

LncRNA-AK007111 affects airway inflammation in asthma via the regulation of mast cell function

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ABSTRACT

Long noncoding RNAs (lncRNAs) are involved in gene transcription and pathophysiological processes of human diseases. Multiple lncRNAs have been shown to play important roles in the occurrence and development of asthma. This study aimed to explore the role of a novel lncRNA, lncRNA-AK007111, in asthma. Overexpression of lncRNA-AK007111 was induced in a mouse model of asthma via viral transfection, followed by the collection of alveolar lavage fluid and lung tissue for the detection of relevant inflammatory factors and pathological analysis of lung sections. Pulmonary resistance and respiratory dynamic compliance were measured using an animal pulmonary function analyzer. The number of mast cells sensitized by immunofluorescence was detected at the cellular level. The degree of degranulation of lncRNA-AK007111 after its knockdown was determined by detecting the level of β -hexosaminidase that was released and quantifying IL-6 and TNF- α using ELISA in a model of RBL-2H3 cells activated by immunoglobulin E plus antigen. Finally, we observed the migration ability of mast cells under a microscope. The results showed that in ovalbumin-sensitized mice, the upregulation of lncRNA-AK007111 promoted the infiltration of inflammatory cells in lung tissue, increased the number of total cells, eosinophils, and mast cells, upregulated IL-5 and IL-6 levels, and increased airway hyper-reactivity. Down-regulation of lncRNA-AK007111 decreased the degranulation ability of IgE/Ag-activated mast cells and inhibited the expression of IL-6 and TNF- α ; moreover, the migration ability of mast cells was significantly weakened. In conclusion, our study revealed that lncRNA-AK007111 plays an important role in asthma by modulating mast cell-related functions.

1. Introduction

Asthma, a chronic inflammatory disease of the airway, results from complex heterogeneous gene-environment interactions. It is characterized by variable respiratory symptoms and reversible airflow restriction, and its mechanisms are often associated with airway inflammation and remodeling [25]. Epidemiological data from the Global Initiative for Asthma have indicated an increasing prevalence of asthma every year [6]. In asthma, mast cells are sensitized to induce their activation, which

leads to bronchial smooth muscle contraction, hypersecretion of mucus, and local infiltration of inflammatory cells in the airway, eventually leading to the occurrence of asthma [7]. The combination of these released compounds induces a large inflammatory response, in which mast cells cascade to amplify allergen signals. Recent studies have shown that mast cells not only initiate an asthmatic response, but also play a key role in the complex mechanism of the disease [18].

Long non-coding RNAs (lncRNAs) are transcripts greater than 200 nucleotides in length that do not have the capacity to code for proteins

Abbreviations: lncRNAs, long non-coding RNAs; IL-6, interleukin-6; AAV6, adeno-associated virus 6; ELISA, enzyme-linked immunosorbent assay; GFP, green fluorescent protein; RT-qPCR, quantitative real time polymerase chain reaction; siRNA, small interfering RNA; PBS, phosphate-buffered saline; H&E, hematoxylin and eosin; PAS, periodic acid-Schiff; BALF, bronchoalveolar lavage fluid; siRNA, short interfering RNA; TNF- α , tumor necrosis factor alpha; IL-5, interleukin-5; Fyn, tyrosine kinase.

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[27]. In the pathogenesis of allergic asthma, lncRNAs participate in not only the regulation of local inflammation and remodeling of the airways, but also in mediating systemic inflammation in the peripheries of the body. The expression of lncRNA is different in children with asthma; plasmacytoma variant translocation is involved in the proliferation of smooth muscle cells and the release of interleukin-6 (IL-6) in patients with asthma [20]. Our previous study demonstrated that a novel lncRNA, lncRNA-AK149641, plays a key role in regulating airway inflammation associated with asthma [12]. Severe asthma is associated with the activation of circulating CD8⁺ T cells; decreased expression of the lncRNA maternally expressed gene 3 was detected in CD8⁺ T cells of patients with severe asthma [5].

Previously, our research group used high-throughput lncRNA chips to screen for differences in lncRNA expression in the lung tissues of asthmatic and normal mice, and obtained expression profiles of differential lncRNAs. We showed that the expression of lncRNA-AK007111 was significantly increased in asthmatic mice. Further validation found that IL-6 secretion was significantly reduced after downregulation of lncRNA-AK007111 in mast cells. IL-6 is closely related to the severity of asthma. In a simultaneous study, we demonstrated that lncRNA-AK007111 has high homology across rats and mice. Based on the key role of mast cells in asthma and the correlation between lncRNA-AK007111 and asthma, the present study aimed to determine the effects of regulating the expression of lncRNA-AK007111 in a mouse model of asthma and in rat mast cell lines on the related airway inflammation, inflammatory factors, and mast cell function.

2. Materials and methods

2.1. Animals and sensitization protocol

Specific pathogen-free, six- to eight-week-old, female BALB/c mice were purchased from the animal core facility of Nanjing Medical University (Nanjing, China) and housed under pathogen-free conditions. All mice were fed ovalbumin (OVA)-free food and water for approximately one week before the commencement of the experiments. The current study complied with the ethical requirements of the Animal Ethical and Welfare Committee of Nanjing Medical University and was approved under the following approval no.: IACUC-1910017, 2019-10-22.

Recombinant adeno-associated virus 6 (AAV6), with strong affinity for lung tissue and permanent expression, was used as a vector. It was constructed and packaged by Hanbio Biotechnology Co., Ltd. (Shanghai, China), with a titer over 1×10^{12} V.g/ml, containing a green fluorescent protein (GFP) expression sequence label and the sequence of interest.

Three days before the first sensitization (day -3), the mice were fully anesthetized (4% chloral hydrate, 0.1 mL/10 g) by intraperitoneal injection and AAV2/6-CMV-lncRNA-AK007111-ZsGreen (ID: AK007111.2) was injected into the trachea via tracheal intubation to upregulate the expression of lncRNA-AK007111 in the lung tissue (virus overexpression group). In the other group (virus control group), AAV2/6-ZsGreen was injected via endotracheal intubation. To establish the mouse model of asthma, the mice in each group were randomly divided into two subgroups: "OVA mice" were sensitized with OVA (V grade) (Sigma-Aldrich, St. Louis, MO, USA) (20 µg OVA, 2 mg Al(OH)₃, and phosphate-buffered saline (PBS) in a total volume of 0.2 mL) on days 0 and 14 via intraperitoneal injection. They were challenged via the airway for 30 min with an aerosol consisting of 1% OVA on three successive days (days 21 to 23), using an ultrasonic nebulizer (PARI GmbH, Starnberg, Germany). In contrast, the "non-OVA mice" were challenged with PBS, instead of OVA, following the same sensitization schedule.

2.2. Enzyme-linked immunosorbent assay

Alveolar lavage fluid and culture supernatant were collected at a specified time and stored at -80 °C. The concentrations of tumor necrosis factor alpha (TNF-α), interleukin-5 (IL-5), and IL-6 were

determined using an enzyme-linked immunosorbent assay (ELISA) kit (FcMACS, Nanjing, China) according to the manufacturer's instructions.

2.3. Fluorescent staining of frozen lung tissue sections

Lung tissues were obtained to evaluate pathological changes in the lung parenchyma. Samples were fixed in 4% neutral-buffered formalin, processed, paraffin-embedded, and sectioned at 4-µm thickness. Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) staining were performed for subsequent light microscopy, as described in previous studies [12]. For comparison, all sample slides were randomly selected with 10–12 field of view and were evaluated at the same magnification (10 × 20). Peribronchial inflammation was determined using a semi-quantitative score [14]. Scoring was performed by a researcher that was blinded to group assignments.

The severity of inflammation was graded according to the following five categories: 0, normal; 1, few inflammatory cells; 2, a ring of inflammatory cells at a depth of one cell layer; 3, a ring of inflammatory cells at a depth of 2–4 cell layers; and 4, a ring of inflammatory cells at a depth of more than 4 cell layers.

To quantify goblet cell hyperplasia, pathological changes were evaluated according to the modified five-point scoring system and were expressed as the percentage of goblet cells in the epithelium, as follows: grade 0, no goblet cells; grade 1, <25%; grade 2, 25%–50%; grade 3, 51%–75%; and grade 4, >75%. The mean score of goblet cell hyperplasia was calculated for each mouse.

2.4. Measurement of airway responsiveness

Mice were anesthetized with 1% pentobarbital sodium (100 mg/kg) via intraperitoneal injection 24 h after the last challenge, and airway hyper-reactivity was measured using the AniRes 2005 Lung Function System (Best Lab International, Inc., Beijing, China) according to the manufacturer's instructions and as per previous studies [11]. Airway responsiveness was examined using airway compliance and expiratory airway resistance values.

2.5. Collection of bronchoalveolar lavage fluid

The mice were sacrificed 24 h after the last OVA stimulation, and their lungs were washed thrice with the slow infusion and extraction of 0.6 mL cold PBS to collect the bronchoalveolar lavage fluid (BALF). The recovery rate was approximately 80%.

2.6. Immunofluorescence staining

Immunofluorescence staining was performed as previously described [4]. Cryosections of the lungs were dried at room temperature (20–25 °C) for 15 min and then rehydrated in PBS for 5 min. To reduce non-specific antibody binding, the sections were incubated at room temperature with 5% normal serum of the host species secondary antibody, diluted in PBS, for 15 min. The sections were incubated with primary antibodies [chicken anti-mouse PGP 9.5, 1:400; Acris Antibodies (Herford, Germany); rabbit anti-mouse tryptase, 1:500; Abcam (Cambridge, UK); goat anti-mouse mMCP-4, 1:400; Acris Antibodies (Herford, Germany)] or appropriate isotype control antibodies for 1 h at room temperature and then overnight at 4 °C. After rinsing twice with 0.1 M PBS, the sections were incubated with secondary fluorescein-conjugated antibodies (Jackson ImmunoResearch, West Grove, PA, USA; donkey anti-rabbit Cy3, 1:400; donkey anti-chicken Cy2, 1:400; donkey anti-goat, 1:400) for 2 h at room temperature. Finally, the sections were washed twice with 0.1 M PBS and once with double-distilled water, mounted with Fluoroshield fluorescence mounting medium (Sigma-Aldrich, St. Louis, MO, USA), and covered with cover slips.

2.7. Transfection and sensitization of RBL-2H3 cells

The rat mast cell line, RBL-2H3, was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured in Dulbecco's Modified Eagle's medium containing 4.5 g/L glucose, and supplemented with 10% fetal bovine serum (WISENT, Nanjing, China), 100 U/mL penicillin, and 100 µg/mL streptomycin in humidified air at 37 °C with 5% CO₂.

Mast cells from 6-well plates were transfected with a single short interfering RNA (siRNA) or a scrambled siRNA control using riboFECTTM CP transfection complex (Guangzhou Ruibo Biotechnology, Guangzhou, China), according to the manufacturer's instructions. The experimental group was then sensitized with 250 ng/mL anti-dinitrophenyl (DNP) immunoglobulin E (IgE) overnight while the control group was left untreated. The cells were washed with Tyrode's buffer twice every alternate day. Thereafter, the IgE plus antigen (IgE/Ag)-mediated cell group was added to 100 ng/mL DNP-HAS (dissolved in Tyrode's buffer), placed in a 37 °C environment, and stimulated in a 5% CO₂ cell culture for 4 h before subsequent collection of the cells.

2.8. Total RNA extraction and quantitative reverse transcription polymerase chain reaction

Total RNA was extracted from cultured cells using TRIzol reagent (Invitrogen, Waltham, MA, USA), according to the manufacturer's instructions. Total RNA (1000 ng) was then reverse-transcribed using a reverse transcription kit (Vazyme, Nanjing, China), in a final volume of 20 µL. Quantitative real-time polymerase chain reaction (RT-qPCR) was performed on a Quant StudioTM 3 PCR instrument (Applied Biosystems, Waltham, MA, USA) using AceQ^R qPCR SYBR Green Master Mix (Vazyme, Nanjing, China), according to the manufacturer's instructions. The expression level of lncRNA was normalized to that of glyceraldehyde-3-phosphate dehydrogenase using the $2^{-\Delta\Delta CT}$ method. The primers were synthesized by Shanghai Sangon Bioengineering Co., Ltd. (Shanghai, China) with the following sequences: mouse lncRNA-AK007111 forward: 5'-AGGTCCAGCCTTTGAGGACAT-3', reverse: 5'-GCCTCAGTGTTCACCCAC-3' and rat lncRNA-AK007111 forward: 5'-AGCCTTTGAGGACAGAGGGA-3', reverse: 5'-CCAACTCCAAGTGTC-CACCG-3'.

2.9. β -hexosaminidase release assay

The extent of degranulation/exocytosis was expressed as the percentage of total β -hexosaminidase that was released, which in turn was determined by an enzyme colorimetric assay. Absorbance was measured at 405 nm using a Multiskan FC microplate photometer (Reco Science, Shanghai, China). The percentage of β -hexosaminidase was calculated using the formula $(s-b)/(t-b) \times 100$, where the letters b, s, and t represent the absorbance of the culture medium, supernatant, and cell lysate, respectively.

2.10. Transwell assay

After routine digestion and centrifugation, cells in each group were collected and the cell density was adjusted. First, 200 µL of cells (cell density 1×10^5 /mL) were added to the upper chamber of each well, and 700 µL of complete medium was added to the lower chamber. After incubation for 24 h, the cells were removed and washed thrice with PBS. The cells were then fixed with 4% paraformaldehyde for 30 min, cleaned thrice with steaming water, and eventually stained with 0.1% crystal violet for 30 min. Microscopic images were obtained (Olympus, Tokyo, Japan) for observation and photography, and five fields ($\times 100$) were randomly selected for cell counting.

2.11. Statistical analyses

Statistical analyses were performed using Prism 8 software (Graph-Pad Software, Inc., La Jolla, CA, USA). Multiple comparisons were carried out using one-way analysis of variance, followed by the Bonferroni method for pairwise comparison between groups. Results yielding a p-value <0.05 were considered statistically significant.

3. Results

3.1. Mouse model of lncRNA-AK007111 overexpression established by AAV2/6-CMV-lncRNA-AK007111

We used high-throughput lncRNA chips to screen for differences in expression of lncRNA in the lung tissues of normal mice and mice with allergic asthma and obtained differential lncRNA expression profiles in the latter. Based on the lung tissue and mast cell lncRNA chip data, as well as bioinformatic analysis results of the mouse model of allergic asthma, we selected a total of 20 candidate lncRNAs for functional research and verified their expression in the model via RT-qPCR [26]. The differences in expression and the results of lncRNA-AK007111 are shown in Fig. S1a. Based on these results, we selected lncRNA-AK007111 for further study.

The AAV2/6 empty and overexpression sequences were introduced into the lung tissue of each group of mice through tracheal intubation, three days before the asthma model. Subsequently, a mouse model of asthma was established by OVA sensitization challenge. After 24 h of the last aerosol inhalation challenge, the lung tissue and alveolar lavage fluid were extracted (Fig. 1A). Frozen sections of the lung tissue were observed under a fluorescence microscope. After the overexpression of lncRNA-AK007111, the expression of GFP increased, indicating successful infection of lung tissue by AAV2/6 (Fig. S1B). Furthermore, RT-qPCR was used to detect the relative expression of lncRNA-AK007111 in the lung tissues. The results showed that the expression of lncRNA-AK007111 in the lung tissue of the OVA group was significantly higher than that in the normal control (NC) group, which was consistent with the chip results (Fig. S1A). In addition, the expression of lncRNA-AK007111 in the overexpression group was significantly higher than that in the control group (Fig. 1B), and the differences were statistically significant ($p < 0.05$).

3.2. Effect of lncRNA-AK007111 overexpression on airway inflammation and secretion of airway mucus in mice

In this experiment, H&E (Fig. 2A) and PAS (Fig. 2B) staining were used to observe the infiltration of inflammatory cells and mucus secretion around the bronchioles. Compared to that in the NC group, the infiltration of inflammatory cells around the bronchioles and in the perivascular space, as well as mucus secretion in the airway, was significantly increased in the OVA group, indicating that the asthma model was successfully established. In addition, inflammatory cell infiltration and mucus secretion in the AK007111 + OVA group was significantly increased compared to that in the OVA group.

3.3. Effect of lncRNA-AK007111 overexpression on airway inflammation and resistance

To investigate the effect of lncRNA-AK007111 overexpression on the airway, lung function was measured and BALF was collected from mice 24 h after the last inhalation of OVA. The total number of inflammatory cells (Fig. 3A) and eosinophils (Fig. 3B) in the BALF of lncRNA-AK007111-overexpressed asthmatic mice was significantly higher than that in normal asthmatic mice. Lung function results showed that the OVA group had significantly lower lung airway compliance (Fig. 3C) and significantly higher airway resistance (Fig. 3D) compared to that in the NC group. Overexpression of lncRNA-AK007111 had no effect on

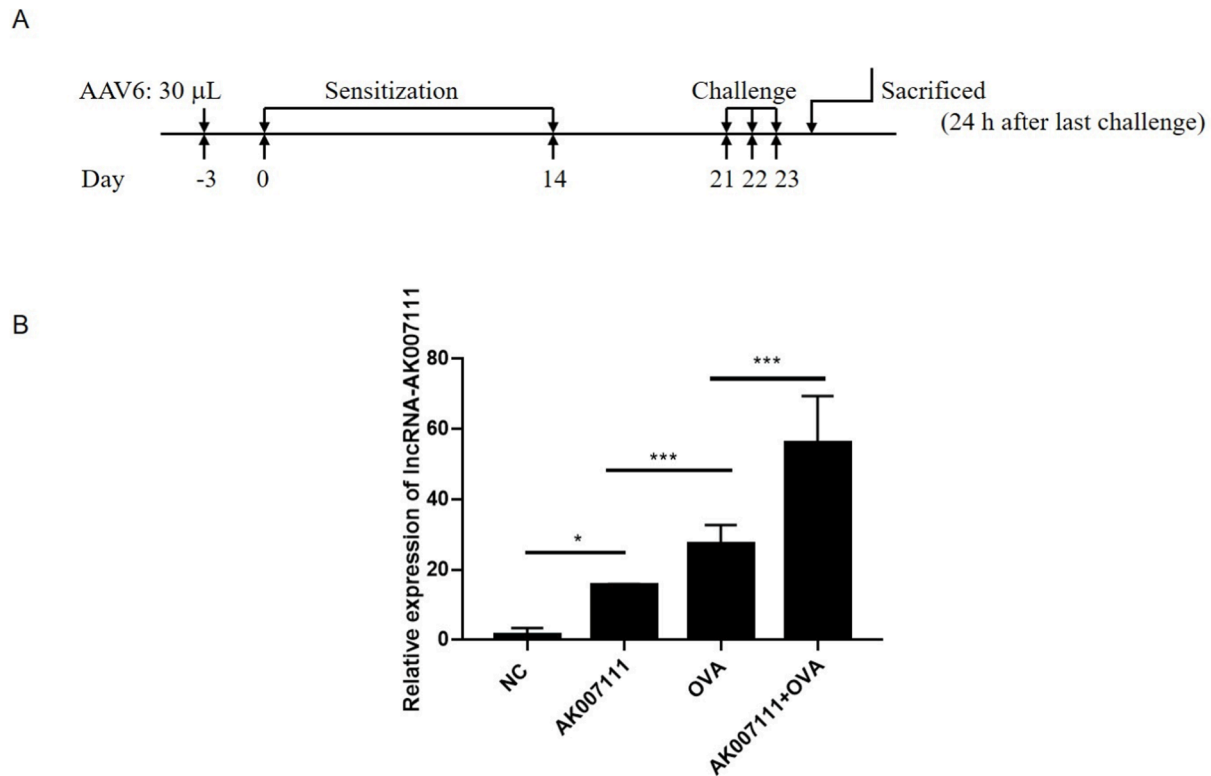


Fig. 1. Infection of the lung tissue by AAV2/6 and upregulation of lncRNA-AK007111 expression by AAV2/6-CMV-lncRNA-AK007111. (A) Brief protocol for the establishment of the ovalbumin (OVA)-induced mouse model of asthma. (B) Quantitative real time polymerase chain reaction (RT-qPCR) was used to measure the relative expression of lncRNA-AK007111 in the lung tissue. Expression in the Ak00711 + OVA group was significantly higher than that in the OVA group. Data are presented as mean $\pm$ standard deviation; $n = 4$. Multiple comparisons were carried out using one-way analysis of variance, followed by the Bonferroni method for pairwise comparison between groups. * $p < 0.05$, *** $p < 0.001$. NC, normal control; OVA, ovalbumin.

non-OVA mice in terms of airway hyper-reactivity. Compared to the asthma group, the upregulation of lncRNA-AK007111 showed significantly lower lung tissue dynamic compliance and significantly higher airway resistance.

3.4. Significant increase in the number of mast cells in lung tissues of lncRNA-AK007111-overexpressed mice

Under a fluorescence microscope, OVA exposure showed significantly increased mast cell infiltration, and the upregulation of lncRNA-AK007111 further increased the number of mast cells (Fig. 4A-B). To investigate the effects of lncRNA-AK007111 overexpression on the secretion of mast cell-associated inflammatory factors, IL-5 and IL-6, in the BALF of asthmatic mice, we detected the expression of these factors in the supernatant of BALF by ELISA. Overexpression of lncRNA-AK007111 significantly increased both IL-5 (Fig. 4C) and IL-6 (Fig. 4D) expression levels in asthmatic mice.

3.5. Effect of lncRNA-AK007111 downregulation on the levels of degranulation, secretion, and chemotaxis in IgE/Ag-mediated rat mast cells

Significantly decreased levels of lncRNA were observed in the lncRNA-AK007111 smart siRNA-transfected cells. We further explored the role of lncRNA-AK007111 in IgE/Ag-mediated mast cell activation. The expression of lncRNA-AK007111 was determined using RT-qPCR, which showed a significant increase in the number of activated cells compared to that in untreated cells. Additionally, we observed a statistically significant ($p < 0.05$) reduction in the number of lncRNA-AK007111 smart siRNA-transduced cells compared to that in the cells of the control group; this decrease was also observed after excitation of

IgE/Ag (Fig. 5A). Based on the experimental results, mast cells were divided into four groups. After siRNA transfection, the mast cells were sensitized and stimulated, and cell supernatant and lysate were collected. First, a Transwell experiment was conducted to explore the effect of lncRNA on mast cell chemotaxis. After lncRNA-AK007111 was downregulated by siRNA, RBL-2H3 migration was found to be significantly lower than that in the control group (Fig. 5B-C). Subsequently, the ratio of the level of β -hexosaminidase in the stimulated cells to the total enzyme in unstimulated cells was calculated. The degranulation ability of lncRNA-AK007111 mast cells was significantly lower than that of the NC cells (Fig. 5D). In addition, the levels of IL-6 and TNF- α (Fig. 5E-F) in the cell supernatant were found to be significantly decreased.

4. Discussion

lncRNAs, important molecules that are possibly involved in the regulation of the inflammatory response, have recently attracted a lot of attention and the number of studies on their role in asthma has been increasing. More than 1300 lncRNAs have been found to be differentially expressed in OVA-induced asthma models both *in vivo* and *in vitro* [23]. Some lncRNAs play an important role in the occurrence and treatment of asthma. For example, lncRNA BCYRN1 is involved in the proliferation and migration of airway smooth muscle cells [24].

OVA is one of the most commonly used allergens to establish animal models of allergic asthma, and the utility of OVA-induced asthma models is widely recognized. These models have been used to evaluate anti-asthmatic effects and the immunological mechanisms involved in the pathogenesis of asthma [8]. In the present study, OVA was used to sensitize and stimulate mice; after atomized OVA stimulation, the mice were seen scratching their heads and breathing more rapidly. The results

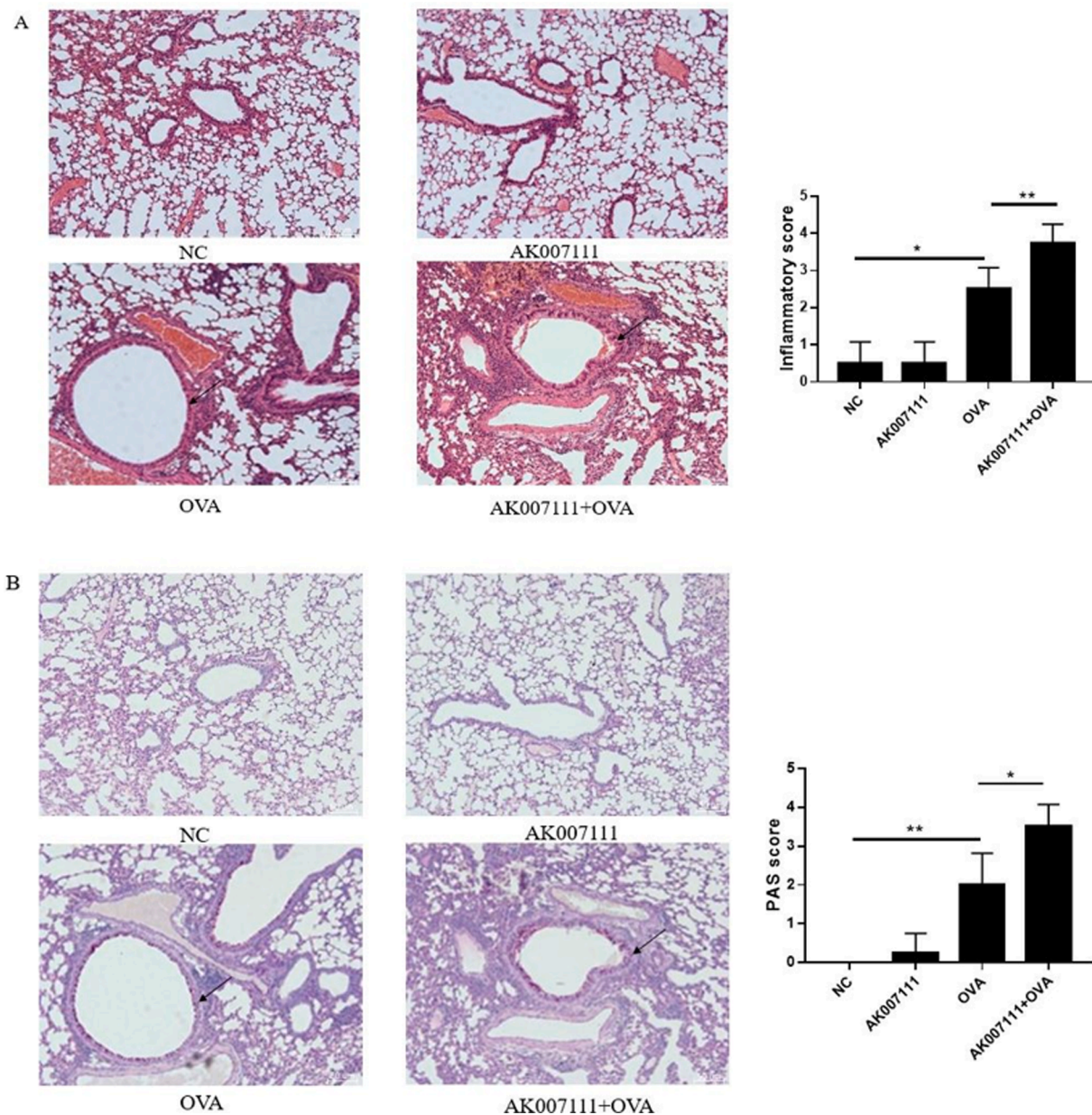


Fig. 2. Effect of lncRNA-AK007111 overexpression on airway inflammation and mucus secretion. (A) Hematoxylin and eosin (H&E) staining ($\times 200$) and (B) periodic acid-Schiff (PAS) staining ($\times 200$). In the AK007111 + OVA group, infiltration of inflammatory cells and mucus secretion around bronchioles increased, and the scores of inflammation and PAS increased significantly ($n = 4$). NC, normal control; OVA, ovalbumin.

of H&E and PAS staining of the lung tissue showed that the asthma model was successfully established. Additionally, we selected AAV6 as a vector to introduce the lncRNA-AK007111 overexpression sequence into the lung tissue of mice. In the third week thereafter, lung tissue was extracted and successful development of the lncRNA-AK007111 overexpression mouse model was verified via the detection of fluorescent protein expression in the lung tissue as well as by RT-qPCR.

Recent studies have shown that the number of eosinophils in the BALF of patients with asthma is significantly high and is associated with high airway response levels and disease severity [9]. Patients with asthma, having persistent eosinophilic airway inflammation, tend to have more severe symptoms and frequent exacerbations [10]. In this study, we showed that the number of total inflammatory cells and eosinophils in the BALF of asthmatic mice were significantly increased when the expression of lncRNA-AK007111 in the lung tissue was upregulated. H&E staining results of the pathological tissues showed that lncRNA-AK007111 overexpression aggravated the infiltration of

inflammatory cells around the bronchioles. Lung function is a powerful indicator of long-term asthma morbidity and mortality. Clinical studies have found that some genes, including *CHI3L1*, *TSLP*, and *GSDMB*, are related to the decline in lung function that is observed in patients with asthma [13,16–17]. In the present study, we used a lung function test to determine airway resistance and compliance. Upregulation of lncRNA-AK007111 expression significantly increased the airway resistance of asthmatic mice and significantly reduced lung tissue compliance. Therefore, lncRNA-AK007111 can indeed affect lung function in asthma, and its expression is closely related to airway hyper-responsiveness in asthma.

Mast cells are activated from the early to the late stage of asthma, and they secrete allergic mediators and various cytokines, including TNF- α , IL-1 β , IL-4, IL-5, IL-6, and IL-33 [3]. TNF- α and IL-1 β contribute to inflammatory response and airway constriction; IL-4 is an essential cytokine for IgE production by B cells and for eosinophil growth; IL-5 affects eosinophil maturation and supports IgE production; and IL-6 affects T

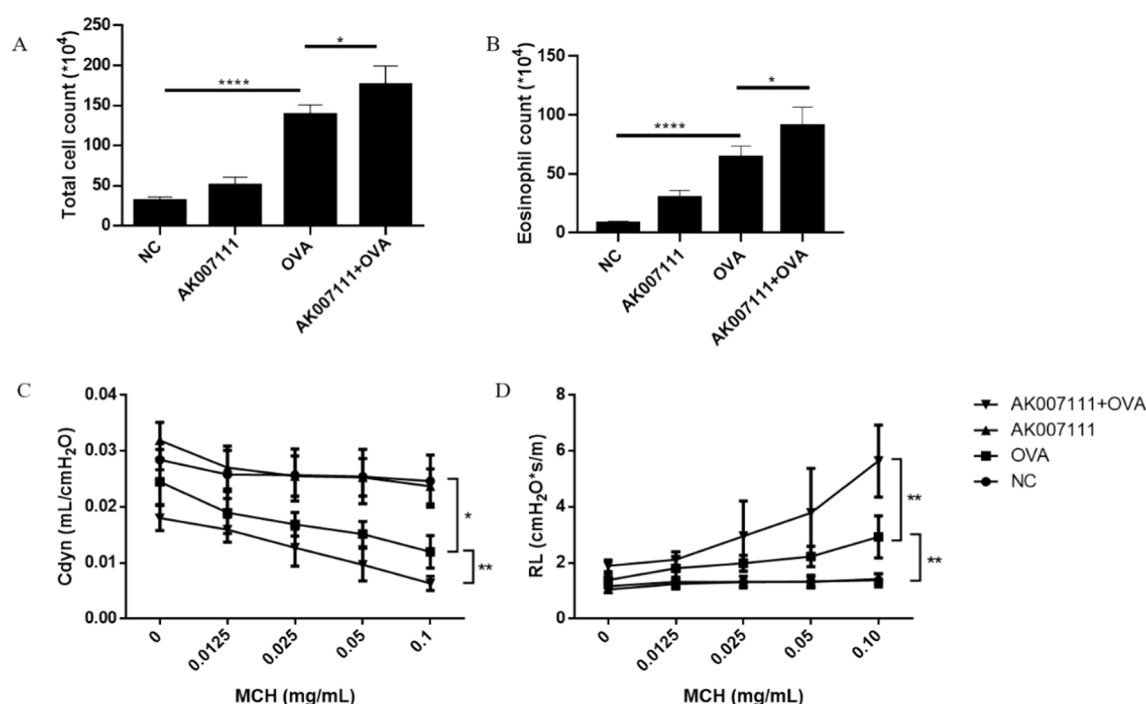


Fig. 3. Effect of lncRNA-AK007111 overexpression on airway inflammation in asthmatic mice. (A) Total inflammatory cell count and (B) eosinophil count in bronchoalveolar lavage fluid (BALF); the number of inflammatory cells and eosinophils in BALF increased after lncRNA-AK007111 overexpression, and the difference was statistically significant. (C) Lung dynamic compliance (Cdyn) and (D) airway resistance (RL); Cdyn decreased, and RL increased in the lncRNA-AK007111 + OVA group compared to those in the ovalbumin (OVA) group, and the differences were statistically significant. Data are presented as mean $\pm$ standard deviation; $n = 4$. Multiple comparisons were carried out using one-way analysis of variance, followed by the Bonferroni method for pairwise comparison between groups. * $p < 0.05$, **** $p < 0.0001$, $n = 4$. NC, normal control; OVA, ovalbumin;

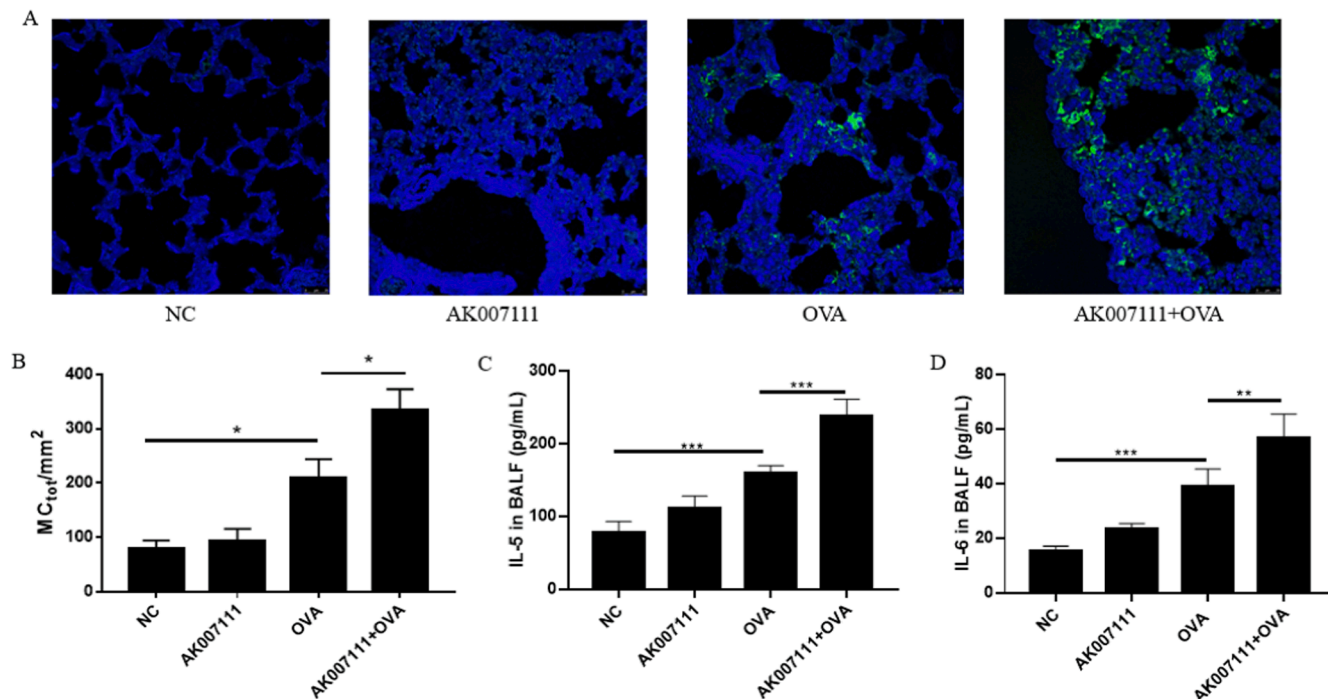


Fig. 4. The number of mast cells and expression of related inflammatory factors in ovalbumin mice. (A-B) Immunofluorescence ($\times 100$); (C) interleukin-5 (IL-5) expression; (D) IL-6 expression. After overexpression of lncRNA-AK007111 in the lung tissue of asthmatic mice, the number of mast cells in the lung tissue and the levels of IL-5 and IL-6 in the bronchoalveolar lavage fluid (BALF) increased significantly. Data are presented as mean $\pm$ standard deviation; $n = 4$. Multiple comparisons were carried out using one-way analysis of variance, followed by the Bonferroni method for pairwise comparison between groups. ** $p < 0.01$, *** $p < 0.001$. NC, normal control; OVA, ovalbumin.

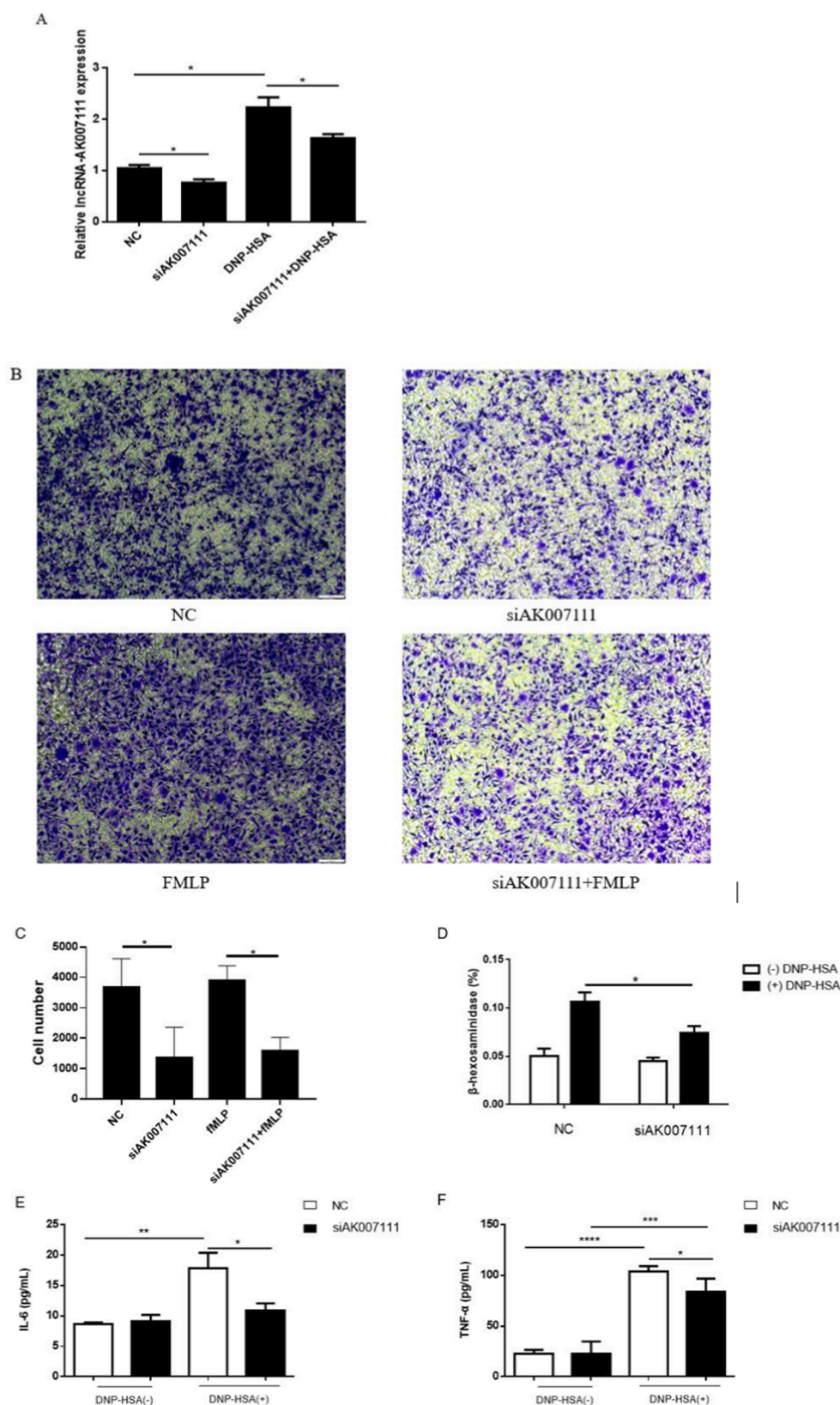


Fig. 5. The levels of degranulation, secretion, and chemotaxis of IgE/Ag-mediated rat mast cells. (A) The relative lncRNA-AK007111 expression detected by quantitative real time polymerase chain reaction (RT-qPCR) of RBL-2H3 cells treated with or without lncRNA-AK007111 smart siRNA or IgE/Ag. Supernatants and lysates were collected after the indicated time points. (B) Transwell experiments (the representative pictures from each group, $\times 100$); cells on the outer surface of the polycarbonate membrane were observed under a microscope and counted randomly in five visual fields. (C) After lncRNA-AK007111 knockdown, mast cell migration was still significantly reduced in the sensitized state. (D) β -hexosaminidase activity was determined by quantifying β -hexosaminidase in cell lysates as representatives of cell-associated granule content. The data are plotted as absorbance (OD value at 405 nm). Levels of interleukin-6 (IL-6) (E) and tumor necrosis factor alpha (TNF- α) (F) released were measured in the supernatants using enzyme-linked immunosorbent assay (ELISA). Data are presented as mean $\pm$ standard deviation; $n = 4$. Multiple comparisons were carried out using one-way analysis of variance, followed by the Bonferroni method for pairwise comparison between groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

and B cell proliferation, as well as IgE production. In previous studies, the number of pulmonary mast cells increased significantly in patients with asthma, especially in the smooth muscle layer of the airway, lung epithelium, and alveolar parenchyma [19]. Abnormal accumulation of mast cells in these lung chambers has been associated with increased

asthma symptoms [1]. In our mouse model of asthma, the number of mast cells increased after the upregulation of lncRNA-AK007111. Subsequently, we found IL-5 and IL-6, released after mast cell activation, to be significantly increased in the asthma model with high expression of lncRNA-AK007111.

Mast cell activation is known to be characterized by the rapid release of numerous pro-inflammatory mediators. Previous studies have shown considerable mast cell migration movement in the alveolar lavage fluid of patients with asthma [15]. By inhibiting lncRNA, various cascade reactions can be inhibited during mast cell activation, thus reducing the inflammatory response and slowing it down during asthma attacks. Based on our experimental results, we hypothesized that lncRNA-AK007111 plays a role in asthma by regulating mast cell function. In our subsequent cytological experiments, we observed that low expression of lncRNA-AK007111 could lead to the migration of mast cells. This is corroborated by the increased local infiltration of lung mast cells in our OVA-induced asthma model. We showed that the related functions of mast cells, including degranulation and secretion abilities (the expression of IL-6 and TNF- α), were weaker than those before transfection.

It is essential to conduct further studies on the pathways by which lncRNA-AK007111 affects mast cell function when it is under-expressed. For example, tyrosine kinase (Fyn) is activated during mast cell desensitization, leading to the eventual degranulation of mast cells [22]. Fyn can directly activate protein kinase C during mast cell degranulation and thus, directly activate mitogen-activated protein kinase, which can integrate intracellular and extracellular signals through four signal transduction pathways: ERK, JNK, P38, and ERK5. Classical downstream signaling pathways, including the PI3K/AKT, NF- κ B, and calcium ion pathways are activated [2,21]. In the future, we plan to conduct a literature review on gene chip technology to enhance our understanding of the specific mechanisms of action behind lncRNA-AK007111.

Our results collectively suggest that lncRNA-AK007111 expression can affect the pneumonia-like response by regulating mast cell function; hence, lncRNA-AK007111 may be a new potential target for the treatment of asthma.

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CRediT authorship contribution statement

Jiejing Xu: Writing – original draft. **Siqing Zhang:** Data curation. **Huilin Li:** Investigation. **Yaqing Bao:** Methodology. **Yi Du:** Formal analysis. **Yao Zhou:** Funding acquisition. **Deyu Zhao:** Conceptualization. **Feng Liu:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

I have shared the link to my data at the Attach File step.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.intimp.2023.110341>.

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