

# MiR-126-5p Down-Regulation Alleviates the Inflammatory Response of Allergic Rhinitis in Children via Inhibiting HIPK2/NF- $\kappa$ B Signaling Pathway

Yunlong Jing, Jiang Xie, Songliang Long, Min Huang

Department of Otolaryngology Head and Neck Surgery, Hunan Children's Hospital, Changsha, 410007, People's Republic of China

Correspondence: Min Huang, Department of Otolaryngology Head and Neck Surgery, Hunan Children's Hospital, No. 86, Ziyuan Road, Changsha, 410007, People's Republic of China, Tel +86-0731-85356311, Email huangmin2005dr@163.com

**Purpose:** The objective of our study was to exploit the potential mechanism of microRNA-126-5p (miR-126-5p) in the occurrence and formation of allergic rhinitis (AR) in children.

**Patients and Methods:** Nasal mucosal tissues were obtained from AR in children and patients with adenoidectomy. Human nasal epithelial cell line (RPMI-2650) and BALB/c mice models were, respectively, established via ovalbumin (OVA) stimulation. Target genes and proteins levels were determined through quantitative real-time polymerase chain reaction (qRT-PCR) and enzyme-linked immunosorbent assay (ELISA) assays. The interaction of miR-126-5p with homeodomain-interacting protein kinase 2 (HIPK2) was confirmed via dual-luciferase reporter detection.

**Results:** MiR-126-5p was memorably increased in nasal mucosal tissue specimens of AR children compared with patients with adenoidectomy, while HIPK2 was distinctly declined (all  $P < 0.05$ ). A negative association was found between miR-126-5p and HIPK2 expression ( $r = -0.5757$ ,  $P < 0.001$ ). Moreover, HIPK2 was predicted to be targeted by miR-126-5p. Proinflammatory cytokines expressions were significantly increased, and anti-inflammatory cytokines were obviously decreased in AR RPMI-2650 cell model ( $P < 0.001$ ). NF- $\kappa$ B signaling pathway was also activated in AR RPMI-2650 cell model. MiR-126-5p inhibitor mitigated the stimulated function by OVA. Silencing HIPK2 recused miR-126-5p inhibitor phenomena in AR RPMI-2650 cell model. Furthermore, in vivo experiments further verified in vitro results, documenting that miR-126-5p inhibitor and si-HIPK2 relieved AR in the mice model.

**Conclusion:** MiR-126-5p down-regulation relieved inflammation response and events of AR in children and mice model of AR through HIPK2/NF- $\kappa$ B signaling pathway, suggesting being a latent therapeutic target in AR.

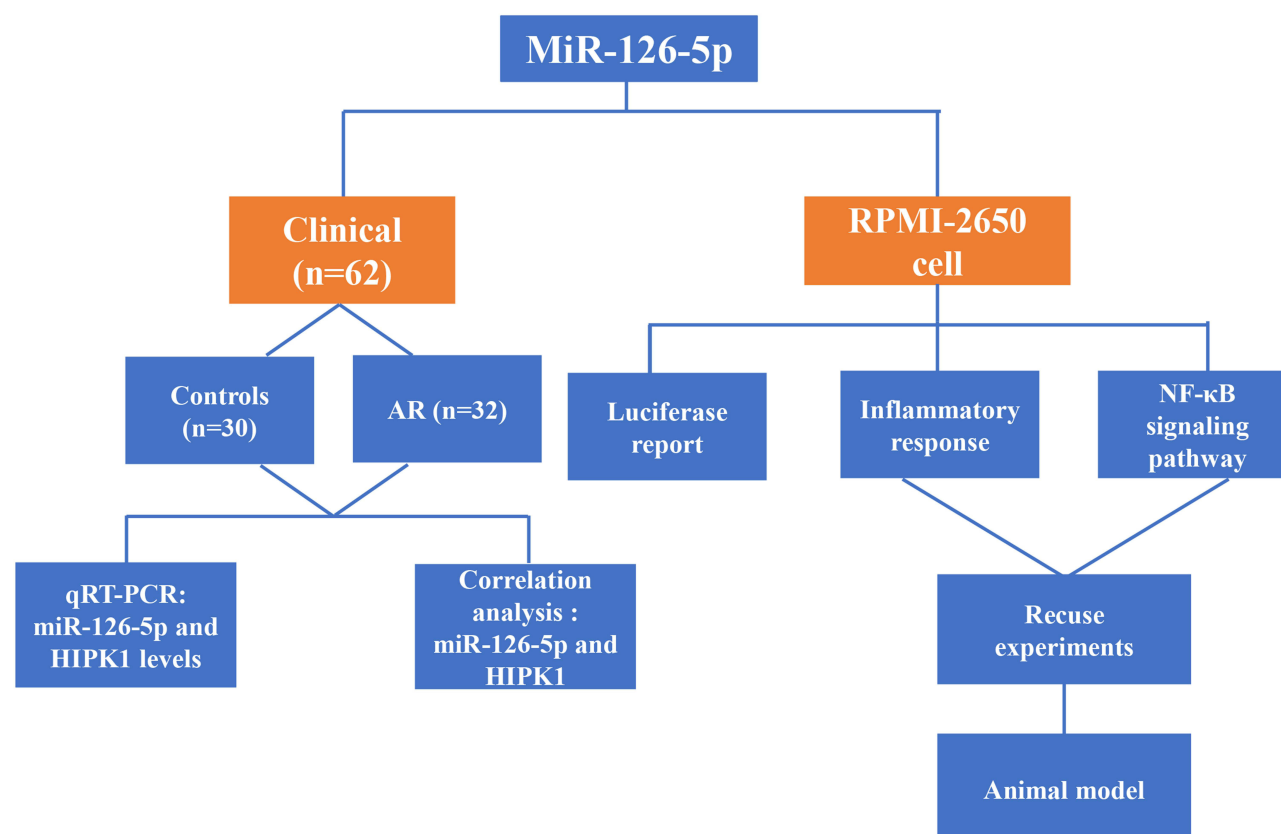
**Keywords:** MiR-126-5p, HIPK2, NF- $\kappa$ B signaling pathway, allergic rhinitis

## Introduction

Allergic rhinitis (AR) is a chronic, non-infectious nasal disorder, mediated by immunoglobulin E (IgE) and immunoglobulin G1 (IgG1), that occurs in atopic individuals following exposure to allergens.<sup>1,2</sup> The last few decades have seen a significant increase in the prevalence of AR, making it one of the most important chronic inflammatory respiratory diseases.<sup>3</sup> According to national epidemiological surveys, AR has a serious impact on people's lives and socio-economic development, and is occurring at increasingly younger ages, especially in children.<sup>4</sup> Common clinical symptoms of AR in children include sudden attacks of itchy, stuffy, and runny nose, discharge, and rhinorrhoea, which seriously impact on children's daily life, learning, sleep, memory, and self-confidence.<sup>5</sup> At present, the use of intranasal corticosteroids is currently the most common treatment AR. However, this only provides temporary relief from the symptoms of AR.<sup>6</sup>

MicroRNAs (miRNAs) are short, single-stranded, endogenous noncoding molecules of approximately 22 nucleotides in length that are involved in post-transcriptional gene regulation.<sup>7,8</sup> As novel and promising biomarkers for disease progression, miRNAs have been documented to play a pivotal role in the control of inflammatory behaviors.<sup>9,10</sup> A large number of reports

## Graphical Abstract



have proven that microRNA-126-5p (miR-126-5p) was abnormally discovered in multiple disorders, such as tumor and inflammation diseases.<sup>11,12</sup> For instance, miR-126-5p was poorly expressed, and played a suppressive role via targeting Bcl2l2 in the development of cervical cancer.<sup>13</sup> Mao et al claimed that plasma miR-126-5p was memorably diminished in sepsis-stimulated acute lung injury, indicated as systemic inflammation and immune function indexes.<sup>14</sup> A few studies have implicated miR-126-5p in AR, with only one study showing that miR-126-5p was elevated in AR sufferers according to microarray and qPCR validation.<sup>15</sup> Interestingly, ENCORI database (<https://rnasysu.com/encori/agoClipRNA.php?source=mRNA>) predicted that the potential binding region of HIPK2 with miR-126-5p. However, whether the regulation of miR-126-5p on inflammation of AR in children is related to HIPK2 has not been studied yet.

In the current research, we hypothesized that down-regulation of miR-126-5p could increase HIPK2 expression status and therefore inhibit NF-κB signaling pathway to alleviate inflammation response of AR. Our study was designed to take advantage of the regulatory effects of miR-126-5p in developing AR and to investigate the possible mechanisms detailed.

## Material and Methods

### Patients and Clinical Sample Collection

Thirty-two children diagnosed with AR were recruited from Hunan Children's Hospital. The diagnostic criteria for AR in children include the following key components: (1) clinical manifestations: children typically present with two or more of the following symptoms: nasal congestion, rhinorrhea, sneezing, and nasal itching. Additional symptoms may include postnasal drip, cough, irritability, and fatigue. Symptoms occur for more than 1h per day and for more than 4 consecutive weeks. (2) physical examination: the presence of pale and edematous nasal mucosa, and watery nasal discharge. (3) laboratory tests: nasal secretion test is positive for eosinophil ratio >0.05, serum-specific IgE, and Skin-Prick

Testing (SPT) of at least one allergen. Moreover, common allergens in children with AR include pollen, dust mites, animal dander, and molds. All children were required to meet the following inclusion criteria: with a diagnosis of AR; aged 2–14 years old, and voluntary participation in this study and consent for nasal mucosal tissue collection. Patients were excluded if they had other inflammatory diseases, patients with other types of nasal inflammatory; positive allergic history except AR. The control group consisted of 30 cases with non-atopic obstructive snorers who received an adenoidectomy over the same time, and these patients did not display the AR-related symptoms. The basic information of AR patients and controls is listed in Table 1. Nasal mucosal tissue was scraped from the surface of the inferior turbinate of each participant and stored in a refrigerator at  $-80^{\circ}\text{C}$ .

All the participants, or their legal guardians, have provided and signed written informed forms for the study. Ethical guidelines endorsed by the Ethics Committee of Hunan Children's Hospital were followed for all procedures carried out in the study. Moreover, this research was conducted following the principles of the Declaration of Helsinki. This study has received documented review and approval from a formally constituted review board (Hunan Children's Hospital). In addition, informed consent has been obtained from the child's parents or legal guardians.

## Cell Culture and Transfection

Human nasal mucosal epithelial cell line RPMI-2650 was obtained from American Type Culture Collection (ATCC, USA) and incubated in a Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum and cultured in a humidified incubator containing 5%  $\text{CO}_2$  at  $37^{\circ}\text{C}$ . The cells were then stimulated with ovalbumin (OVA) to establish an AR cell model.

Cell transfection was conducted in the RPMI-2650 cells with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). RPMI2650 cells were transfected or co-transfected with miR-126-5p mimic, miR-126-5p inhibitor and silencing HIPK2 (si-HIPK2) vectors, or their negative controls (mimic NC, inhibitor NC, si-NC). All lentiviral vectors were provided by GenePharma Co., Ltd (Shanghai, China).

## Dual-Luciferase Reporter Detection

Using ENCORI (<https://rnasysu.com/encori/agoClipRNA.php?source=mRNA>), the latent binding site of miR-126-5p with HIPK2 was predicted. On the basis of the predictive outcome, wild-type (WT) and mutant (MUT) sequences of the binding sites (HIPK2-WT and HIPK2-MUT) were designed and produced. They were inserted into the luciferase reporter gene vectors (pmirGLO, Promega, Madison, WI, USA). The indicated vector and miR-126-5p mimic/inhibitor or negative control (mimic NC/inhibitor NC) of miRNA mimic/inhibitor were co-transfected into RPMI-2650 cell with

**Table 1** Basic Information of AR Patients and Control Subjects

Characteristics	AR Patients (n=32)	Control (n=30)	P Value
<b>Age (years)</b>	9.94±2.00	10.70±1.75	0.116
<b>BMI(kg/m<sup>2</sup>)</b>	16.83±1.20	17.10±1.18	0.377
<b>Female (n%)</b>	17 (53.13)	16 (53.33)	0.904
<b>Disease duration (months)</b>	15.37±4.10	/	/
<b>INSS score, mean±SD</b>			
<b>Nasal rhinorrhea</b>	0.94±0.24	/	/
<b>Itching</b>	1.60±0.50	/	/
<b>Sneezing</b>	1.78±0.54	/	/
<b>Congestion</b>	1.25±0.66	/	/
<b>TNSS score</b>	5.57±1.03	/	/
Specific IgE levels (kU/L)	10.58±3.10	0.28±0.10	<0.001
Eosinophil counts ( $\times 10^9/\text{L}$ )	0.45±0.02	0.12±0.01	<0.001
CRP (mg/L)	58.22±8.42	6.91±1.87	<0.001

**Notes:** Data were reported as mean ± SD (standard deviation).  $P < 0.05$  means statistically significant.

**Abbreviations:** AR, allergic rhinitis; BMI, body mass index; INSS, individual nasal symptom score; TNSS: total nasal symptom score; CRP: C-reactive protein.

Lipofectamine 3000. Fluorescence intensity was calculated via a Dual-Luciferase® Reporter Assay System (Promega, Madison, WI, USA) after transfection for 48h.

## Animals and Treatment

All the animal experimentation was conducted in strict compliance with the National Institutes of Health (NIH) guidelines for the Care and Use of Laboratory Animals. They were supported by Hunan Children's Hospital.

Male BALB/c mice were 6–8 weeks old. They were obtained from Shanghai Laboratory Animal Center (Shanghai, China) and fed freely at room temperature for one week for testing. The mice were then randomized into 6 groups and underwent corresponding treatments. Control group mice were sensitized and administered by saline alone (control group,  $n=5$ ) without OVA or aluminum. AR mice group was sensitized and administered by OVA (AR group,  $n=5$ ). AR treated with inhibitor NC group, and the mice were sensitized and administered by OVA followed by an infection of inhibitor NC (AR+inhibitor NC group,  $n=5$ ). AR treated with miR-126-5p inhibitor group, and the mice were sensitized and challenged by OVA followed by an infection of miR-126-5p inhibitor sequence (AR+miR-126-5p inhibitor group,  $n=5$ ). AR treated with miR-126-5p inhibitor, and si-NC group, mice were sensitized and challenged by infection of miR-126-5p inhibitor plus si-NC (AR+miR-126 inhibitor+si-NC group,  $n=5$ ). AR treated with miR-126 inhibitor and si-HIPK2 group, and the mice were sensitized and challenged by OVA followed by infection of miR-126-5p inhibitor plus si-HIPK2 (AR+miR-126 inhibitor+si-HIPK2 group,  $n=5$ ). Obvious changes in the nasal structure and function were observed 7 days after the mice model was established. Behavioral observations were then carried out.

## Assessment of Nasal Symptoms and Sample Preparation

Two hours after the last stimulation with OVA, blood samples were obtained and then centrifuged. The serum samples were preserved at  $-80^{\circ}\text{C}$  for next analysis.

On the basis of the previous grading standards,<sup>16</sup> allergic symptoms (rubbing number and sneezing frequencies) were observed and recorded within 10 min of the final OVA challenge by observing in a double-blinded manner. According to the severity, the frequency of rubbing and sneezing ranges from 1 to 3.

## Enzyme-Linked Immunosorbent Assay (ELISA)

The concentrations of specific IgE and IgG1, interleukin 6 (IL-6), tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), interleukin 2 (IL-2), interferon- $\gamma$  (IFN- $\gamma$ ), ECP (eosinophil cation protein), LTC4 (leukotriene C4), PGD2 (prostaglandin D2) in the supernatant of RPMI-2650 cells or serum of AR mice model were calculated using matched ELISA kits (Abcam) (R&D Systems, MN, USA) following the instruction provided by the manufacturer. A microplate reader (Bio-Tek Synergy 2; Bio-Tek Instruments, Winooski, VT, USA) was employed to determine the optical density (OD) of the cells in each well at 450nm. Each reaction was independently performed three times.

## Quantitative Real-Time Polymerase Chain Reaction (QRT-PCR) Assay

Following the directions of TRIzol reagent (Invitrogen Inc., Carlsbad, CA, USA), total RNA was isolated from nasal mucosa tissues or cells. RNA samples were assayed for concentration and purity. Qualified RNA was reversed-transcribed into complementary DNA (cDNA) with the PrimeScript RT Kit (Takara, Dalian, China). QRT-PCR was conducted with SYBR Green Premix Ex Taq (TaKaRa, Dalian, China) on an ABI 7500 Real-Time PCR system (Applied Biosystems, NY, USA) and appropriate primers designed and synthesized by Invitrogen. U6 and GAPDH were regarded as the endogenous genes for miRNA and mRNA, respectively. The fold changes were determined via the relative quantification equation of  $2^{-\Delta\Delta\text{Ct}}$ .

## Statistical Analysis

Statistical analysis was analyzed via GraphPad Prism 9.0 (GraphPad software), and measurement variables were reported as mean  $\pm$  SD (standard deviation). Independent Student's *t*-test was utilized for comparison of the difference between the two groups. One-way analysis of variance (ANOVA) was applied to analyze the difference between more than two groups. Statistically significant difference was  $P<0.05$ .

## Results

### MiR-126-5p and HIPK2 are Both Abnormally Expressed in AR Patients

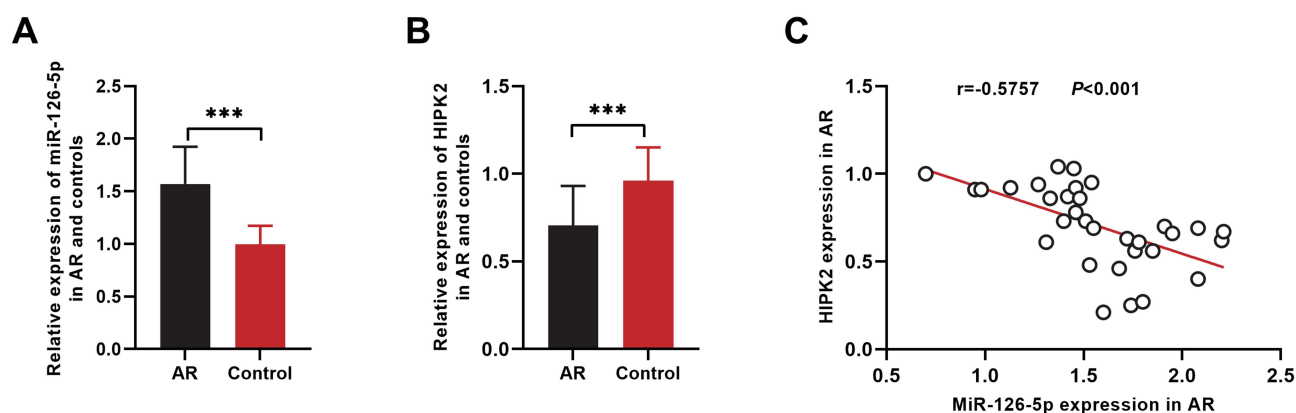
To assess the function of miR-126-5p, as well as HIPK2 in the occurrence and development of AR, the relative abundances of miR-126-5p and HIPK2 were detected in the nasal mucosal tissue samples of children with AR and controls using qRT-PCR assay. Relative to controls, miR-126-5p was memorably up-regulated in AR patients ( $P<0.05$ , Figure 1A). However, HIPK2 was significantly diminished in nasal mucosal tissue samples of AR patients in children in comparison to controls ( $P<0.05$ , Figure 1B). On the basis of Pearson's correlation analysis, an adverse correlation was disclosed of miR-126-5p expression with HIPK2 expression of AR in children ( $r=-0.5757$ ,  $P<0.001$ , Figure 1C). Our findings disclosed that aberrant expressions of both miR-126-5p and HIPK2 might be involved in the formation and development of AR.

### Direct Interaction Between miR-126-5p and HIPK2

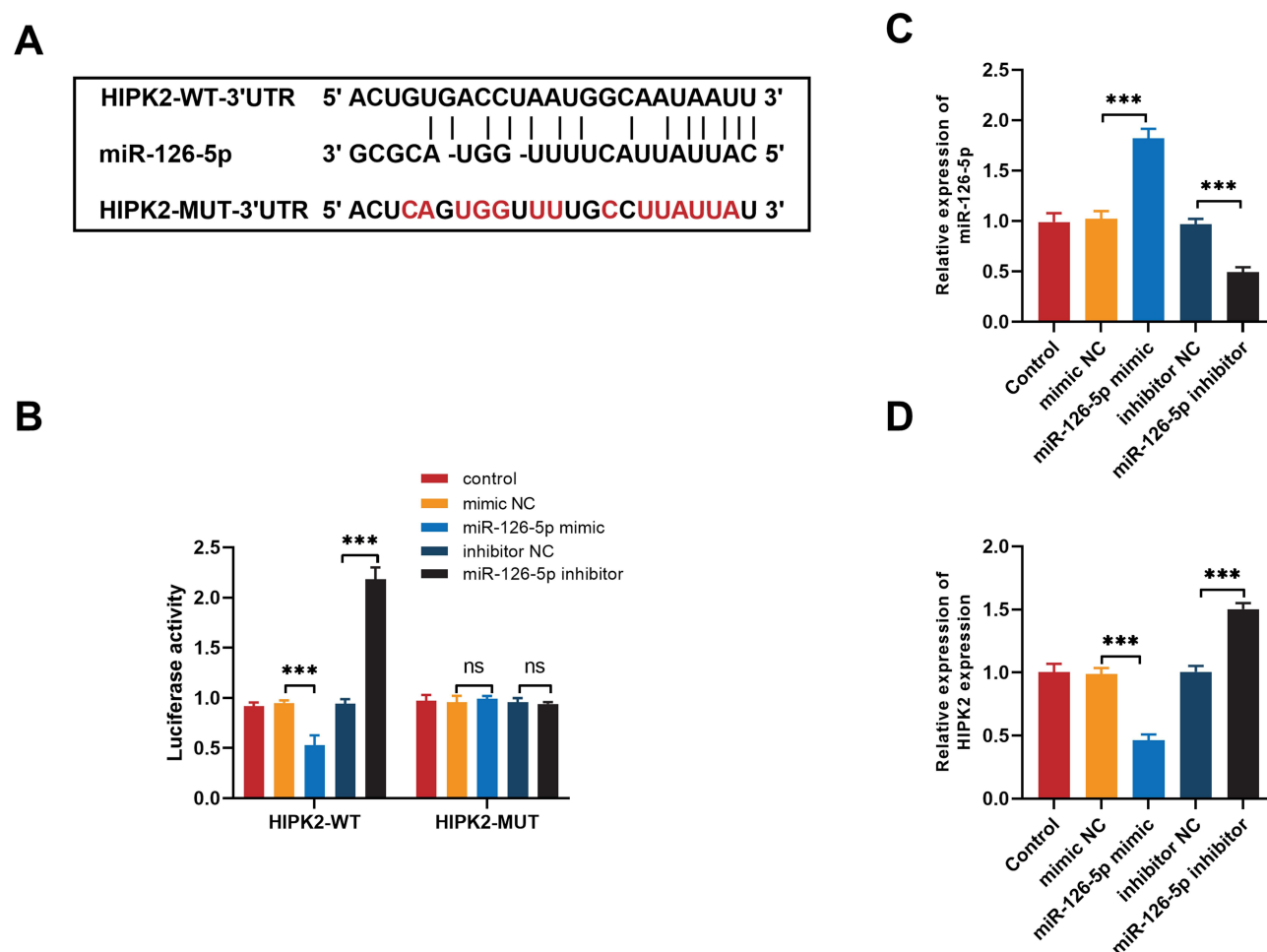
To further verify the correlation of miR-126-5p with HIPK2 in AR, the candidate binding sites were predicted using the ENCORI dataset (Figure 2A). According to the dual-luciferase reporter detection, miR-126-5p mimic memorably declined the relative luciferase activity of HIPK2-WT reporter, likewise miR-126-5p inhibitor substantially enhanced the relative luciferase activity of HIPK2-WT reporter ( $P<0.001$ ). Nevertheless, no obvious differences were discovered in the HIPK2-MUT group (Figure 2B). As indicated in Figure 2C and D, up-regulation of miR-126-5p could substantially reduce HIPK2 expression, while silencing miR-126-5p obviously increased HIPK2 expression via qRT-PCR measurement (all  $P<0.05$ ). Our results disclosed that miR-126-5p might negatively interact with HIPK2.

### Effects of Down-Regulation miR-126-5p on Inflammatory Cytokines Production in AR RPMI-2650 Cell Model

Next, an in vitro cell model was established by inducing RPMI-2650 with OVA for 48h. Then, miR-126-5p inhibitor or inhibitor NC were transfected in AR RPMI-2650 cell model. We analyzed the mRNA and protein levels of inflammatory cytokines, including IL-5, TNF- $\alpha$ , IL-2, IFN- $\gamma$ , ECP, LTC4, and PGD2, in AR RPMI-2650 cell model transfected with inhibitor NC or miR-126-5p inhibitor. Data indicated that OVA elevated the levels of IL-5 and TNF- $\alpha$ , and diminished IL-2 and IFN- $\gamma$  levels in RPMI-2650 cells, whereas IL-5, TNF- $\alpha$ , ECP, LTC4, and PGD2 levels were substantially reduced, IL-2 and IFN- $\gamma$  levels obviously elevated, when down-regulation miR-126-5p by its inhibitor sequence in OVA-induced RPMI-2650 cells (Figure 3A and B). Consistently, ELISA documented that silencing miR-126-5p could cause reduced protein levels of pro-inflammatory cytokines and basophil- and mastocyte-associated inflammatory cytokines, including IL-5, TNF- $\alpha$ , ECP, LTC4, and PGD2, and facilitated protein levels of anti-inflammatory cytokines, including IL-2 and IFN- $\gamma$  in OVA-induced RPMI-2650 cells (Figure 3C and D). The data documented that miR-126-5p down-regulation mitigated inflammatory response in the in vitro cell model of AR.



**Figure 1** Relative abundances of miR-126-5p (A) and HIPK2 (B) in AR of children and controls. Correlation of miR-126-5p with HIPK2 was assessed by means of Pearson's correlation analysis (C). \*\*\*,  $P<0.001$ .



**Figure 2** MiR-126-5p directly targeted HIPK2. The candidate binding site prediction of miR-126-5p with HIPK2 was carried out and the wide type (WT) and mutant type (MUT) binding sequences were indicated via ENCORI database (A). The direct interaction was confirmed between miR-126-5p and HIPK2 via dual-luciferase reporter detection (B). Relative abundances of miR-126-5p were examined in RPMI-2650 cells transfected with miR-126-5p mimic or inhibitor via qRT-PCR assay (C). Relative abundances of HIPK2 after miR-126-5p mimic or inhibitor transfection were examined in RPMI-2650 cells via qRT-PCR assay (D). ns:  $P>0.05$ ; \*\*\*:  $P<0.001$ .

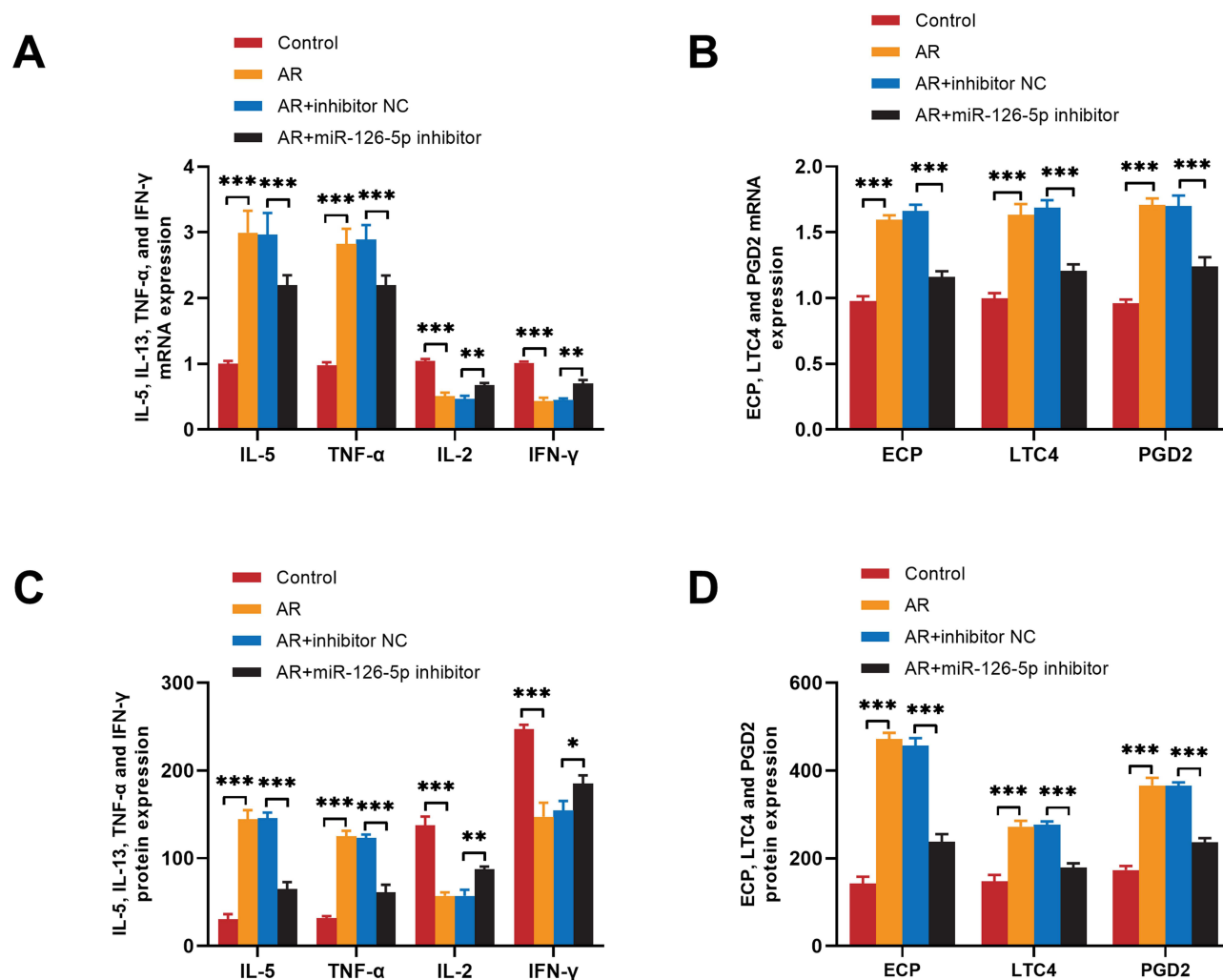
## MiR-126-5p Down-Regulation Suppressed NF- $\kappa$ B Signaling Pathway in AR RPMI-2650 Cells

Published literature has disclosed that NF- $\kappa$ B signaling pathway plays a central role in the occurrence of inflammatory response.<sup>17</sup> To investigate miR-126-5p function in NF- $\kappa$ B signaling pathway in OVA-stimulated RPMI-2650, the phosphorylation levels of I $\kappa$ B $\alpha$  and p65 were compared in cells treated with miR-126-5p inhibitor. AR RPMI-2650 cell model exhibited a distinct increase in the levels of phosphorylated I $\kappa$ B $\alpha$  (p-I $\kappa$ B $\alpha$ ) and phosphorylated p65 (p-p65), while miR-126-5p inhibitor decreased the levels of p-I $\kappa$ B $\alpha$  and p-p65 without affecting I $\kappa$ B $\alpha$  and p65 levels (Figure 4A and B). The above outcomes documented that miR-126-5p down-regulation suppressed NF- $\kappa$ B pathway activation in OVA-stimulated RPMI-2650 cells.

## Knockdown HIPK2 Reversed the Effects of Down-Regulation miR-126-5p on OVA-Induced Inflammation Response

We simultaneously transfected miR-126-5p inhibitor and si-HIPK2 in AR RPMI-2650 cells in order to further appraise the relationship of miR-126-5p with HIPK2 in AR. It was claimed that levels of pro-inflammatory factors, including IL-5 and TNF- $\alpha$ , and basophil- and mastocyte-related inflammatory cytokines, including ECP, LTC<sub>4</sub>, and PDG2, were notably elevated in AR RPMI-2650 cells in relative to RPMI-2650 cells, while anti-inflammatory factors, including IL-2 and IFN- $\gamma$ , were significantly diminished (Figure 5A and B). Meanwhile, AR+miR-126-5p inhibitor group inhibited the pro-



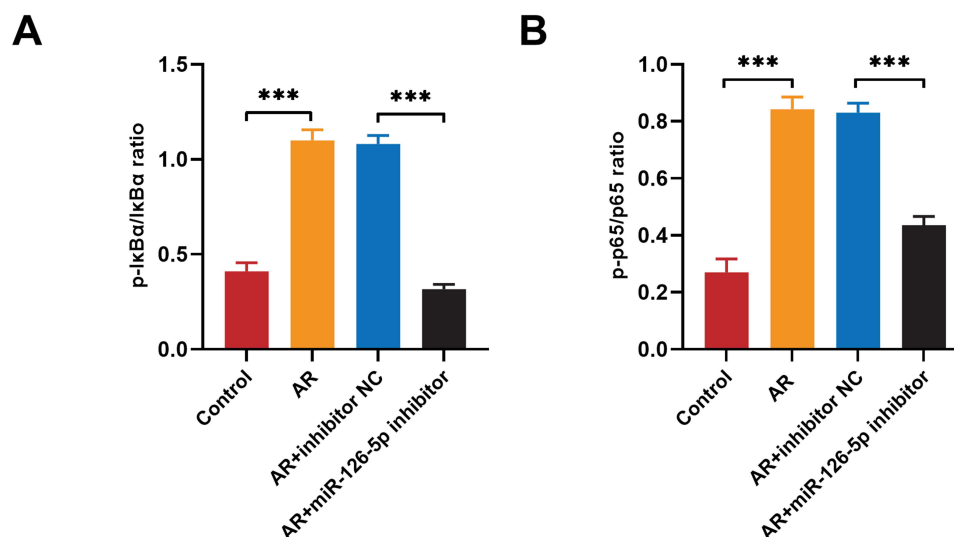


**Figure 3** Impacts of miR-126-5p inhibitor on OVA-stimulated increased inflammatory factors in RPMI-2650 cells. Down-regulation of miR-126-5p inhibited the relative abundances of IL-5 and TNF- $\alpha$  and facilitated levels of IL-2 and IFN- $\gamma$  in AR RPMI-2650 cell model using qRT-PCR assay (A). Down-regulation of miR-126-5p inhibited ECP, LTC4 and PGD2 levels via qRT-PCR assay (B). ELISA assay was applied to examine the protein levels of IL-5, TNF- $\alpha$ , IL-2, IFN- $\gamma$  (C), ECP, LTC4 and PGD2 (D) in AR RPMI-2650 cell model. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

inflammatory cytokines of IL-5 and TNF- $\alpha$ , as well as basophil- and mastocyte-related inflammatory cytokines of ECP, LTC4, and PGD2, and facilitated the anti-inflammatory factors of IL-2 and IFN- $\gamma$ , but silencing HIPK2 recused these effects in comparison to AR + inhibitor NC group (Figure 5A and B). Similarly, si-HIPK2 also reserved the significant reduction of p-IkBa and p-p65, causing by miR-126-5p inhibitor in AR cell model (Figure 5C and D). The results revealed that knockdown miR-126-5p might relieve the inflammatory events through regulating HIPK2/NF- $\kappa$ B axis.

## HIPK2 Knockdown Recused the Effects of Down-Regulation MiR-126-5p on IgE, IgG1 Levels and Allergic Response in AR Mice Model

To assess whether miR-126-5p affects IgE and IgG1 levels and allergic responses via HIPK2/NF- $\kappa$ B signaling pathway, AR mice were challenged with inhibitor NC, miR-126-5p inhibitor, si-NC, or si-HIPK2. As indicated Figure 6A, miR-126-5p was elevated, and HIPK2 was diminished in serum samples from AR mice in comparison to normal mice. Moreover, Figure 6B–D disclosed that the reduction in rubbing numbers, sneezing frequencies, and symptom scores in miR-126-5p inhibitor infected AR mice. Additionally, serum IgE and IgG1 levels were substantially diminished in AR mice administered by miR-126-5p inhibitor (Figure 6E and F). However, the above phenomena were abolished after si-



**Figure 4** Decreased miR-126-5p expression inhibits NF-κB signaling pathway in RPMI 2650. Relative to control, phosphorylation of IκBα and p65 (p-IκBα and p-65) were distinctly increased in AR group, while both p-IκBα (**A**) and p-65 (**B**) levels were memorably reduced in miR-126-5p inhibitor group in comparison to inhibitor NC group without affecting IκBα and p65 expression. \*\*\*:P<0.001.

HIPK2 administration, suggesting that knockdown miR-126-5p mitigated the serum IgE, IgG1 levels and allergic events in AR mice model by targeting HIPK2.

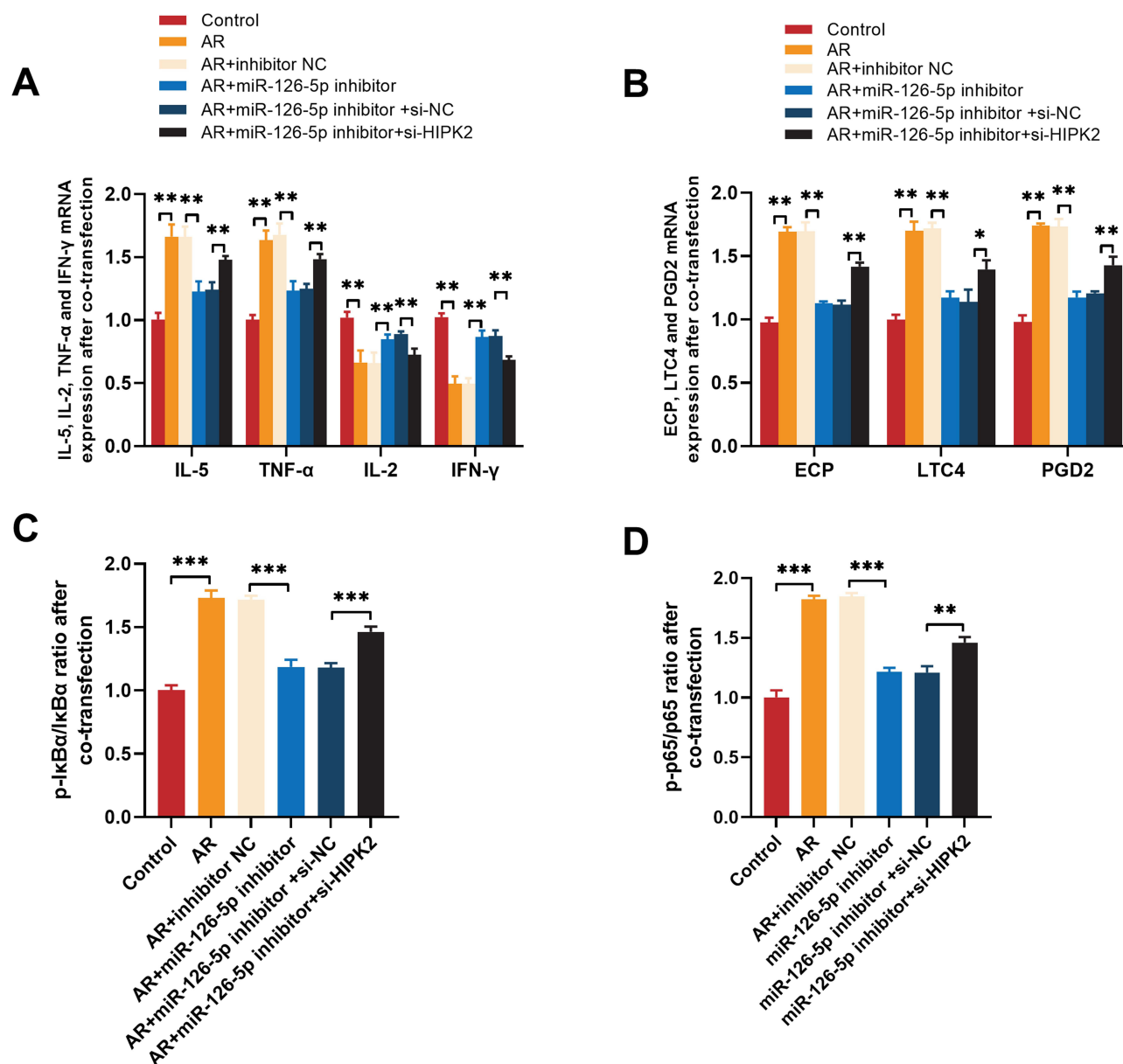
## Discussion

AR is a common disease in otorhinolaryngology. In general terms, there are two types of AR that occur in the clinic, one of which has obvious seasonal correlation and is named seasonal AR.<sup>18,19</sup> Another type of AR has no obvious seasonal correlation, and patients may suffer from it all year round, also known as perennial AR. AR is not an immediate life-threatening disease. However, the sufferer's quality of life is greatly affected, including sleep, cognitive function, school performance, and so on.<sup>20,21</sup> Traditional treatment approaches of AR mainly include avoiding contact with allergens, drug therapy, and immunotherapy.<sup>3</sup> What is worse, drugs are still bound to cause adverse reactions and complications of treatments. In recent years, the prevalence rate of AR has increased sharply, and there are no safe and effective treatments at present. It is therefore important to exploit new therapies that can effectively stop AR from progressing.

A growing number of reports have elucidated the function of miRNAs in several pathophysiological behaviors, such as infection and injury, which was involved in the onset and occurrence of inflammatory events.<sup>22,23</sup> As a promising miRNA, miR-126-5p has been indicated to display pro-inflammatory roles in multiple inflammation-associated diseases. For instance, in atherosclerosis, miR-126-5p inhibited apoptosis of endothelial cell through NF-κB-regulated PI3K/AKT/mTOR signaling pathway.<sup>11</sup> Xiao et al claimed that miR-126-5p participated in the inflammatory symptoms in the onset and formation of rheumatoid arthritis.<sup>24</sup> However, miR-126-5p's effect on AR is poorly understood. Using AR RPMI-2650 cell and mice models, this study aimed to evaluate the therapeutic significance of miR-126-5p on AR-associated inflammatory events and latent molecular mechanisms. In the present study, we examined and analyzed inflammatory cytokines in AR cell model and mice model. Reduced miR-126-5p expression suppressed inflammatory response and mitigated AR events. Importantly, knockdown miR-126-5p promoted HIPK2 expression levels to suppress NF-κB pathway activation.

For the first time, miR-126-5p was observed to be enhanced, and HIPK2 was poorly expressed in nasal mucosal tissue samples from AR sufferers in comparison to controls. A negative correlation was disclosed between miR-126-5p and HIPK2 in AR of children. Moreover, according to bioinformatics analysis, prospective binding sites were predicted between miR-126-5p and HIPK2 via ENCORI database, which was confirmed by means of dual-luciferase reporter gene detection. Our findings are consistent with the published articles. Han et al claimed that miR-126-5p was involved in inflammatory responses induced by 1-methyl-4-phenylpyridinium in Parkinson's disease model cells.<sup>25</sup> What is more, miR-126-5p was a forward-correlated mediator of lipopolysaccharide-induced inflammatory events via inhibiting cylindromatosis in chronic human

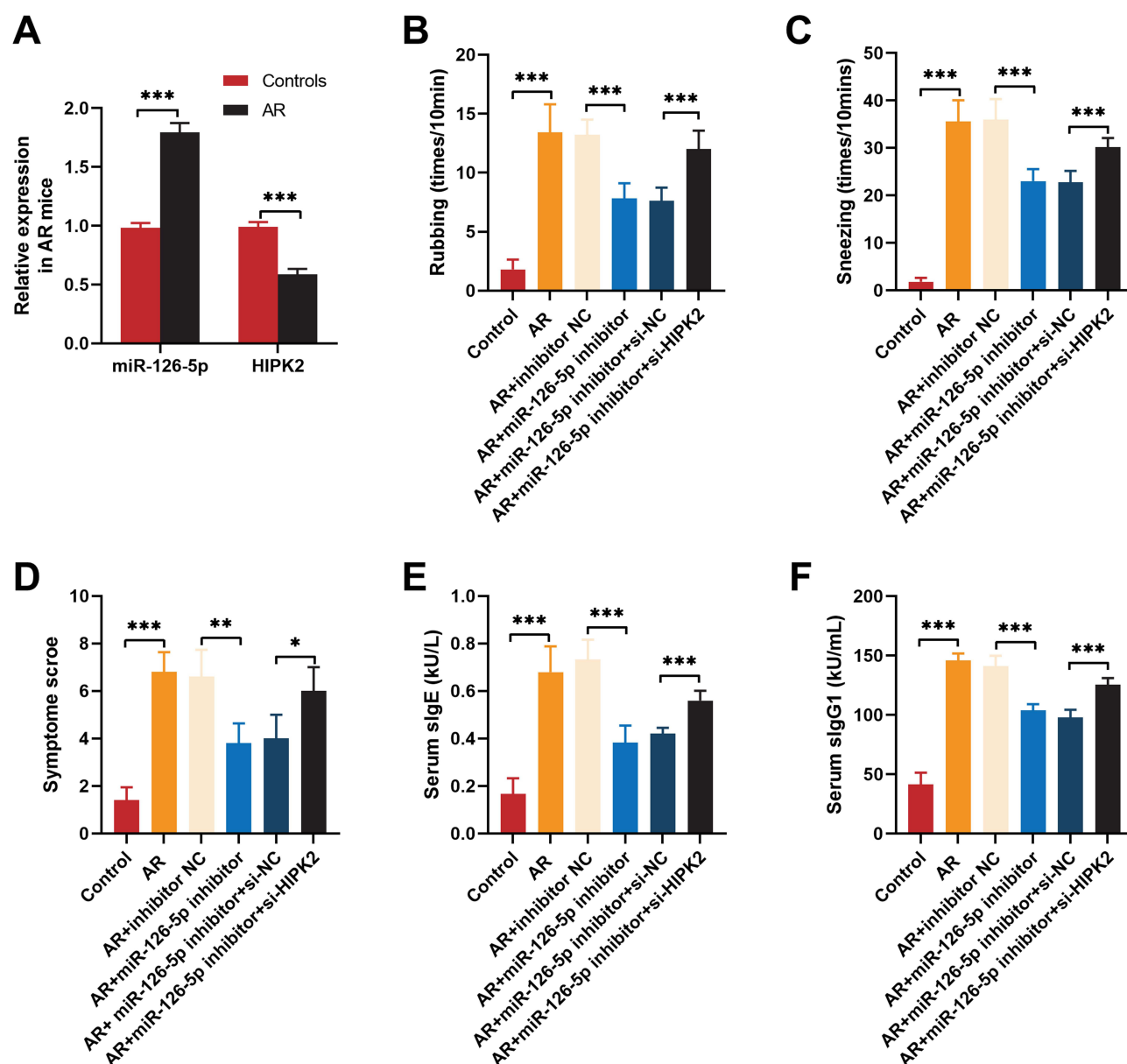




**Figure 5** Silencing HIPK2 declined the impacts of miR-126-5p inhibitor on OVA-stimulated inflammation. MiR-126-5p inhibitor impacted on the production of inflammatory factors, including IL-5, TNF- $\alpha$ , IL-2 and IFN- $\gamma$ , knockdown HIPK2 reserved the effects (A). Levels of ECP, LTC4 and PGD2 was distinctly decreased after transfection of miR-126-5p inhibitor, and knockdown HIPK2 could reserve the effect in AR RPMI-2650 cell model (B). Relative abundances of p-IkBa (C) and p-65 (D) was declined after transfection of miR-126-5p and increased when transfection with si-HIPK2 in AR RPMI-2650 cells. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

immunodeficiency virus type 1 patients.<sup>26</sup> Additionally, in kidney injury, HIPK2 C-terminal domain was implicated in renal inflammation through the modulation of nuclear NF- $\kappa$ B signaling expression.<sup>27</sup> Furthermore, a published article claimed a substantial increase of miR-126-5p in hypoxia-stimulated human umbilical vein endothelial cells, and associated with hypoxia stimulated endothelial injury by regulating HIPK2.<sup>28</sup> Using our obtained data together with the published excellent articles on the topic, we analyzed the miR-126-5p's effects on HIPK2 expression level in nasal mucosal tissues. Decreased miR-126-5p expression yielded obviously suppressive impacts on inflammatory symptoms by means of elevating HIPK2 levels in AR.

To exploit the potentiality of down-regulation miR-126-5p on molecular mechanism in AR, the levels of inflammation cytokines and target-related genes were detected by means of qRT-PCR and ELISA assays. Our findings showed that miR-126-5p inhibitor diminished the secretion of pro-inflammatory cytokines (IL-5 and TNF- $\alpha$ ), basophil- and



**Figure 6** Impacts of knockdown HIPK2 on OVA-induced allergic responses and IgE, IgG1 concentration in AR mice. Relative abundances of miR-126-5p and HIPK2 in normal mice and AR mice model (A). Within 10 minutes of AR mice, the number of rubbing (B) and the frequency of sneezing (C) were recorded and pooled. Symptom scores (D) were statistically calculated. ELISA detection was applied to measure the levels of IgE (E) and IgG1 (F) stimulated by OVA. \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ .

mastocyte-associated inflammatory factors (ECP, LTC<sub>4</sub>, and PGD<sub>2</sub>) and contributed to the production of anti-inflammatory factors (IL-2 and IFN- $\gamma$ ) in AR RPMI-2650 cell model. Meanwhile, reduced miR-126-5p expression diminished the relative abundances of p-IkBa and p-p65 without alteration of IkBa and p65 expression. Transfection of si-HIPK2 could abolish the effects of the above results in OVA-induced RPMI-2650 cells. In the AR mice model, miR-126-5p inhibitor down-regulated HIPK2 and attenuated IgE and IgG1 concentrations and inflammatory symptoms. The findings showed that knockdown miR-126-5p expression played an anti-inflammatory role through the adjustment of inflammatory cytokine secretion via HIPK2/NF- $\kappa$ B signaling pathway in AR.

Several shortcomings of our present study should be addressed. First of all, the sample size was slightly small, which might lead to the bias of our findings. On the other hand, although RPMI-2650 cell line is applicable in simulating the inflammatory response of AR to some extent, its origin may lead to certain differences compared to the primary nasal epithelial cells of AR patients, which may affect the extrapolation of our research results. Moreover, the lack of

categorization of subtypes of AR in children, including local AR, perennial AR, and seasonal AR, is a shortcoming that will be addressed at a later stage. Further analysis is required to evaluate whether the application of miR-126-5p can be effective in preventing AR from developing and improving clinical outcomes.

## Conclusion

Overall, it was documented that miR-126-5p was enhanced and HIPK2 was decreased in AR of children. Reduced miR-126-5p expression diminished the abundances of inflammatory cytokines in AR cell and mice models, and silencing HIPK1 could preserve the inhibitory effects. Notably, miR-126-5p knockdown suppressed NF- $\kappa$ B pathway activation through the regulation of HIPK2. Thus, miR-126-5p might be emphasized to be a latent target for therapeutic approaches for AR in the future.

## Ethics Approval

This experiment was conducted with approval of the Animal Ethics Committee of Hunan Children's Hospital. All institutional and national guidelines for the care and use of laboratory animals were followed. Appropriate measures were taken to minimize the number and suffering of animals.

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## Disclosure

The author(s) report no conflicts of interest in this work.

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