily characterized by the high levels of serum allergen-specific IgE and several inflammatory factors and the infiltration of inflammatory cells in the nasal mucosa. $CD4^+$ T helper cells serve a critical role in immune responses by differentiating into Th1, Th2, Th9, Th17 and Treg cells.[28] The Th1/Th2 imbalance has been believed as a significant immunological mechanism of AR.[29] In addition, Th17 cells that primarily produce IL-17 are of great significance in autoimmune diseases and in the defense reaction of the body.[30]

In the current study, we first explored the effect of miR-10b-5p on OVA-induced AR mice through evaluating allergic symptoms, eosinophils infiltration, Th17 cell differentiation and the levels of serum OVA-specific IgE, IL-4, IL-6, and IL-17. IgE is associated with allergic inflammation in allergic responses.[31] The inflammatory response of the nasal mucosa of AR subjects is characterized by the recruitment of eosinophils, basophils and T cells expressing Th2 cytokines.[32] Recruitment of T cells and inflammatory cells such as eosinophils leads to the further secretion of histamine, leukotrienes, and pro-inflammatory cytokines, and these secretions maintain the allergic response and accelerate the late phase response.[33,34] A previous study suggested that hsa-miR-10b-5p has a possible effect on the immunological processes in the early phase of type 1 diabetes in newly diagnosed children and adolescents.[35] In this study, we found that miR-10b-5p agomiR decreased the numbers of nose-scratching and sneezing, alleviated eosinophils infiltration, and decreased the level of serum OVA-specific IgE and the production of the Th2 cytokine IL-4; moreover, it decreased the level of the pro-inflammatory factor IL-6, the production of the Th17 cytokine IL-17, and the percentage of Th17 cells in AR mice. Similarly, Chen et al.[15] suggested that miR-10b overexpression suppresses IL-17A production by both total CD4 and differentiating Th17 cells in ankylosing spondylitis. Emerging evidence has suggested that inflammatory mechanisms play a critical role in the progression of AR.[4] Inflammatory response is related to the increased number of eosinophils and high levels of pro-inflammatory cytokines.[1] Th2 cells have the ability to activate eosinophils.[36] IL-4 is released by Th2 cells in response to allergens and is reported to contribute to the release of IgE by B cells.[37] IL-17 coordinates tissue inflammation by promoting the secretion of a variety of pro-inflammatory factors (such as IL-6 and TNFα), which mediate tissue infiltration and tissue destruction.[38] IL-17 is able to promote Th2 cell activation and induce eosinophil and neutrophil accumulation as well as serum IgE production in asthma.[7] In this study, miR-10b-5p agomiR suppressed inflammatory response through inhibiting eosinophils infiltration, pro-inflammatory cytokine IL-6 secretion, and IL-4 production that might result from IL-17 induced Th2 cell activation. Thus, the data suggested that miR-10b-5p agomiR promoted allergic inflammation and symptoms by inhibiting Th17 immune response.

Numerous studies have demonstrated that MIAT exerts its regulatory effects on physiological or

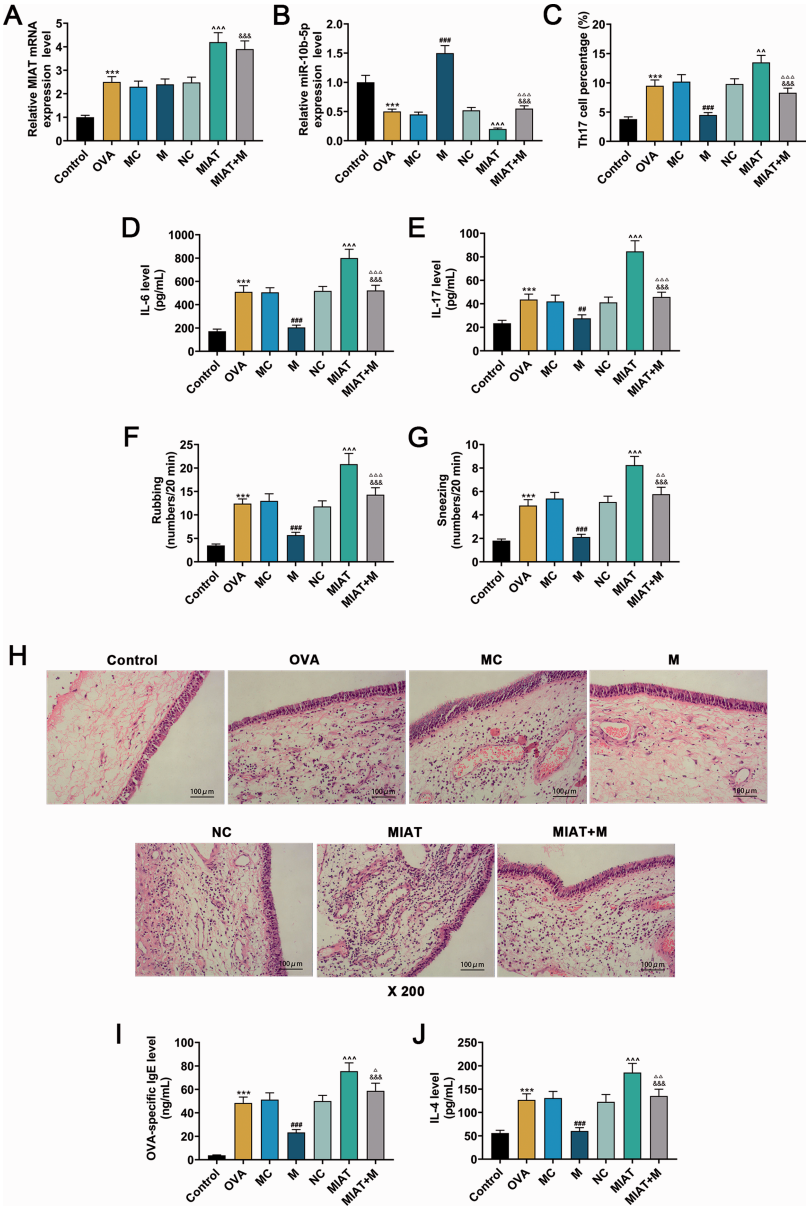


Figure 4. MIAT promoted allergic phenotypes and inflammatory response in allergic rhinitis (AR) mice through miR-10b-5p. (A and B) Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed to determine the expression levels of MIAT (A) and miR-10b-5p (B) in the nasal mucosa of AR mice treated with miR-10b-5p agomiR or/and MIAT. (C) Flow cytometry analysis was performed to quantitate the percentage of Th17 cells in $CD4^+$ T cells of AR mice treated with miR-10b-5p agomiR or/and MIAT. (D and E) ELISA was used to evaluate the levels of IL-6 (D) and Th17 cell differentiation (IL-17) (E) in serum of AR mice treated with miR-10b-5p agomiR or/and MIAT. (F and G) The numbers of rubbing (F) and sneezing (G) of AR mice treated with miR-10b-5p agomiR or/and MIAT. (H) Hematoxylin and eosin (H&E) staining was used to observe the infiltration of eosinophils in the nasal mucosa of AR mice treated with miR-10b-5p agomiR or/and MIAT. (I and J) ELISA was used to determine the levels of serum ovalbumin (OVA)-specific IgE (I) and IL-4 (J) in AR mice treated with miR-10b-5p agomiR or/and MIAT. M, miR-10b-5p agomiR; MC, agomiR NC. $^{***}p < 0.001$ vs. Control; $^{\#\#\#}p < 0.001$ vs. MC; $^{\wedge\wedge\wedge}p < 0.001$ vs. NC; $^{\triangle}p < 0.05$ or $^{\triangle\triangle}p < 0.01$ or $^{\triangle\triangle\triangle}p < 0.001$ vs. MIAT. GAPDH and U6 were used as an endogenous control. Data are shown as the mean ± standard deviation (SD).

pathological processes through targeting miRNAs.[20,39,40] According to the data from ENCORI, miR-10b-5p possesses binding sites for MIAT. Additionally, dual-luciferase activity was reduced in cells co-transfected with miR-10b-5p mimic and MIAT-WT; however, it remained stable in cells co-transfected with miR-10b-5p mimic and MIAT-MUT compared with the mimic control and MIAT-MUT groups. The present data also suggested that MIAT was up-regulated in the nasal mucosa of AR mice,

while miR-10b-5p was down-regulated. Furthermore, overexpression of MIAT significantly inhibited the expression levels of miR-10b-5p in the nasal mucosa of AR mice. These results confirmed that miR-10b-5p was a target of MIAT. As expected, the present study showed that MIAT overexpression counteracted the effect of miR-10b-5p overexpression on allergic symptoms, eosinophils infiltration, Th17 cell differentiation and the levels of serum OVA-specific IgE, IL-4, IL-6, and IL-17 in AR mice, which suggested that MIAT overexpression promoted allergic inflammation and symptoms by activating Th17 immune response via miR-10b-5p inhibition.

Conclusion

Taken together, the present study provides new mechanistic insights into the roles of miR-10b-5p and MIAT in the pathogenesis of AR and establishes a theoretical basis for the treatment of AR. Nonetheless, further research is still required to identify some unknown complex regulatory processes of AR, which will help to clarify the occurrence and development of AR and develop novel ideas and approaches for its prevention and treatment.

Availability of Data and Materials

The analysed data sets generated during the study are available from the corresponding author on reasonable request.

Author Contributions

(i) Zhiqi Ma and Haijuan Lian made a substantial contribution to the concept or design of the work; or acquisition, analysis or interpretation of data,
(ii) Zhiqi Ma, Haijuan Lian , Xiaoyan Lin and Yong Li drafted the article or revised it critically for important intellectual content,
(iii) All authors approved the version to be published,
(iv) Each author should have participated sufficiently in the work to take public responsibility for appropriate portions of the content.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethics Statement

All animal experiments were performed in compliance with the guidelines of the China Council on Animal Care and Use. This research was recommended by the Committee of Experimental Animals of Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine (AH + 20,190, 722,002). Every effort was made to minimize animal discomfort and suffering. The animals' experiments were performed in Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine.

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