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Silencing SOX11 Alleviates Allergic Rhinitis by Inhibiting Epithelial-Derived Cytokines

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Background: Allergic rhinitis is a chronic inflammatory disease of the nasal mucosa affecting the quality of life of patients. SRY-box transcription factor 11 (SOX11) was reported to play important roles in inflammatory responses, but its role in AR is poorly understood.

Aims: To explore the role of SOX11 in the development of allergic rhinitis.

Study Design: Cell culture and animal study.

Methods: An in vivo murine allergic rhinitis model was established using ovalbumin treatment in female mice. Interleukin-13-stimulated human nasal mucosa epithelial cells were used for in vitro studies. Expression levels of SOX11, epithelial-derived cytokines, and mucin were determined in both modesls.

Results: SOX11 was highly expressed in allergic rhinitis mice. Allergy symptoms, serum ovalbumin-specific IgE, histamine, eosinophils, goblet cells, and type 2 cytokine secretion were increased in ovalbumin-treated mice. Furthermore, allergic rhinitis mice exhibited overproduction of epithelial-derived cytokines (thymic stromal lymphopoietin, interleukin-25, interleukin-33), C-C motif chemokine ligand 26 (CCL26), and mucin 5 AC (MUC5AC). Silencing SOX11 alleviated the behavioral symptoms and upregulation of epithelial-derived cytokines, CCL26, and MUC5AC. In human nasal mucosa epithelial cells, interleukin-13 enhanced SOX11 expression in a time-dependent manner, and signal transducer and activator of transcription 6 (STAT6) was involved in the interleukin-13-mediated expression of SOX11 by regulating transcription. Knockdown of SOX11 reduced epithelial-derived cytokine expression and MUC5AC levels in interleukin-13-treated human nasal mucosa epithelial cells.

Conclusion: SOX11 plays a critical role in allergic rhinitis development by regulating epithelial-derived cytokines and might be a new therapeutic target for allergic rhinitis.

INTRODUCTION

Allergic rhinitis (AR) is an inflammatory disorder of the nasal mucosa characterized by nasal obstruction, pruritus, and sneezing. AR can profoundly impact the quality of life of patients, especially in those with moderate to severe disease.[1] The clinical manifestations of AR are caused by a series of inhalant allergen-induced type 2 helper T (Th2) cell responses, including excessive allergen-specific immunoglobulin E (IgE) release, mast cell activation, eosinophil infiltration, and goblet cell hyperplasia.[2] Pharmacotherapy, allergen-specific immunotherapy (AIT), and other non-pharmacological measures are currently available as options for AR treatment. Among these, AIT is the only therapy that targets the causative factors of AR.[3] However, the widespread application of AIT is still limited by its risk of side effects, high cost, and low patient compliance arising from cumbersome, lengthy treatment courses.[4] Therefore, novel therapeutic methods and AR targets urgently need to be explored.

The histological feature of AR is typical type 2 inflammation is induced mainly by the type 2 cytokines interleukin (IL)-4, IL-15, and IL-13.[5] In addition to these Th2 cell-secreted molecules, damaged airway epithelial cells participate in the sensitization process via the secretion of cytokines, including thymic stromal lymphopoietin (TSLP), IL-25, and IL-33, to trigger Th2 immune responses.[6] AR histopathology is also characterized by mucosal eosinophil infiltration. C-C motif chemokine ligand 26 (CCL26) acts as a

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potent chemotactic agent of eosinophils and is overproduced in the nasal mucosa and lavage fluid of AR patients.[7]

SRY-box transcription factor 11 (SOX11) is a member of the SOX C protein family and is implicated in multiple biological processes, such as neurogenesis, cell differentiation, and embryonic development.[8] A recent study indicated that targeted inhibition of SOX11 alleviated inflammatory responses in a spinal cord injury mouse model.[9] In lung fibroblasts, expression of IL-13-induced inflammation-related factors (such as CCL26) was downregulated by silencing SOX11.[10] These studies demonstrate the ability of SOX11 to regulate inflammatory responses and epithelial-derived cytokines. However, whether SOX11 functions in the pathogenesis of AR remains unclear.

Given these findings, we hypothesized that SOX11 knockdown might alleviate AR by inhibiting epithelial-derived cytokines. To test this hypothesis, we used lentiviral systems to silence SOX11 in a murine AR model and IL-13-stimulated human nasal epithelial cells (HNEpCs). Knockdown of SOX11 alleviated AR symptoms and suppressed the expression and production of epithelial-derived cytokines and mucins. Moreover, signal transducer and activator of transcription 6 (STAT6) participates in IL-13-induced SOX11 expression by regulating SOX11 transcription.

MATERIALS AND METHODS

Lentiviral Systems

We generated the lentiviral systems Lv-Mus-SOX11-shRNA and Lv-Homo-SOX11-shRNA, which produced shRNA targeting SOX11, for the in vitro and in vivo studies, respectively. The lentivirus producing the shRNA targeting a non-specific sequence (Lv-NC) was used as a negative control.

Lentiviral vectors were obtained from Hunan Fenghui Biotechnology Co., Ltd (Hunan, China). shRNA sequences were synthesized and inserted into the vectors by Generalbiol (Chuzhou, China). Information on the lentiviral systems is presented in Table 1. 293T cells were used to produce lentiviral particles. Cells were grown in 10 cm dishes to reach 70% confluence and transfected with lentiviral vectors and packaging mixture. The medium was changed at 6 h and the lentiviral particles were collected after 48 h. The supernatant collected after centrifugation was filtered with 0.45 μm filters and then dispensed. Viral titers were determined by the fluorescence counting method.

Murine AR Model

Female BALB/c mice of 6-8 weeks were used as experimental animals in this study. They were housed at an ambient temperature of 22 °C ± 1 °C, humidity of 45%-55%, and a 12 h diurnal light cycle. The mice were then randomly allocated to the control (saline treatment), OVA (OVA-induced AR), OVA + shNC (OVA-induced AR + control shRNA lentivirus infection), and OVA + shSOX11 (OVA-induced AR + SOX11 shRNA lentivirus infection) groups.

To establish an AR mouse model, mice were sensitized 3 times (days 1, 8, and 15) by intraperitoneal injection of 0.2 ml suspension consisting of 0.5 mg/ml ovalbumin (OVA; Aladdin, Shanghai, China) and 20 mg/ml $Al(OH)_3$. From days 22 to 29, mice were challenged by intranasal instillation of 0.02 ml of OVA solution (40 mg/ml) once daily (Figure 1a). Mice receiving the same volumes of saline solution served as controls.

SOX11 silencing in mice was mediated by the Mus-SOX11-shRNA (shSOX11) lentivirus. Briefly, mice were instilled intranasally with SOX11-shRNA lentivirus (0.02 ml, $1x10^8$ TU/ml) once at day 19 and daily from days 22 to 29 (3 h before OVA treatment). Mice treated with Lv-NC lentivirus (shNC) served as negative controls. Nasal mucosal tissue, lavage fluid, and serum were collected one day after the last session of OVA challenge.

Air-Liquid Interface Cultures and Stimulation Experiments In Vitro

The human nasal mucosa epithelial cell line HNEpC was procured from iCell Bioscience (Shanghai, China). Cells were grown in PneumaCult-ALI-S (NovoBiotechnology Ltd., Beijing, China) with 10% fetal bovine serum and placed in an incubator at 37 °C and 5% CO_2.

When cells reached 75% to 80% confluence, 200 μl of cell suspension ($1.1x10^5$ cells) was added to the transwell inserts. Cells were grown submerged until a confluent monolayer was formed. The media in the upper and lower layers were replaced with medium containing 500 nM retinoic acid. After 48 h, all media were decanted and the lower layer was supplemented with 700 μl of PneumaCult-ALI medium. Cells were maintained in the air-liquid interface for 21 days to allow differentiation.

HNEpCs were incubated in 50 ng/ml IL-13 (Sino Biological Inc., Beijing, China) for 1, 3, 6, and 24 h. For SOX11 silencing experiments, HNEpCs were cultured with Homo-SOX11-shRNA (shSOX11) lentivirus for 72 h, followed by stimulation with IL-13 for 24 h. For STAT6 blocking experiments, HNEpCs were treated with the STAT6-specific inhibitor AS1517499 (100 nM; Shanghai yuanye Bio-Technology Co., Ltd, Shanghai, China) for 30 min prior to adding IL-13. At various time points after IL-13 treatment, cells or supernatants were collected for analysis.

TABLE 1. Information on the Lentiviral Systems Used

	Target sequence (5′-3′)	Vector	Endonuclease site
Lv-Mus-SOX11-shRNA	CGATGAAGACGACGACGAAGA	pLKO.1-EGFP-puro	AgeI/EcoRI
Lv-Homo-SOX11-shRNA	GCTTCAAGAACATCACCAAGC	pLKO.1-EGFP-puro	AgeI/EcoRI
Lv-NC	TTCTCCGAACGTGTCACGT	pLKO.1-EGFP-puro	AgeI/EcoRI

Nasal Symptoms Evaluation

After the final session of OVA treatment, the frequency of sneezing and nasal rubbing in the animals were immediately recorded for 10 min, as previously described.[11]

Immunofluorescence

Fixed HNEpCs were permeabilized using 0.1% polyethylene glycol tert-octylphenyl ether (Beyotime, Shanghai, China), followed by blocking in goat serum (Solarbio, Beijing, China). A primary antibody against SOX11 (Affinity, Changzhou, China) was added to the section at a dilution ratio of 1:100 with further incubation at 4 °C overnight. Cy3-labeled secondary antibody (Beyotime, Shanghai, China) was used after three washes. The nucleus was stained using 4′,6-diamidino-2-phenylindole (Aladdin, Shanghai, China). Photographs were taken under fluorescence microscopy (Olympus, Tokyo, Japan) at 400x magnification.

Immunohistochemistry

Fixed nasal mucosal tissue was embedded and sectioned to 5 μm thickness, then deparaffinized and rehydrated. Following soaking in PBS for 5 min, the sections were subjected to antigen retrieval by microwaving. Incubation with 3% H_2O_2 for 15 min was used to block the endogenous peroxidase activity. Following blocking by 1% bovine serum albumin (Sangon, Shanghai, China), the sections were incubated overnight at 4 °C with primary antibody against SOX11 (BIOSS, Beijing, China) and MUC5AC (ABclonal, Shanghai, China). The sections were incubated with HRP-conjugated goat anti-rabbit IgG (ThermoFisher Scientific, Pittsburgh, USA) for 1 h. Expression of SOX11 and MUC5AC were visualized by using 3,3-diaminobenzidine. Sections were re-stained with hematoxylin (Solarbio, Beijing, China), followed by observation under a microscope at 400x magnification.

Histopathological Examination

The tissue samples from the murine nasal mucosa were fixed and dehydrated. Fixed tissues were embedded in paraffin and sectioned at 5 μm for histopathological analysis. Section staining was performed using hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). Independent histopathologists, who were blinded to group allocation, counted the eosinophils and goblet cells in four randomly selected fields of view of each section under a light microscope at 400x magnification.

Quantitative real-time PCR (qRT-PCR) Analysis

Total RNA was extracted by TRIpure (BioTeke, Beijing, China). M-MLV reverse transcriptase (Beyotime, Shanghai, China) was employed to reverse transcribe mRNA to cDNA. qRT-PCR analysis was run on Exicycler 96 RT-PCR instrument (Bioneer, Daejeon, Korea) with SYBR Green (Solarbio, Beijing, China), following standard protocols. The relative expression of each target gene was determined using the $2^{-\Delta\Delta Ct}$ method. β-actin served as an internal control. The mRNA primers for qRT-PCR analysis are listed in Table 2.

Western Blotting

RIPA lysis buffer (Beyotime, Shanghai, China) with 1% PMSF (Beyotime) was utilized for total protein extraction. The proteins were denatured at a high temperature and separated by SDS-PAGE. The primary antibodies used are as follows: SOX11 (1:1000; Affinity, Changzhou, China), p-STAT6 (1:1000; Affinity), STAT6 (1:500; Affinity), and β-actin (1:2000; Proteintech, Wuhan, China). Antibody binding was visualized using secondary antibodies (Proteintech). The blots were analyzed using Gel-Pro-Analyzer software with the ECL reagent (7sea biotech, Shanghai, China).

Dual luciferase Assay

SOX11 luciferase reporter plasmids (Figure 5a) and STAT6 overexpression vector (STAT6-OE) were constructed for luciferase assay and transfected into HEK293T cells using Lipofectamine 3000 (Invitrogen, Carlsbad, USA). After 24 h, cells were treated with IL-13 (50 ng/ml) for 1 h. Dual Luciferase Reporter Gene Assay Kit (KeyGEN, Jiangsu, China) was used to measure luciferase activity, following the manufacturer's instructions.

Enzyme-linked Immunosorbent Assay (ELISA)

Serum histamine, OVA-specific IgE, and mucin 5 AC (MUC5AC) in the culture supernatants were detected using ELISA kits from Wuhan Fine Biotech Co., Ltd. (Wuhan, China) based on the protocols prepared by the manufacturers. Mouse nasal lavage fluid IL-4, IL-5, IL-13, TSLP, IL-25, and IL-33 levels were measured by ELISA kits procured from MultiSciences Biotech (Hangzhou, China). CCL26 was detected using the ELISA kit from CUSABIO (Wuhan, China).

Statistical Analysis

Statistical analyses were conducted using GraphPad Prism 8.0 (GraphPad Software, La Jolla, USA). Normality was assessed

TABLE 2. mRNA Primers Used for qRT-PCR Analysis

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
homo SOX11	CATCACCAAGCAGCACCCG	CTCGCTGAACGAATCCAAATCC
mus SOX11	GCGTGTCCACCTCCTCATCC	AAGTTCGCCTCCAGCCAGTC
mus MUC5AC	CGTGGTCTGGAAGGATGCTAT	GAACTGTTGCCCGTTGTAGGT
homo CCL26	CTCCTTGGCCTCTGCTGT	GGCTTGTGGCTGTATTGG
homo IL-25	GGGAGCGACCCAGATTAG	AGGTGTCCTGCCCTTTGC
homo TSLP	TCCAGAGCCTAACCTTCA	TTGCCTGAGTAGCATTTATC
homo IL-33	TACCACTGGAGAAGGAATGAGAG	ATCTCTTTAGAATGTTGACCGTT

using Shapiro-Wilk's test and equality of variances was tested using F test or Brown-Forsythe test. Based on the results of the tests for normality and homoscedasticity, the appropriate statistical tests were conducted to compare collected data from the experimental groups. To compare the means between two groups, unpaired *t*-test with or without Welch's correction was employed depending on the homoscedasticity. To compare the means in more than two groups, one-way analysis of variance (ANOVA), Welch ANOVA tests, and Kruskal-Wallis tests were applied for data with normal distribution with equal variances, normal distribution without equal variances, and non-normal distribution, respectively. Data were presented as mean ± satdard deviation. *P*-values < 0.05 were considered significant.

RESULTS

Allergic Symptoms and Increased SOX11 Expression Were Observed in OVA-induced AR Mice

The experimental schematic diagram of the in vivo AR model is depicted in Figure 1a. As shown in Figure 1b,c, sneezing and nasal rubbing were more frequent in OVA-treated mice. OVA-specific IgE and histamine measured using ELISA showed markedly increased levels in OVA-treated mice (Figure 1d,e). Similarly, the IL-4, IL-5, and IL-13 levels in nasal lavage fluid of OVA-treated mice were increased (Figure 1f-h). The SOX11 expression was upregulated in the nasal mucosa of OVA-treated mice (Figure 1i,j), with similar results detected using immunohistochemistry (Figure 1k). Western blotting showed increased phosphorylate

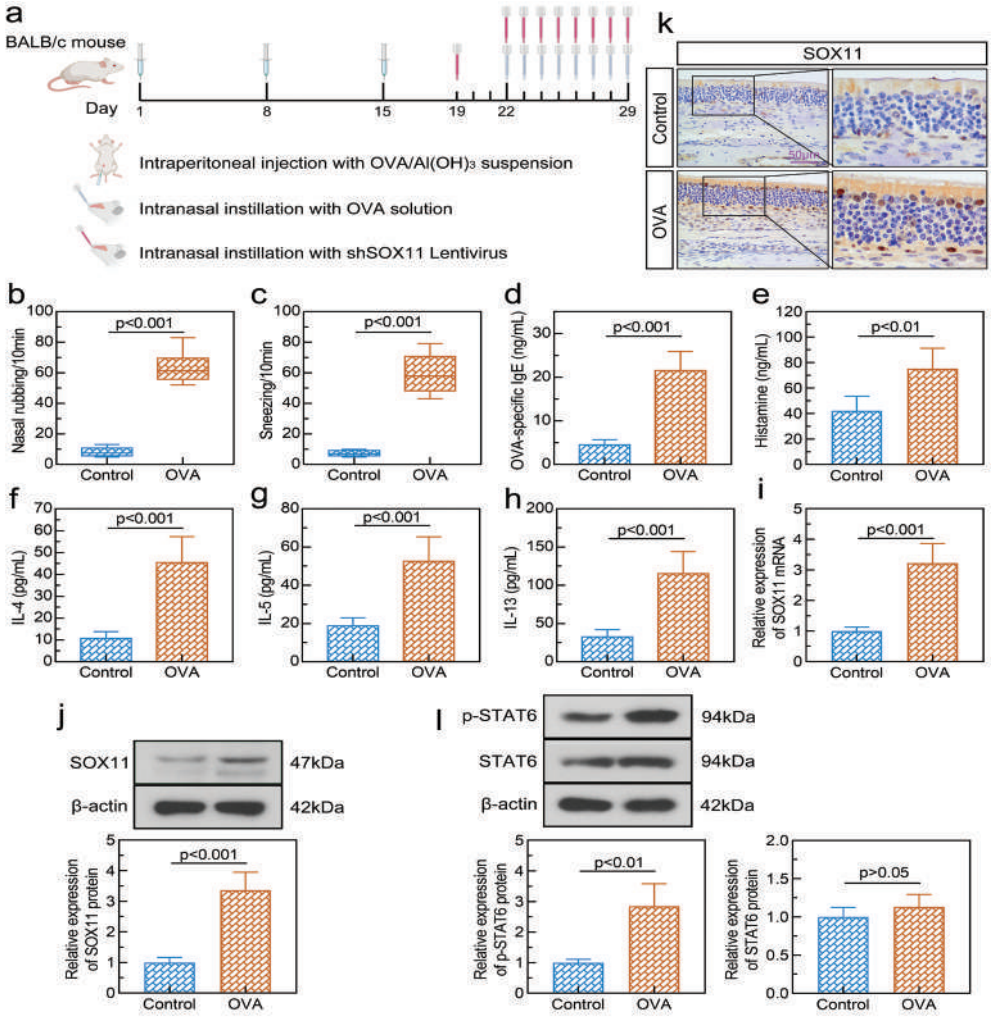

FIG. 1. Allergic symptoms and increased expression of SOX11 were observed in OVA-induced AR mice. (a) Schematic diagram of the murine AR model and shSOX11 lentivirus treatment. (b-c) Number of sneezes and nasal rubbing in mice recorded within 10 min after the last OVA treatment session. (d-e) Levels of OVA-specific IgE and histamine in serum were detected by ELISA. (f-h) Levels of IL-4, IL-5, and IL-13 in nasal lavage fluid detected by ELISA. (i) *SOX11* gene expression in nasal mucosa tissues measured by qRT-PCR. (j) SOX11 protein levels in nasal tissues determined by Western blotting. (k) Immunohistochemical staining for SOX11 in nasal mucosa tissues. (l) p-STAT6 and STAT6 protein expression in the nasal tissues determined by Western blotting.

STAT6 (p-STAT6) protein levels in OVA-treated mice (Figure 1l), whereas the protein expression of STAT6 did not differ between groups. These data indicate that OVA-treated mice developed AR symptoms and SOX11 expression was upregulated.

Silencing SOX11 Attenuated Allergic Symptoms in AR Mice

We further examined the effect of silencing SOX11 in AR mice. The frequent sneezing and nasal rubbing seen in AR mice were relieved upon silencing SOX11 (Figure 2a,b). Immunohistochemistry demonstrated that shSOX11 attenuated the expression of SOX11 in AR mice (Figure 2c). We then performed H&E and PAS staining of nasal mucosal tissues. As shown in Figure 2d and e, AR histopathological features observed in the nasal mucosa of OVA-treated mice, such as cilia shedding and increased eosinophils and goblet cells, were ameliorated in SOX11-silenced mice. These results suggested that silencing SOX11 attenuated the allergic symptoms of AR mice.

Silencing SOX11 Decreased Epithelial-derived Cytokine Production and Mucin Expression in AR Mice

As indicated in Figure 3a–d, the levels of epithelial-derived cytokines (TSLP, IL-25, and IL-33) and CCL26 in nasal lavage fluids were increased in AR mice and were decreased after SOX11 silencing. MUC5AC expression quantified using qRT-PCR and immunohistochemistry indicates mucin upregulation in AR mice (Figure 3e,f), which was decreased by silencing SOX11. These data imply that downregulating SOX11 inhibits epithelial-derived cytokine secretion and mucin expression.

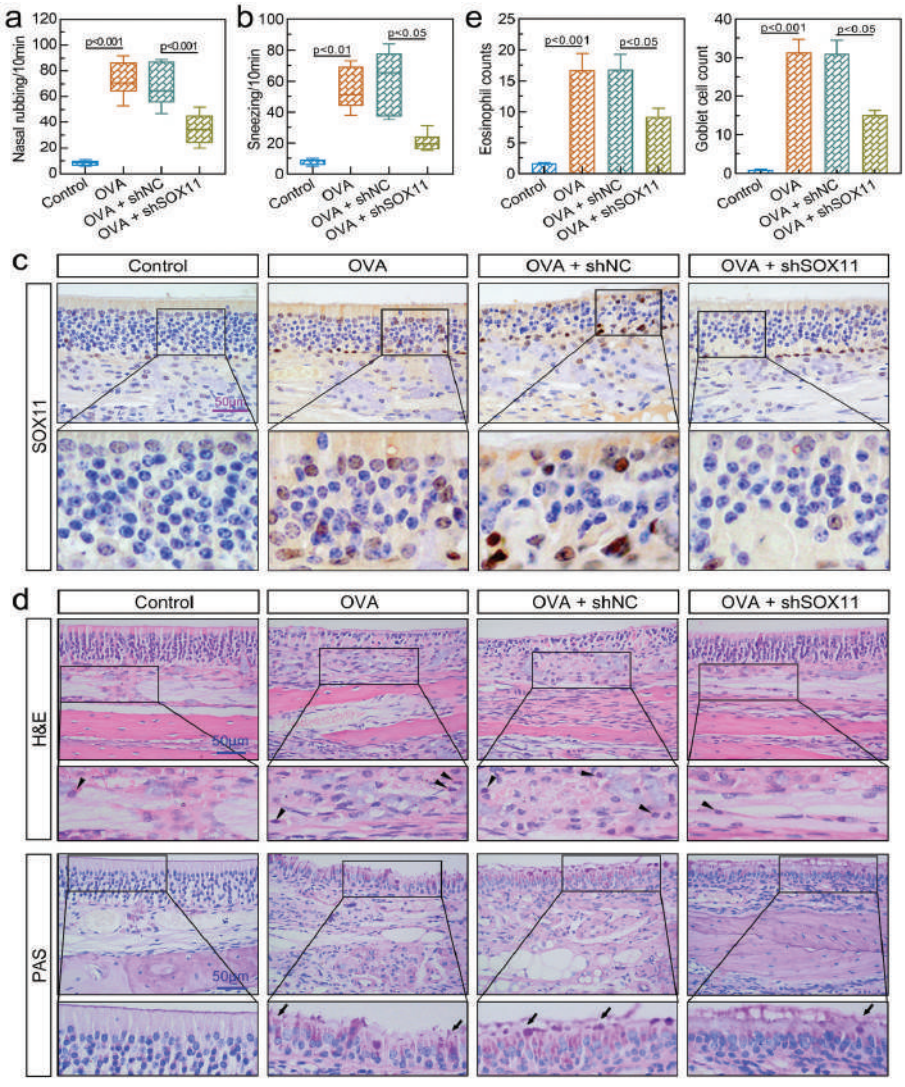

FIG. 2. Silencing SOX11 attenuated allergic symptoms of AR mice. (a-b) Number of sneezes and nasal rubbing in mice recorded within 10 min after the last OVA treatment session. (c) Immunohistochemical staining for SOX11 in nasal mucosa tissues. (d) Eosinophils and goblet cells in nasal mucosa tissues. Triangles indicate eosinophils. Arrows indicate goblet cells. (e) The number of eosinophils and goblet cells was counted.

SOX11 and STAT6 Expression were Upregulated in IL-13-treated HNEpCs

To examine the SOX11 expression in nasal mucosa epithelial cells, cultured HNEpCs were treated with IL-13 (Figure 4a). The transcript and protein levels of SOX11 were increased with increasing IL-13 treatment time (Figure 4b,c). These results were further confirmed by immunofluorescence to detect SOX11 (Figure 4d). p-STAT6 levels were upregulated by IL-13 stimulation (Figure 4e), while STAT6 expression was not affected by IL-13 treatment. These findings demonstrate that SOX11 and p-STAT6 expression were upregulated by IL-13 treatment in HNEpCs.

STAT6 was Involved in the IL-13-mediated Expression of SOX11

To explore whether STAT6 participates in the regulation of SOX11 expression, an array of plasmids encoding SOX11 promoter regions from -1757 to +20 bp sites were used. We then co-transfected them with STAT6 overexpression vector into HEK293T cells for dual luciferase assays. These regions contain six STAT6-binding sites, as presented in Figure 5a. Luciferase activity was enhanced in the promoter region of -1757 to +20 bp in comparison to the empty vector group (Figure 5b), suggesting that STAT6 was able to promote the transcription of SOX11. Significant reductions in luciferase activity were observed when the -1757 to -1334 bp and -1334 to -658 bp fragments were lost, indicating that the region of -1757 to -658 bp in the SOX11 contains the key elements for STAT6 binding. To verify the regulatory effect of STAT6 on SOX11, we used AS1517499 to inhibit STAT6 in HNEpCs. We also determined whether STAT6 is involved in SOX11 expression mediated by IL-13. IL-13 treatment enhanced the promotion of SOX11 transcription by STAT6" to explain the results in Figure 5b. In qRT-PCR, SOX11 expression was elevated by IL-13 treatment and decreased following treatment with 100 nM AS1517499 (Figure 5c). The same changes were detected in the protein levels of SOX11 (Figure 5d). Altogether, these findings demonstrate that STAT6 promoted SOX11 transcription and STAT6 inhibition reduced IL-13-enhanced SOX11 expression.

Silencing SOX11 Reduced Epithelial-derived Cytokine Expression and Mucin Levels in IL-13-treated HNEpCs

We subsequently determined if silencing SOX11 alleviates IL-13-induced changes in HNEpCs. As demonstrated in Figure 6a and b, SOX11 expression in IL-13-treated HNEpCs was reduced at both mRNA and protein levels upon shSOX11 lentiviral infection. Consistently, TSLP, IL-25, IL-33, and CCL26 mRNA expression were upregulated in HNEpCs after treatment with IL-13 (Figure 6c-f). This enhancement was mitigated by silencing SOX11. In cell supernatants, the IL-13-induced overproduction of MUC5AC was also ameliorated with SOX11 silencing (Figure 6g). These findings indicate that silencing SOX11 inhibits IL-13-induced upregulation of TSLP, IL-25, IL-33, and CCL26, as well as overproduction of MUC5AC in HNEpCs.

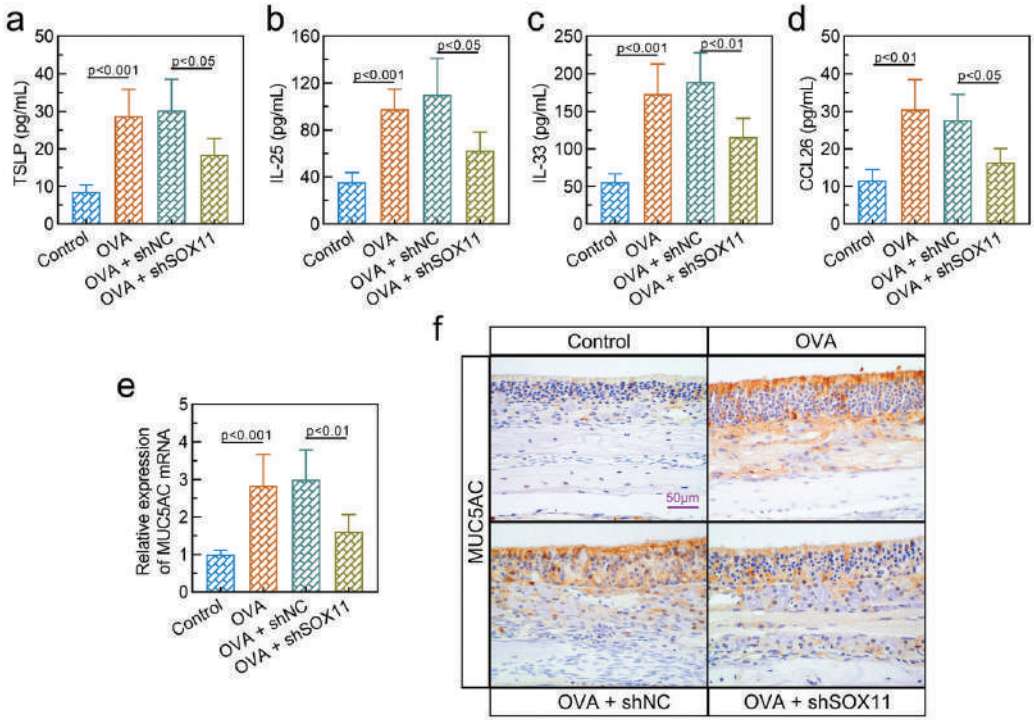

FIG. 3. Silencing SOX11 decreased epithelial-derived cytokine production and mucin expression in AR mice. (a-d) Levels of TSLP, IL-25, IL-33, and CCL26 in nasal lavage fluid detected by ELISA. (e) MUC5AC expression in nasal mucosa tissues measured by qRT-PCR. (f) Immunohistochemical staining for MUC5AC in nasal mucosa tissues.

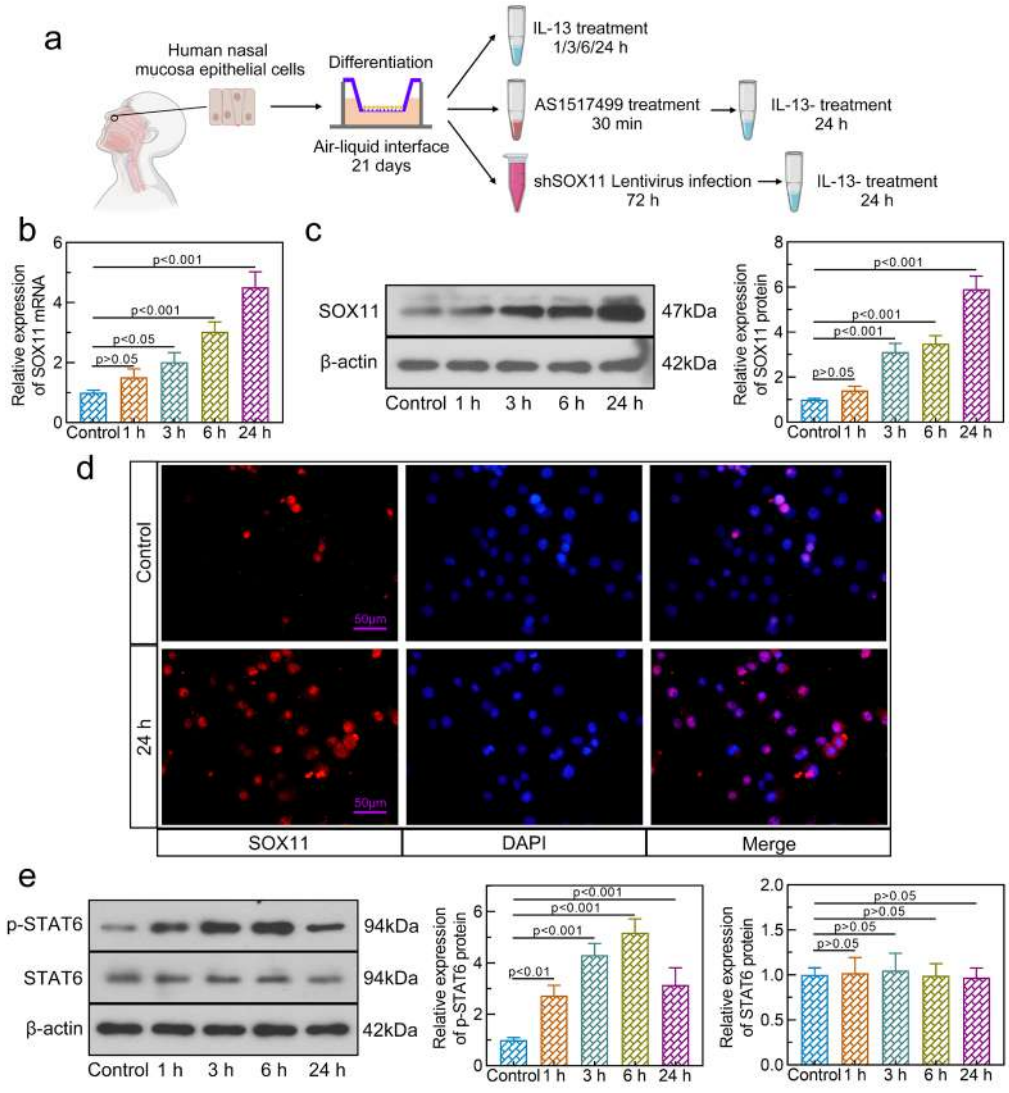

FIG. 4. SOX11 and STAT6 expression were upregulated in IL-13-treated HNEpCs. (a) Schematic diagram of the culture and treatment of HNEpCs. (b) SOX11 expression measured by qRT-PCR. (c) SOX11 protein expression determined by Western blotting. (d) Immunofluorescence staining for SOX11 expression. (e) Representative images and relative protein levels of p-STAT6 and STAT6 obtained by Western blotting analysis.

DISCUSSION

In this study, the expression levels of SOX11 and p-STAT6 were increased in OVA-induced AR mouse model and IL-13-treated HNEpCs. Allergic symptoms, nasal mucosal eosinophils, and goblet cell infiltration were evidently reduced in SOX11-silenced AR mice. Downregulating SOX11 decreased the overproduction of epithelial-derived cytokines and mucin. Mechanistically, STAT6 bound to the promoter of SOX11 to enhance SOX11 expression.

Upon allergen stimulation, IgE on mast cells in the nasal mucosa crosslinks with high-affinity receptors and activates mast cells, leading to the release of chemical mediators, such as histamine, into the nasal mucosal tissues and thereby causing AR.[12] Previous studies found that the typical type 2 inflammatory cytokines that account for local IgE and mucus production and eosinophil recruitment could be detected in tissues and nasal secretions following allergen exposure.[6,13,14] In the current study, typical allergic symptoms, including increased OVA-specific IgE, histamine, and type 2 cytokines, as well as eosinophil and phagocytic infiltration, were demonstrated in the OVA-treated mice.

Epithelial-derived cytokines, including IL-25, IL-33, and TSLP, are secreted in response to exogenous danger signals. These molecules are key regulators of the pathologic immune response associated with AR.[15] TSLP is a critical mediator of allergic inflammation and its expression is induced by IL-13 in a STAT6-dependent manner in mouse nasal tissue and epithelium.[16] The immune mediator CCL26 serves as a key factor in the recruitment of Th2

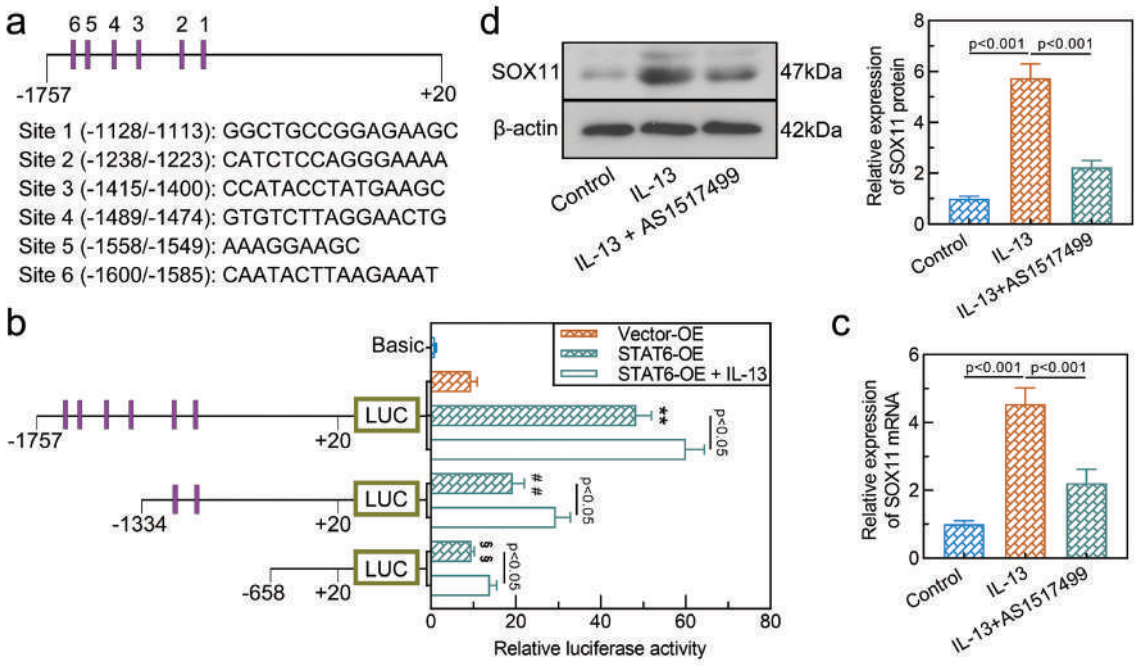

FIG. 5. STAT6 enhanced the transcription of SOX11 gene. (a) Schematic model of predicted binding sites of STAT6 on SOX11 promoter. (b) Effects of STAT6 on the transcriptional activity of truncated SOX11 promoter fragments. (c) SOX11 expression in AS1517499-treated HNEpCs measured by qRT-PCR. (d) SOX11 protein levels in AS1517499-treated HNEpCs obtained by Western blotting analysis. $^{**}P < 0.01$ vs. SOX11 promoter (−1757 to +20) + Vector-OE group; $^{\#\#}P < 0.01$ vs. SOX11 promoter (−1757 to +20) + STAT6-OE group; $^{\S\S}P < 0.01$ vs. SOX11 promoter (−1334 to +20) + STAT6-OE group.

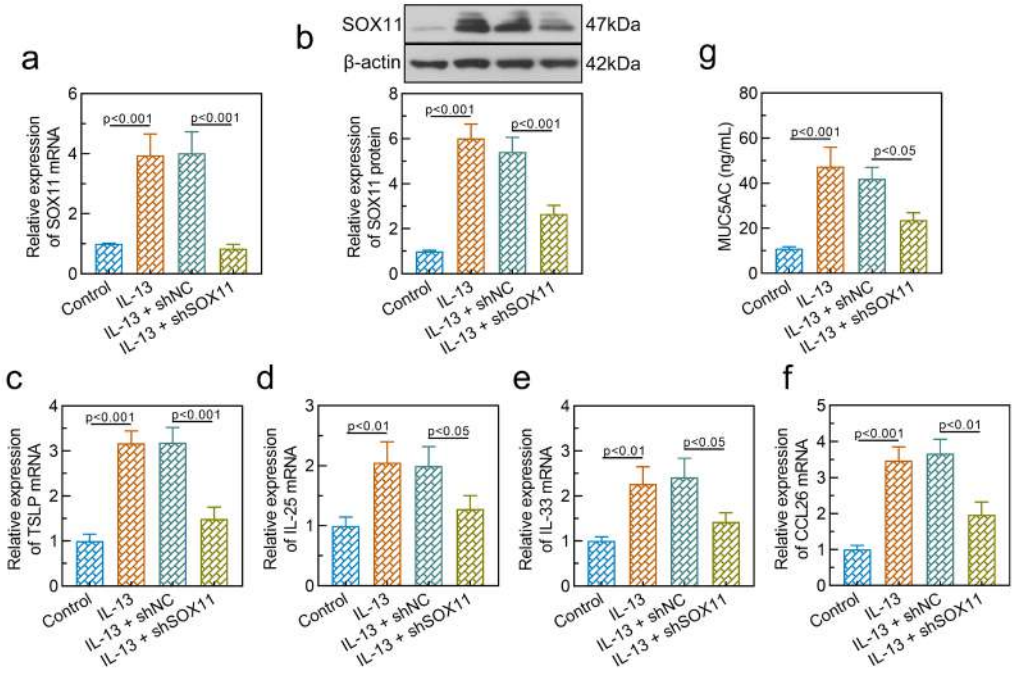

FIG. 6. Silencing SOX11 reduced the epithelial-derived cytokine expression and mucin level in IL-13-treated HNEpCs. (a) SOX11 expression in IL-13-induced HNEpCs measured by qRT-PCR. (b) SOX11 protein levels obtained by Western blotting analysis. (c-f) Expression of epithelial-derived cytokines measured by qRT-PCR. (g) MUC5AC levels in cell supernatants detected by ELISA.

cells and eosinophils to inflammatory sites in allergic disorders.[17] In lung fibroblasts, CCL26 expression was increased upon IL-13 stimulation, while knockdown of SOX11 downregulated CCL26 expression.[10] MUC5AC is a major mucin that is abundantly expressed and secreted in respiratory disorders.[18,19] Our results revealed that inhibiting SOX11 attenuated the overproduction of TSLP, IL-25, IL-33, CCL26, and MUC5AC in AR mice or IL-13-treated HNEpCs.

The role of SOX11 in the modulation of immune responses has attracted increasing interest.[9] A recent study demonstrated a positive correlation between SOX11 expression and infiltration of T lymphocytes in inflammation.[20] Additionally, SOX11 is the only member of the SOX family of transcription factors that can be induced by IL-13 in lung fibroblasts.[21] The functions of the SOX family members vary depending on their companion factors and the DNA sequence to which they bind. SOX proteins require the binding of companion transcription factors to adjacent regions on their DNA-binding sites to elicit transcriptional activation or repression.[8] SOX11 mediates certain inflammation and fibrosis-related genes in the IL-13/STAT6 pathway.[10] STAT6 is normally expressed in airway epithelial cells and is closely associated with allergic inflammation.[22] STAT6 has been shown to induce type 2 cytokines by activating GATA-binding protein 3.[23] Moreover, STAT6 acts as an essential signal molecule in the inflammatory response induced by IL-13. Expression of TSLP, CCL26, and MUC5AC induced by IL-13 in epithelial cells during airway inflammation relies on STAT6 activation.[16,24] In our study, we found that STAT6 enhanced the transcription of SOX11 and inhibition of STAT6 repressed IL-13-induced SOX11 expression.

In summary, our results suggest that SOX11 may be involved in AR progression as a downstream target of STAT6. We demonstrated that SOX11 silencing could effectively attenuate allergic symptoms and Th2-mediated changes such as epithelial-derived cytokine and mucin overproduction. Targeted inhibition of SOX11 may be a promising novel approach toward the development of AR therapy.

Ethics Committee Approval: The First Affiliated Hospital of Harbin Medical University 2022024.

Data Sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author Contributions: Concep- M.L.; Design- L.J., M.L.; Data Collection or Processing- L.J., C.W., R.Z., J.C., Y.L.; Analysis or Interpretation- L.J., J.C.; Supervision- L.T.; Critical Review- L.T.; Literature Search- L.J.; Writing- L.J.

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