



MiR-146a mimic attenuates murine allergic rhinitis by downregulating TLR4/TRAFF6/NF- κ B pathway

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Aim: This study aims to investigate whether microRNA (miR)-146a exerts therapeutic potential to allergic rhinitis (AR). **Methods:** Female BALB/c mice were randomly divided into the AR and miR-146a groups. miR-146a was administered into the nostril before ovalbumin (OVA) challenge daily on days 21–28. **Results:** The expression level of miR-146a in AR nasal mucosa was significantly decreased. miR-146a mimic markedly attenuated sneezing and nasal rubbing events, decreased the levels of ovalbumin-specific IgE as well as mastocyte- and basophil-related inflammatory cytokines, reduced inflammatory cells, affected the T-helper 2-released cytokines. Furthermore, miR-146a mimic inhibited the expressions of proteins relevant to Toll-like receptor 4 (TLR4)/TRAFF6/NF- κ B signaling pathway. **Conclusion:** The anti-inflammatory effects of miR-146a on AR might exert through the inhibition of the toll-like receptor 4 (TLR4)/TRAFF6/NF- κ B signaling pathway to some extent.

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Allergic rhinitis (AR) is one of the most common chronic disorders worldwide, characterized by sneezing, rhinorrhea and rhinobyon [1,2]. Evidences illustrate that mast cell infiltration, lymphocytes imbalance and goblet cell hyperplasia are involved in the pathogenesis of AR [3,4]. AR, type I allergic disorder mediated by IgE humoral immune response, is accompanied by an influx of eosinophils and T-helper 2 (Th2) cells secreting pro-inflammatory cytokines, IL-4, IL-5 and IL-13 [5]. Nowadays, the conventional therapy for AR focuses on disease control through anti-inflammatory, antihistamines, antileukotrienes agents as well as glucocorticoid-based drugs. These drugs only alleviate AR symptoms in short-term, whereas inevitable side effects limit their long-term clinic application. Thus, it is urgent to develop novel therapeutic agents targeting AR pathogenesis with low-side effects or compliance with allergen avoidance.

MicroRNAs (miRNAs) are small noncoding RNA molecules, usually composed 21–25 nucleotides. It is acknowledged that miRNAs participate in many biological processes such as cell differentiation, immune defense and apoptosis [6]. In recent years, microRNA (miR)-146a has become one of the hotspots in the research field of inflammatory disorders for potent and extensive anti-inflammatory performance [7,8]. Liu *et al.* indicated that miR-146a might have therapeutic potential in AR immunotherapy through inducing antigen-specific regulatory T cells [9], but the concrete molecular mechanisms underlying its anti-AR roles have not been well elucidated yet.

Toll-like receptor 4 (TLR4)/NF- κ B signaling pathway is essential to regulate cell proliferation, apoptosis, oxidative stress and inflammatory responses [10–12]. Tumor necrosis factor receptor-associated factor 6 (TRAFF6) is an important target gene of miR-146a in the negative regulation of inflammatory responses [13,14]. Strikingly, Liu *et al.* reported that miR-146a suppresses inflammatory responses by inhibiting TLR4/TRAFF6/NF- κ B signaling in rheumatoid arthritis [15].

The present study aimed to explore the role of miR-146a in AR pathogenesis and underlying mechanisms to evaluate the feasibility to be potential therapeutic agent. It is presumed that miR-146a exerts anti-inflammatory roles via inhibiting TLR4/TRAF6/NF- κ B signaling pathway in AR mice.

Methods & materials

Animals

Female BALB/c mice (6 weeks old) were purchased from Shanghai Laboratory Animal Center (Shanghai, China) and maintained in horizontal laminar flow cabinets at 21–23°C, 40–60% humidity in a 12-h light/dark cycle with *ad libitum* access to sterile food and water in a specific pathogen-free facility. Five to six mice were housed per cage. All the animal-involved experiments were conducted according to the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals and approved by the First Affiliated Hospital of Zhengzhou University.

Participants

AR patients were recruited in the First Affiliated Hospital of Zhengzhou University. All study complied with the Declaration of Helsinki and approved by the Ethical Committee in the First Affiliated Hospital of Zhengzhou University.

Ovalbumin-induced AR modeling & intranasal miR-146a treatment

The AR murine model was established as previously described [16] with minor modifications. The miR-146a mimic (5' to 3') (UGAGAACUGAAU UCCAUGGGUU) was purchased from Shanghai Zimmer Pharmaceutical Company, Shanghai, China. Briefly, female mice were randomly divided into four experimental groups, namely the control (Ctrl), AR, miR control (miR-ctrl) and miR-146a mimic (miR-146a) groups. For the AR group, mice were sensitized with intraperitoneal injection of 100 μ g ovalbumin (OVA, grade V; Sigma-Aldrich, MO, USA) and 2 mg aluminium hydroxide [Al[3]₃] (Sigma-Aldrich) in 100 μ l normal saline on day 0, 7 and 14, then challenged daily with an OVA solution (40 mg/ml in normal saline, 20 μ l/mouse) into the nostrils on days 21–28. For the control group, normal saline [2] plus Al[3]₃ without OVA was injected, then challenged with normal saline instead of the OVA solution on days 21–28. For the miR-ctrl and miR-146a groups, mice were sensitized with intraperitoneal injection of 100 μ g OVA and 2 mg Al[3]₃ in 100 μ l normal saline on day 0, 7 and 14, then intranasal administration of scrambled miR (negative control) or miR-146a mimic (0.1 mg/ml in normal saline, 20 μ l/mouse) was performed 30 min at a rate of 1 μ l/min before OVA challenge daily on days 21–28. Then mice were anesthetized with 50 mg/kg and tissues were collected for further experiments.

Assessment of nasal signs & sample preparation

Within 20 min after the final nasal challenge with OVA on day 28, sneezing frequencies and number of nasal rubbing events of all the mice were recorded up to 10 min by observers in a double-blinded manner. Blood and nasal lavage fluid (NLF) were collected from each mouse 2 h after the final challenge under anesthesia, followed by centrifugation at 4000 r.p.m. for 15 min. The supernatants of blood and NLF were stored at -80°C for subsequent assays. The sediment of NLF was used for cell counting. To obtain nasal mucosa tissues for Western blotting, the skull was sagittally bisected to remove septal and turbinate mucosa. Samples of the nasal mucosa were also stored at -80°C for further study.

Reverse transcription-quantitative PCR

The total RNA of isolated nasal mucosa was extracted using TRIzol reagent (Life technologies, CA, USA), transcribed into complementary DNA (cDNA) using PrimeScript First Strand cDNA synthesis kit (Takara, Dalian, China) according to the manufacturers' instructions. Quantitative PCR was performed using SYBR Green Premix Ex Taq (TaKaRa) to determine the mRNA expression of target genes on a 7900HT fast real-time PCR system (Applied Biosystems, NY, USA). The primers used were as follows (5' to 3'): miR-146a, CAGGATGAGAACTGAAT (forward), GTGCAGGGTCCGAGGT (reverse); TRAF6, TCATTATGATCTGGACTGCCCAAC (forward), TGCAAGTGTCTGCGCCAAGTG (reverse); β -actin, GGCTGTATTCCTCCATCG (forward), CCAGTTGTGTAACAATGCCATGT (reverse). The amplification protocol was as follows: pre-incubation at 98°C for 1 min, followed by 40 cycles at 98°C for 10 s, 56°C for 20 s and 72°C for 30 s. The relative mRNA expressions of targeted genes were calculated using the comparative 2^{- $\Delta\Delta$ C_t} method. β -actin was used as the inner control.

Western blot analysis

Total protein was extracted from the murine nasal mucosa with radioimmunoprecipitation buffer supplemented with protease inhibitor (Sigma, MO, USA). BCA Protein Assay kit (Beyotime Institute of Biotechnology, Shanghai, China) was used to quantify the protein concentration. Equal amount of protein (30 µg) from each sample was isolated using 10% SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane. After blocking with 5% nonfat milk for 1 h at room temperature, the membrane was incubated with primary antibodies against TRAF6 (1:2000, Cell Signaling Technology, MA, USA), TLR4 (1:2000, Abcam, MA, USA), p-IκBα (1:1000, Abcam), IκBα (1:1000, Abcam), p65 (1:2000, Cell Signaling Technology), β-actin (1:2000, Cell Signaling Technology), Lamin B (1:1000, Abcam) at 4°C overnight, respectively. On the next day, after three PBST washes, the membrane was incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies (1:3000, Bioworld, MN, USA) for 2 h at room temperature. The enhanced chemiluminescence kit (Millipore, MA, USA) was applied to visualize proteins of interest on the UVP BioSpectrum Imaging system (BioSpectrum, CA, USA). Quantification of Western blot bands was performed using Quantity one 4.6.2 (Bio-Rad, CA, USA) software. β-actin and Lamin B served as inner control for normalization.

Enzyme-linked immunosorbent assay

The concentrations of related proteins, including OVA-specific IgE, leukotriene C4 (LTC4), prostaglandin D2 (PGD2), eosinophil cation protein (ECP), IL-2, IL-4, IL-5, IL-13 and IFN-γ, in the NLF and serum samples were determined using corresponding ELISA kits (R&D Systems, MN, USA), respectively, according to the manufacturer's protocols. For the detection of OVA-specific IgE, after samples were incubated with purified antimouse IgE monoclonal antibody, washed by PBST (0.05% Tween 20 in phosphate-buffered saline) for three-times, biotin-labeled OVA was added, followed by the addition of horseradish peroxidase-labeled antibiotin (American Qualex, Inc.). Then the reactions were triggered with tetramethylbenzidine (Moss, Inc., ME, USA) and stopped with H₂SO₄. MTP-32 microplate reader (Corona Electric Co., Ltd., Ibaraki, Japan) was applied to record the absorbance at 450 nm. Each sample was analyzed in triplicate.

Histologic assessment

The 24 h after the last OVA challenge at day 28, mice of different groups were anesthetized and sacrificed by exsanguination to collect nasal mucosa tissues. Then tissues were fixed in 4% paraformaldehyde for 48 h, followed by embedded in paraffin. The 3-µm sections were deparaffinized and stained with 10% Giemsa solution (Solarbio, Beijing, China) to investigate leukocytes and mast cell infiltration in the nasal mucosa. Cells were counted under a microscope at ×400 magnification.

Inflammation cells counting

After configuration, the sediment of NLF sample from each group was resuspended in 1 ml 100 mM phosphate-buffered saline supplemented with 1% bovine serum albumin. Total number of leukocyte cells was counted in a hemocytometer (Mindray 3000, Shenzhen, China). The eosinophils, neutrophils and lymphocytes were stained by Wright's–Giemsa assay for cell counting.

Statistical analysis

Statistical software SPSS Version 12.0 (SPSS, Inc., IL, USA) was used for statistical analysis. All data were expressed as the mean ± standard deviation (SD) and analyzed by the one-way analysis of variance (ANOVA) or Student's test. The $p < 0.05$ was considered to be statistically significant.

Results

miR-146a is substantially downregulated in AR mice & AR patients

To explore the role of miR-146a in AR development, the expression of miR-146a in nasal mucosa under different conditions was detected. As shown in Figure 1A, the mRNA expression level of miR-146a in AR nasal mucosa was significantly downregulated compared with that in control group. TRAF6 is reported to be directly suppressed by miR-146a to regulate inflammatory cytokines secretion [14]. Then the expression of TRAF6, miR-146a target gene, was detected. Results showed that the mRNA and protein expression levels of TRAF6 were both markedly increased in AR group compared with the control group (Figure 1B), indicating that the inhibition of TRAF6 by miR-146a in AR mice was attenuated. Besides, the expression patterns of miR-146a and TRAF6 in the nasal

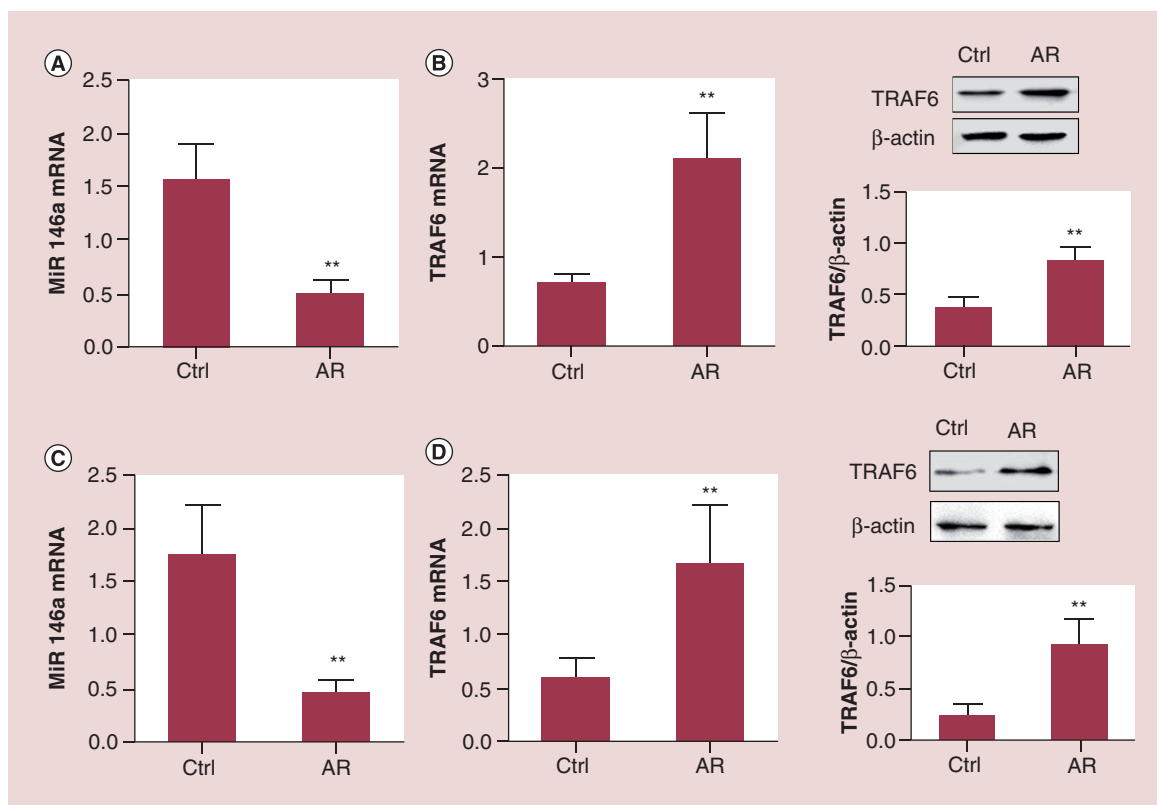


Figure 1. miR-146a was decreased in the nasal mucosa of allergic rhinitis mice and allergic rhinitis patients. (A) Relative miR-146a mRNA levels in the nasal mucosa of AR mice. **(B)** TRAF6 mRNA levels and protein expressions in the nasal mucosa of AR mice. **(C)** Relative miR-146a mRNA levels in the nasal mucosa of AR patients. **(D)** TRAF6 mRNA levels and protein expressions in the nasal mucosa of AR patients. mRNA levels were examined by RT-qPCR and normalized to control. β-actin was applied as an internal control. Protein expressions were analyzed by western blotting. β-actin was applied as a loading control. Each value represents the mean ± SD of eight mice/patients in each group.

***p* < 0.01 versus Ctrl group.

AR: Allergic rhinitis; RT-qPCR: Reverse transcription-quantitative PCR; SD: Standard deviation.

mucosa of AR patients were similar with those in AR mice, which solidifying the finding miR-146a is substantially downregulated under AR conditions.

miR-146a mimic attenuated sneezing & nasal rubbing events in AR mice

To investigate whether miR146a possesses anti-inflammatory roles to AR, miR146a mimic was administered into the nostril before OVA challenge daily on days 21–28, and the sneezing and nasal rubbing in 10 min were recorded on day 28 of AR modeling. It was found that, the untreated-AR mice suffered from severe sneezing and nasal rubbing compared with the control, while excessive miR-146a treatment significantly decreased sneezing and nasal rubbing events by $37.44 \pm 15.62\%$ (Figure 2A) and $33.73 \pm 13.88\%$ (Figure 2B), respectively. Additionally, miRNA control failed to attenuate sneezing and nasal rubbing events in AR mice (Supplementary Figure 1). These results suggest that miR-146a attenuates AR-related nasal symptoms. Here, budesonide aqueous nasal spray was applied as the positive control, which represented obvious attenuation of AR-related nasal symptoms, sneezing and nasal rubbing (Supplementary Figure 1). Strikingly, miR-146a mimic has similar effect with budesonide aqueous nasal spray (Supplementary Figure 1).

miR-146a mimic decreased the levels of OVA-specific IgE as well as mastocyte- & basophil-related inflammatory cytokines in AR mice

To further explore the mechanisms underlying the anti-inflammatory roles of miR146a in AR, the levels of OVA-specific IgE as well as mastocyte- and basophil-related inflammatory cytokines, including LTC₄, PGD₂ and

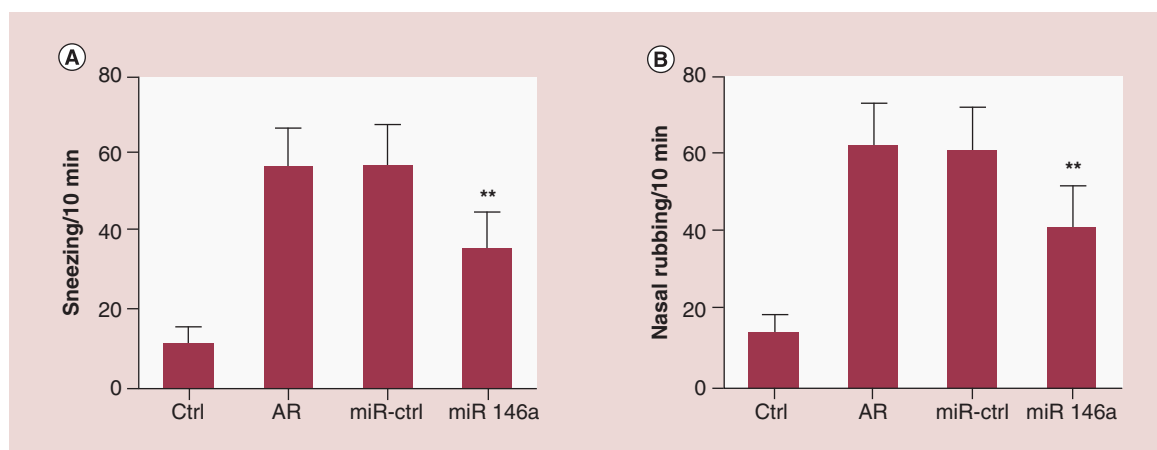


Figure 2. miR-146a mimic attenuated sneezing and nasal rubbing events in Allergic rhinitis mice. Quantification analyses of sneezing (A) and nasal rubbing events (B) were performed between four groups (n = 8). miR-146a, OVA-induced AR mice were administered miR-146a mimic (0.1 mg/ml, 20 μ l/mouse) into the nostril 30 min before OVA challenge daily on days 21–28. Each value represents the mean $\pm$ SD of eight mice in each group.

**p < 0.01 versus AR group.

AR: Allergic rhinitis; SD: Standard deviation.

ECP in AR mice were evaluated. In AR NLF samples, the levels of OVA-specific IgE, LTC₄ and PGD₂ were obviously higher than the control, while miR146a mimic treatment to AR mice corrected the abnormal increases to significant extents, with OVA-specific IgE being decreased by $57.35 \pm 25.07\%$ (Figure 3A), LTC₄ by $23.89 \pm 11.68\%$ (Figure 3B), and PGD₂ by $26.94 \pm 12.35\%$ (Figure 3C). The levels of OVA-specific IgE, LTC₄ and ECP in AR serum represented the similar pattern under miR146a intervention, with OVA-specific IgE being decreased by $37.01 \pm 15.73\%$ (Figure 3D), LTC₄ $23.09 \pm 14.42\%$ (Figure 3E) and ECP by $48.23 \pm 28.31\%$ (Figure 3F). Combining both, miR-146a mimic decreased the levels of OVA-specific IgE as well as mastocyte- and basophil-related inflammatory cytokines in AR mice.

miR-146a mimic reduced inflammatory cells in AR mice

As shown in Figure 4A, compared with AR group, the number of mast cells in the nasal mucosa of miR-146a group was significantly enhanced. Also, the number of eosinophils in nasal mucosa of miR-146a treated mice was higher than that of the AR group (Figure 4B). The number of eosinophils, lymphocytes, as well as neutrophils in the NLF of AR mice was markedly greater than that of control mice (Figure 4C–E), while in the NLF of miR146a-treated AR mice these cells experienced significant decrease in comparison with untreated-AR mice, suggesting that miR-146a alleviates the infiltration of inflammatory cells under AR challenge.

miR-146a mimic affected the secretion of inflammatory cytokines in AR serum

To uncover the influence of miR-146a on AR-associated inflammation, the levels of several inflammation-associated cytokines in the serum were determined via ELISA. As shown in Figure 5A–C, the pro-inflammatory cytokines IL-4, IL-5 and IL-13 were remarkably increased in AR mice compared with the control mice, and the administration of exogenous miR-146a significantly decreased their expression levels in comparison with AR mice. On the contrary, the downregulated levels of anti-inflammatory cytokines IFN- γ and IL-2 in AR mice were corrected to some extent as a result of the intervention of miR-146a mimic (Figure 5D & E). These results suggest that miR-146a might exert anti-AR role through affecting the secretion of inflammatory cytokines.

miR-146a relieved inflammatory phenotypes via TLR4/TRAF6/NF- κ B signaling pathway

Evidences revealed that miR-146a attenuated inflammatory responses induced by a variety of damages via inhibiting TLR4/NF- κ B signaling to a large extent [15,17]. To illustrate the molecular mechanisms underlying the attenuation of miR-146 on AR symptoms, key molecules along the TLR4/NF- κ B signaling pathway were detected in the murine nasal mucosa from different groups via western blotting analysis. As shown in Figure 6A–C, the expression levels of TLR4 and its downstream molecule TRAF6 were significantly increased in AR nasal mucosa compared

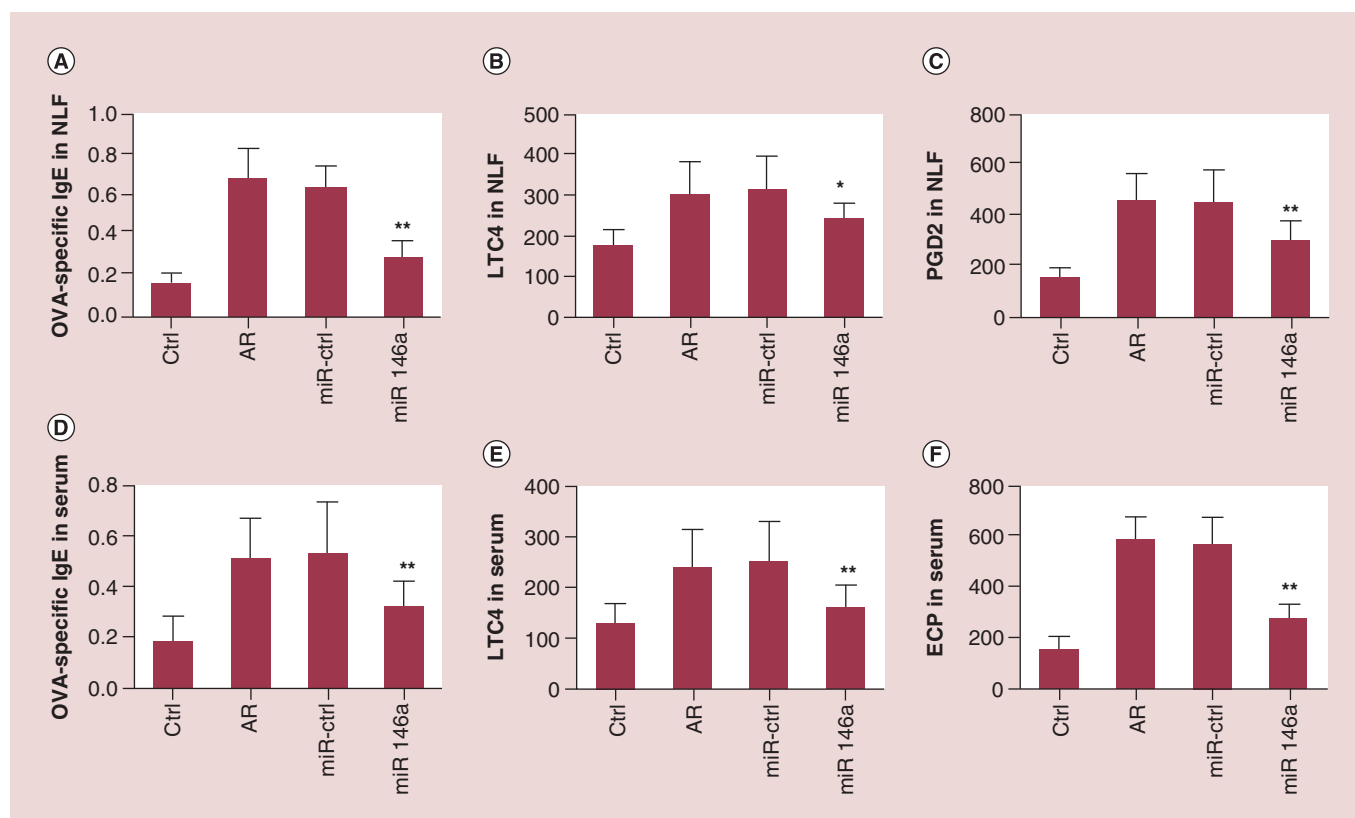


Figure 3. miR-146a mimic attenuated inflammatory response in AR mice. miR-146a mimic decreased OVA-specific IgE, LTC4 and PGD2 in the NLF (A–C), and OVA-specific IgE, LTC4 and ECP in the serum (D–F) of AR mice. Samples were collected at day 28 after the first OVA challenge. Protein levels were detected using ELISA and expressed as optical density at 450 nm. Each value represents the mean $\pm$ SD of eight mice in each group.

* $p < 0.05$; ** $p < 0.01$ versus AR group.

AR: Allergic rhinitis; ECP: Eosinophil cation protein; NLF: Nasal lavage fluid; OVA: Ovalbumin; SD: Standard deviation.

with the control group, while they were markedly downregulated in miR-146a-treated AR mice. Also, NF- κ B was significantly activated in AR group as evidenced by an enhanced p-I κ B α /I κ B α ratio in cytoplasm and p65 expression level in the nucleus (Figure 6D & E). As expected, miR-146 mimic markedly attenuated these abnormalities in AR mice. Results of this portion suggest that miR-146a might exert its anti-inflammatory roles via inhibition TLR4/TRAF6/NF- κ B signaling pathway in AR nasal mucosa.

Discussion

The prevalence of AR is known to sharply rise in recent years with no safe and effective therapeutics nowadays. AR is a chronic inflammatory disorder caused by T cells and related T-helper 2 (Th2) cytokines. Evidences indicate that miR-146a exert potent anti-inflammatory roles in a variety of inflammation-involved disorders [8,13,18]. However, little is known about the influence of miR-146a on AR. This study is designed to investigate the therapeutic potential of miR-146a on AR-related inflammation responses and the underlying mechanisms using an OVA-induced AR mouse model.

In this study, the mRNA expression level of miR-146a in AR nasal mucosa was significantly decreased compared with the control group, suggesting that miR-146a might possess some relationship with AR. It has been reported that miR-146a regulates innate immune responses via directly inhibiting TRAF6 [19]. Intriguingly, the expression levels of TRAF6 were markedly increased in AR mice, which stands the initial hypothesis that miR-146a might correlate with AR pathogenesis.

To investigate whether miR-146a exerts anti-inflammatory roles on AR, miR-146a mimic was administered into the nostril before OVA challenge daily on days 21–28. It was found that exogenous miR146a significantly alleviated allergic symptoms, sneezing and nasal rubbing, further pointed that it might have therapeutic potential to AR.

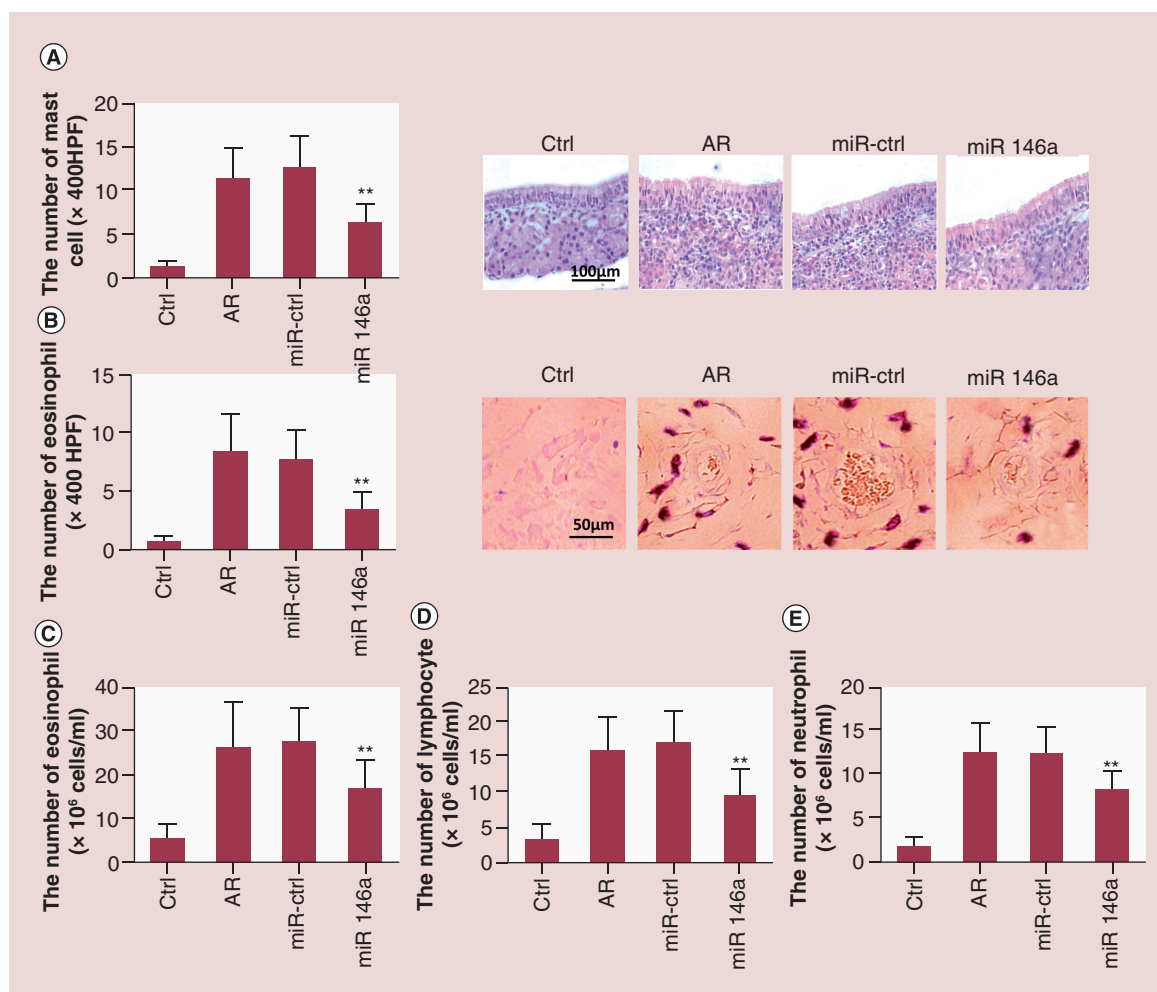


Figure 4. miR-146a mimic reduced leukocytes and mast cells infiltration in Allergic rhinitis mice. Samples were collected 24 h after the last OVA challenge at day 28. **(A & B)** Histological analysis for infiltration of mast cells **(A)** and eosinophils **(B)** in nasal mucosa of mice. They were stained with Giemsa solution and counted under a microscope at $\times 400$ magnification. **(C–E)** The number of eosinophil **(C)**, lymphocyte **(D)** and neutrophil **(E)** in the NLF. Each value represents the mean $\pm$ SD of eight mice in each group.

** $p < 0.01$ versus AR group.

NLF: Nasal lavage fluid; OVA: Ovalbumin; SD: Standard deviation.

IgE-mediated immune response, inflammatory cell disorders and Th1/Th2 imbalance-induced abnormal cytokines are reported to be closely related with AR development, which crosslink with each other during AR pathogenesis [16,20]. Thus, the following experiments were conducted according to these three aspects, respectively. First, miR-146a mimic significantly decreased the levels of OVA-specific IgE as well as mastocyte- and basophil-related inflammatory cytokines LTC₄, PGD₂ and ECP in AR mice, suggesting that miR-146a could inhibit IgE-mediated immune response in AR mice. Second, miR-146a reduced the numbers of mast cells and eosinophils in the nasal mucosa, as well as eosinophils, lymphocytes and neutrophils that were enhanced in NLF of AR mice, which means that exogenous miR-146a could rectify the inflammatory cell disorders in AR to some extent. Third, miR-146a mimic decreased the secretion of the pro-inflammatory cytokines (IL-4, IL-5 and IL-13) mainly produced by Th2 cells while increased the secretion of anti-inflammatory cytokines (IFN- γ and IL-2) mainly produced by Th1 cells in AR mice, indicating that miR-146a might exert anti-inflammatory role through rectifying the Th1/Th2 imbalance to adjust the secretion of inflammatory cytokines in AR mice. Taking the above results into account that miR-146a alleviated allergic symptoms, inhibited IgE-mediated immune response, decreased inflammatory cells as well as impacted abnormal secretion of inflammatory cytokines, it is confirmed that miR-146a possesses therapeutic effects on experimentally induced AR.

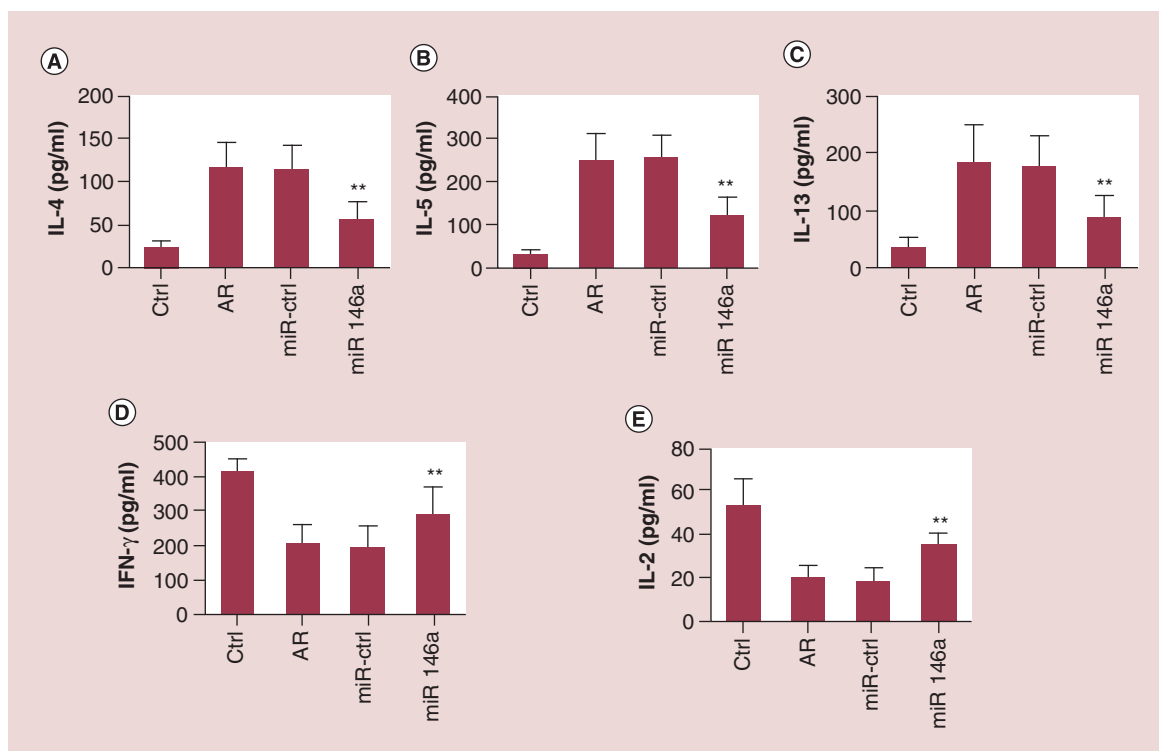


Figure 5. Effects of miR-146a mimic on the production of inflammatory cytokines. miR-146a mimic impacted the secretion of inflammatory cytokines, IL-4 (A), IL-5 (B), IL-13 (C), IFN- γ (D), and IL-2 (E), in the serum of AR mice. ELISA was performed to detect the above protein cytokines. Each value represents the mean $\pm$ SD of 8 mice in each group. ** $p < 0.01$ versus AR group. AR: Allergic rhinitis; SD: Standard deviation.

Studies have demonstrated that many agents exert anti-inflammatory effects by suppressing TLR4/TRAF6/NF- κ B signaling pathway [14,17,21,22]. TLRs are germline-encoded pattern recognition receptors and facilitate the initiation of innate immune responses such as inflammation [23,24]. As a key member of the TLR family TLR4 can be activated by lipopolysaccharide and nonbacterial agonists [25,26]. It is demonstrated that TLR4 plays pivotal roles in the modulation of immune response during AR pathogenesis [27]. TLR4 signaling activation triggers the synthesis and release of inflammatory cytokines and mediators. Meanwhile, TLR4-activated TRAF6 further activates the inhibitor of κ B kinase and initiates the activation of NF- κ B [28]. The NF- κ B pathway has been suggested to play critical roles in TLR4-mediated inflammatory regulation [29]. In AR murine models, local NF- κ B inhibition suppresses the allergic response [30]. In the present study, results showed that TLR4 signaling was activated by the increased expression of TLR4, TRAF6 and NF- κ B in the nasal mucosa of AR mice. However, miR-146a treatment suppressed the TLR4 signaling activation, indicating that the inhibition of TLR4/TRAF6/NF- κ B signaling pathway might underlie the anti-inflammatory effect of miR-146a on AR. To our knowledge, this study demonstrates for the first time that miR-146a might exert therapeutic effects on experimental AR via downregulating TLR4/TRAF6/NF- κ B signaling pathway. In recent years, agents that target TLR4 signaling have been reported to attenuate AR by regulating inflammatory cytokines or cells [31,32]. Combined with our finding, a cocktail of these agents inhibiting TLR4 signaling might be a novel therapeutic point for AR treatment.

There are several limitations exist in this study. On one hand, the dosage of miR-146a was chosen according to previous described, and miR-146a at higher doses would be tested to find out the most effective dosage that could even cure the AR abnormalities in the future. On the other hand, the antagonists and agonists specific to TLR4/TRAF6/NF- κ B signaling pathway or related knockout transgenic mice would be applied to illustrate the concrete molecular mechanisms underlying the anti-inflammatory effects of miR-146a on AR.

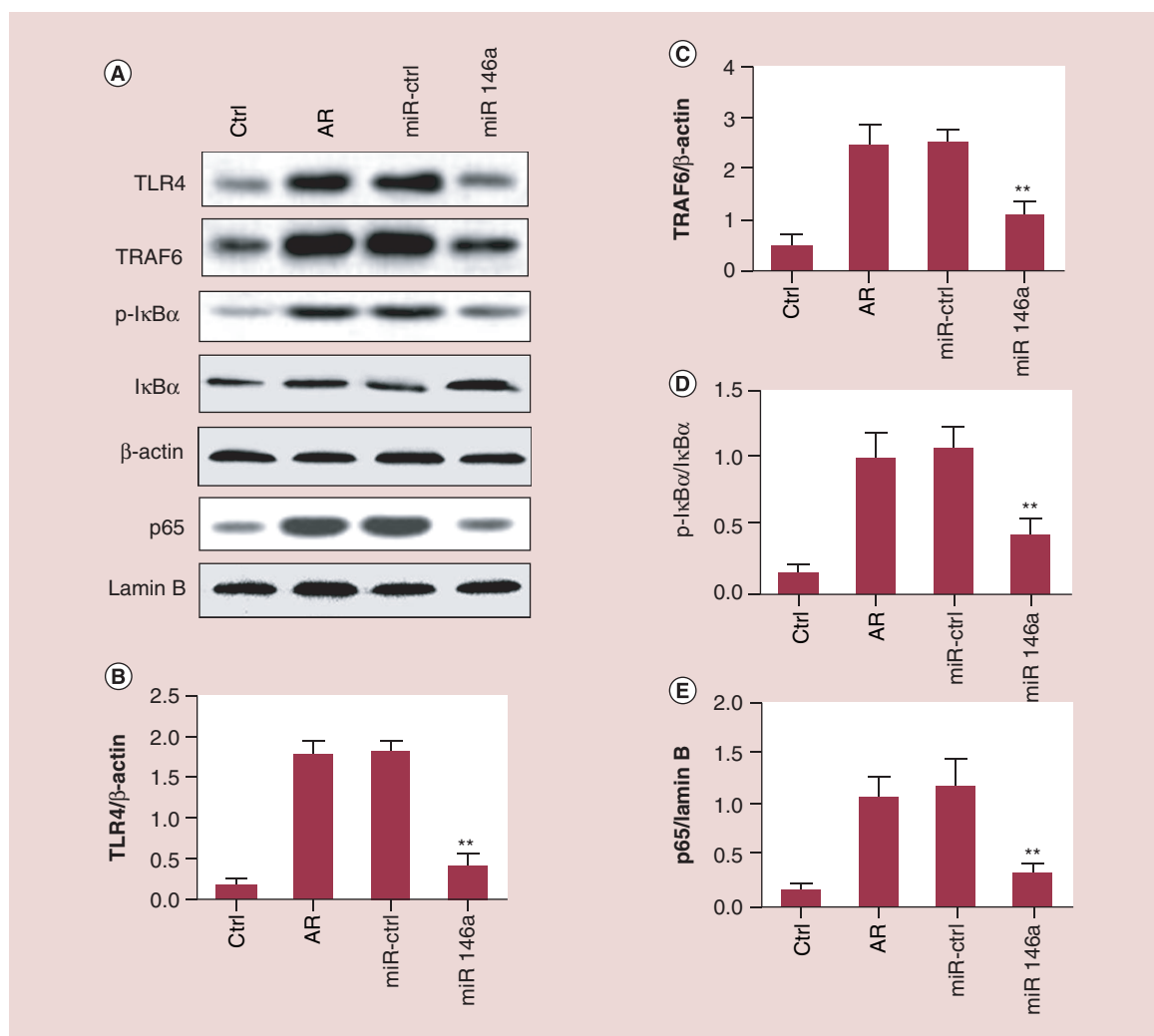


Figure 6. miR-146a mimic downregulated the proteins relevant to toll-like receptor 4 (TLR4)/TRAF6/NF-κB signaling pathway. (A) Western blotting analysis of key effectors associated with TLR4/TRAF6/NF-κB signaling pathway in the nasal mucosa of AR mice. (B–E) Semi-quantitative analyses of TLR4/β-actin, TRAF6/β-actin, p-IκBα/IκBα in cytoplasm and p65/Lamin B in nucleus. β-actin and Lamin B served as inner control, respectively. Each value represents the mean ± SD of eight mice in each group.

**p < 0.01 versus AR group.

AR: Allergic rhinitis; SD: Standard deviation; TLR: Toll-like receptor.

Conclusion

In short, this study indicates that miR-146a mimic alleviates the allergic symptoms, reduces inflammatory cells and regulates the production of pro-inflammatory mediators predominantly produced by Th2 cells in AR mice. The anti-inflammatory effects of miR-146a on AR might exert through the inhibition of TLR4/TRAF6/NF-κB signaling pathway to some extent. Thus, miR-146a could be considered as a potential therapeutic agent for AR.

Future perspective

AR is one of the most common disorders in airway allergic inflammation with no effective and curative treatments. miRNA-146a has been shown to possess potent anti-inflammatory effects. The anti-inflammatory effects of miR-146a on AR might exert through the inhibition of the TLR4/TRAF6/NF-κB signaling pathway to some extent.

Summary points

- The expression level of miR-146a in Allergic rhinitis (AR) nasal mucosa was significantly decreased.
- miR-146a mimic markedly attenuated sneezing and nasal rubbing events, decreased the levels of ovalbumin-specific IgE as well as mastocyte- and basophil-related inflammatory cytokines, reduced inflammatory cells, affected the T-helper 2-released cytokines.
- miR-146a mimic inhibited the expressions of proteins relevant to toll-like receptor 4 (TLR4)/TRAF6/NF- κ B signaling pathway. miR-146a mimic alleviates the allergic symptoms, reduces inflammatory cells and regulates the production of pro-inflammatory mediators predominantly produced by T-helper 2 cells in AR mice.
- The anti-inflammatory effects of miR-146a on AR might exert through the inhibition of the Toll-like receptor 4 (TLR4)/TRAF6/NF- κ B signaling pathway to some extent.

Financial & competing interests disclosure

This work was funded by Jilin Electric Research Funding. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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