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RESEARCH PAPER

Roles of Clara cell 10-kD protein and type 2 innate lymphoid cells in allergic rhinitis

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ABSTRACT

This study examined the potential roles of CC10 (Clara cell 10-kD protein) and ILC2s (type 2 innate lymphoid cells) in allergic rhinitis (AR). After ovalbumin was used to construct the AR model, microarray analysis was performed to reveal the key differentially expressed genes. The phenotypic changes of nasal mucosa were examined by H&E staining. Western blot analysis, qRT-PCR, ELISA and immunohistochemistry were performed to identify the levels of cytokines. The lineage markers (CD127 and CD117) of ILC2s were detected using immunofluorescence. The microarray analysis and qRT-PCR results showed that CC10 overexpression inhibited the expression of A20, BAFF, and IL-4 R *in vivo*. Also, CC10 overexpression was found to ameliorate the damage of nasal mucosa in AR mice. Investigations revealed that the ILC2s were activated in AR mice and AR patients with high levels of IgE, IgG1, IL-4, IL-5, IL-13, IL-25, and IL-33. Moreover, CD127+ was found to activate ILC2s. However, CC10 overexpression suppressed the activation of ILC2s. In conclusion, this research suggested that CC10 could suppress the activation of ILC2s to attenuate the damage of nasal mucosa and that CD127+ may be a biomarker of the activation of ILC2s in AR mice and AR patients.

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Introduction

Allergic rhinitis (AR) is a chronic nasal mucosal inflammatory disease that depends on the immunoglobulin E (IgE)-mediated hypersensitivity reaction, and it is characterized by a runny nose, sneezing, nasal itching, and other abnormal symptoms [1,2]. Previous studies indicated that allergen exposure was regulated by antigen-specific T helper 2 (Th2) cells and that secreted cytokines resulted in mucus hypersecretion, intense eosinophil infiltration, and airway damage [3,4]. Ovalbumin (OVA) is a natural protein in egg white, and it has been used to construct an AR model that could induce the infiltration of inflammatory cells and increase the epithelial layer thickness in mice [5].

Type 2 innate lymphoid cells (ILC2s) are newly discovered cells, including natural helper cells, monocytes, and Th2 cells, but they cannot express known surface markers for T, B, NK, or NKT cells [6–9]. Nonetheless, ILC2s can generate Th2 cytokines (IL-4, IL-5, and IL-13) under the stimulation of IL-33, IL-25, thymic stromal lymphopoietin (TSLP) or leukotriene D4 [10–12]. In humans, ILC2s were found to be widely distributed in peripheral blood, GI tract, lung, BAL, and nasal polyps [13]. They were also observed to promote allergen-induced airway hyperresponsiveness and type-2 lung inflammatory responses in animal models [14]. Increased levels of ILC2s were found in AR patients' peripheral blood [15]. These findings indicated that ILC2s could play a positive role in AR development.

Like ILC2s, Clara cell 10-kD protein (CC10) has the potential to regulate AR. It belongs to the secretoglobin family. Found only in mammals, CC10 acts as an anti-inflammatory and immunomodulatory effector [16–19]. Several studies reported that the level of CC10 decreased in patients with allergic airway diseases, including AR and asthma, and that it significantly inhibited Th2 responses in patients [16,20,21]. Similarly, OVA sensitization in CC10 knockout mice increased the activity of Th2 cytokines (IL-4, IL-5, and IL-13) [22]. Therefore, we hypothesized that CC10

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might block Th2-mediated responses in AR by suppressing the activity of ILC2s. In this study, we investigated the interaction between CC10 and ILC2 in AR patients and OVA-mediated AR mice models.

Materials and methods

Human nasal mucosa clinical samples and animals

A total of 6 human nasal mucosa clinical samples, including normal nasal mucosa tissues (n = 3) and AR nasal mucosa tissues (n = 3), were collected from patients at Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology. AR diagnostic tests were performed on patients based on their medical history, physical examination, and positive skin prick test responses to appropriate aeroallergens (i.e. dust mites, ragweed extract, cat, and dog). These clinical samples were processed for qRT-PCR, immunohistochemistry, and immunofluorescence. A total of 42 female C57BL/6 mice were obtained from Teng Xin Biotechnology Co., Ltd. (Chongqing, China). All the mice were fed under specific-pathogen-free (SPF) conditions with free water and clean food. These mice were used in subsequent experiments. All research protocols were reviewed and approved by the Ethics Committee of Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science and Technology.

CC10-overexpression vector construction

The CC10-overexpression vector (Cat#: P1691) was constructed by and purchased from Miaolingbio Co., Ltd. (China). Briefly, the complete CC10 open reading frame (ORF) was amplified. The CC10-overexpression vector was then generated by inserting the amplified ORF sequences into the pDC316-mCMV-eGFP vector. Next, the CC10-overexpression vector (pDC316-mCMV-CC10- eGFP) and adenovirus genomic plasmid pBHGloxΔE1 were co-transfected into 293 T cells using Lipofectamine 2000 Reagent (Promega, Madison, USA). The transfected 293 T cells were subsequently lysed with three consecutive freeze/thaw cycles. After that, the viral particles were isolated from the supernatant by centrifugation. Finally, CC10 adenoviral titers were determined using a fluorescence microscope.

AR model

The female C57BL/6 mice were randomly divided into four groups: the normal group, AR group, AR+NC (negative control) group, and AR+OE (overexpression) group. Then, OVA (Sigma, St. Louis, USA) was used to produce an AR model. As for the AR model groups, the C57BL/6 mice were first intraperitoneally immunized with 0.5 mL mixture of 20 μg OVA and 0.4% aluminum hydroxide gel on day 1, 3, 5, and 8. On day 15 ~ 24, after initial immunity, the mice received a nasal drip of 20 μL or 500 μg OVA. After the last allergen exposure, the mice were watched for 30 min. Mice's symptoms indicated that the AR model was successfully constructed, such as a runny nose, sneezing, and nasal itching. The mice in the normal group were intraperitoneally immunized with 0.5 mL 0.4% aluminum hydroxide gel at initial immunity, and they received a nasal drip of 20 μL PBS. The mice in the AR+NC group were nasal-dripped with 20 μl virus particles with no CC10 ORF insertion. However, those in the AR+OE group were transfected with 1.5×10^8 PFU CC10-overexpression virus particles by nasal drip, 3 days before the first immunity.

Microarray analysis

To perform microarray analysis, the total RNA was isolated from nasal mucosa tissues of mice in AR+NC and AR+OE groups. The Agilent Bioanalyzer 2100 (Agilent Technologies) was employed to assess the RNA integrity. Then, the total RNA was synthesized into cDNA and labeled with Cyanine-3-CTP. The labeled cDNAs were hybridized onto the Agilent SurePrint G3 Mouse GE V2.0 microarray. Array images were analyzed using Feature Extraction software (version10.7.1.1, Agilent Technologies) to get raw data. After completing the basic analysis with the raw data using GeneSpring (version14.8, Agilent Technologies), the raw data were normalized with the quantile algorithm. Differentially expressed genes (DEGs) were then identified with the fold change ≥ 2.0. Afterward, GO analysis and KEGG analysis were applied to determine the key biological processes and pathways of DEGs, respectively.

qRT-PCR

The total RNA was isolated from the nasal mucosa tissues of mice or humans using TRIzol Reagent (Beyotime Biotechnology, Co., Ltd., Shanghai, China). After the total RNA (1 μg) was reverse-transcribed, qRT-PCR was performed using SYBR® Premix Ex Taq II (TaKaRa Biotechnology, Dalian, China). The specific primer sequences are shown in Table 1. For normalization, β-actin was used as the housekeeping gene. The gene expression was finally analyzed using the $2^{-\Delta\Delta CT}$ technique [23].

Western blot assessment

Tissues were first collected and treated with the CytoBuster Protein Extraction Reagent (Merck Millipore, Darmstadt, Germany). The protein concentration was then determined with the Mic BCA Protein Assay Kit (Pierce). According to the standard procedure, the protein was separated with 10% SDS-PAGE gel and then imprinted onto the nitrocellulose membrane. The membrane was subsequently incubated overnight at 4°C with the following antibodies: IL-17RB (Cat#: ab229320; 1:1000, Abcam, UK), CD127 (Cat#: ab180521; 1:2000, Abcam, UK), c-Kit (Cat#: ab256345; 1:1000, Abcam, UK), TNFAIP3 (Cat#: ab74037; 1:1000, Abcam, UK), BAFF (Cat#: ab224710; 1:1000, Abcam, UK), IL-4 R (Cat#: ab61099; 1:1000, Abcam, UK), IL-4 (Cat#: ab34277; 1:1000, Abcam, UK), IL-5 (Cat#: ab34277; 1:1000, Abcam, UK), IL-13 (Cat#: ab133353; 1:3000, Abcam, UK) and β-actin (Cat#: ab11577; 1:200, Abcam, UK). After that, the blot was incubated with the horseradish peroxidase-conjugated anti-rabbit secondary antibody (Cat#: ab181658; 1:5000, Abcam, UK) for 1 h at room temperature. Next, the protein bands were observed with the enhanced chemiluminescence detection system, and the β-actin antibody was used to confirm the comparable load. Finally, the density of protein bands was measured using the ImageJ software.

Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed to detect the levels of IgE, IgG1, IL-4, IL-5, and IL-13 in the mice serum. Briefly, the mice serum was collected from the supernatant with the aid of a laboratory centrifuge working at 2000 r/min for 20 min. Then, the levels of IgE, IgG1, IL-4, IL-5, and IL-13 were detected by using commercially available ELISA kits according to the manufacturer's instructions. All the ELISA kits in this study were purchased from Like Chuangxin Biotechnology Co., Ltd. (Beijing, China). The optical density was read at 450 nm on a microplate absorbance reader.

Hematoxylin-eosin staining (H&E staining)

The nasal mucosa tissues from mice were stained for 10 min with hematoxylin solution (Nanjing Jiancheng Bioengineering Institute, China). After that, they were then rinsed in tap water for 5 min until the water was colorless. Next, a mixture (1%) of hydrochloric acid and ethanol was used to remove the excess hematoxylin staining from the cytoplasm. After the staining

Table 1. Primers used in this study.

Gene Name	Primer Sequence (5′-3′)
mCC10	Forward AGATCGCCATCACAATCACTG
	Reverse AGGGATGCCACATAACCAGAC
mβ-actin	Forward GAGACCTTCAACACCCCAGC
	Reverse ATGTCACGCACGATTTCCC
mc-kit	Forward GCTTGATTAAGTCGGATGCTG
	Reverse GGACCTTCAGTTCCGACATTAG
mCD127	Forward AGCCAGAGGAGTTGGAGACAC
	Reverse GGAGGGGCATTGCAGGTAC
mIL-17RB	Forward AGCAAGCGGCAGATAAAGTG
	Reverse GCTGCTGAAATCACTACAAAAGAG
hA20	Forward AATGCTAAGAAGTTTGGAATCAGG
	Reverse CCGAGAACAATGGGGTATCTG
hBAFF	Forward CCTCACGGTGGTGTCTTTCTAC
	Reverse TGGTGGTTCAAAGATTTTCAGTC
hIL-4 R	Forward CCAGGGCTCTGGAAAATCAG
	Reverse AGGCGATGCACAGAAGCTC
hβ-actin	Forward TGACGTGGACATCCGCAAG
	Reverse CTGGAAGGTGACAGCGAGG

was added to eosin solution (Beyotime Biotechnology, Shanghai, China) for 30 s, it was rinsed in tap water for 3 min until the water was colorless. The images were finally obtained at 100× and 400× magnification. The cell nucleus was stained blue, while the cytoplasm was stained red or pink.

Immunofluorescence

After deparaffinization and rehydration, the human and mice nasal mucosa sections were heated in citrate buffer to 92°C for 10 min for antigen retrieval. The goat serum (Hyclone, South Logan, USA) was used to block the sections for 60 min at room temperature. After blocking, the sections were incubated with the primary antibody against CD127 (Rabbit origin, Cat#: 501230, Zen BioScience, China) and CD117 (Rabbit origin, Cat#: 3074, Cell Signaling Technology, USA) at 4°C overnight. After washing the sections in PBS, they were incubated for 60 min with an HRP-conjugated anti-FITC antibody (Cat#: ab6717, Abcam, UK). The washed sections were then stained in the dark for 15 min with DAPI solution (Beyotime Biotechnolo gy, Shanghai, China). The imaging at 400× and 600× magnification was performed using a laser scanning confocal microscopy (Leica, Germany).

Immunohistochemistry

After deparaffinization and rehydration, the human nasal mucosa sections were heated in citrate buffer to 92°C for 10 min for antigen retrieval. The sections were then immersed in 3% H_2O_2 to block endogenous peroxidase activity for 15 min. The goat serum (Hyclone, South Logan, USA) were later used to incubate the sections for 1 h at room temperature. Next, the sections were incubated in primary antibody against IL-25 (mouse origin, Cat#: ab115672, Abcam, USA) or IL-33 (mouse origin, Cat#: 66235-1-Ig, Proteintech, USA) at 4°C overnight. After incubating and washing them with PBS, the sections were incubated with the HRP-conjugated goat anti-mouse IgG antibody (Cat#: ab6789, Abcam, USA) for 0.5 h at room temperature. Color development was achieved with DAB-Peroxidase Substrate Solution (Zsbio Biotechnology Co., Ltd., Beijing, China). This solution rendered positive cells brown. The sections were then counterstained with the hematoxylin solution (Nanji ng Jiancheng Bioengineering Institute, China). Finally, the sections were observed at 100× and 400× magnification.

Statistical analysis

In this study, three repeats were performed for each test, and the data collected were expressed as mean ± SD (standard deviation). SPSS 19.0. was used to perform correlation and ANOVA analyses. P-values <0.05 were considered statistically significant.

Results

Microarray analysis revealed the overexpression of CC10 in AR mice

Microarray analysis was performed to assess the effect of CC10 overexpression on AR mice. The distribution of AR mice samples transfected with negative control or CC10-overexpression vectors was first examined to ensure a proper dynamic range with normalized intensity (Figure 1a). Scatter plot data suggested that the AR mice transfected with CC10-overexpression vectors generated aberrantly expressed genes (Figure 1b). The heat map and hierarchical cluster analysis displayed the good clustering of the samples, and 3223 DEGs (1327 upregulated DEGs and 1896 downregulated DEGs) were identified (Figure 1c). Among the 3223 DEGs, zinc-finger molecule A20 (A20), B cell-activating factor (BAFF), and interleukin 4 receptor α-chain (IL-4 R) were the most significantly downregulated DEGs. The top 20 biological processes and pathways are shown in Figure 1d and e, respectively. The biological process enrichments indicated that the immune-related biological processes were the key biological processes such as immune response, inflammatory response, innate immune response, positive regulation of T cell proliferation, and mast cell activation. The KEGG enrichment results also displayed that immune-related pathways were the key pathways such as cytokine-cytokine receptor interaction, antigen processing and presentation, and Th17 cell differentiation. As for the microarray analysis, it was found that CC10 overexpression could significantly downregulate the expression of A20, BAFF, and IL-4 R, which was associated with the immune response. Overall, the results

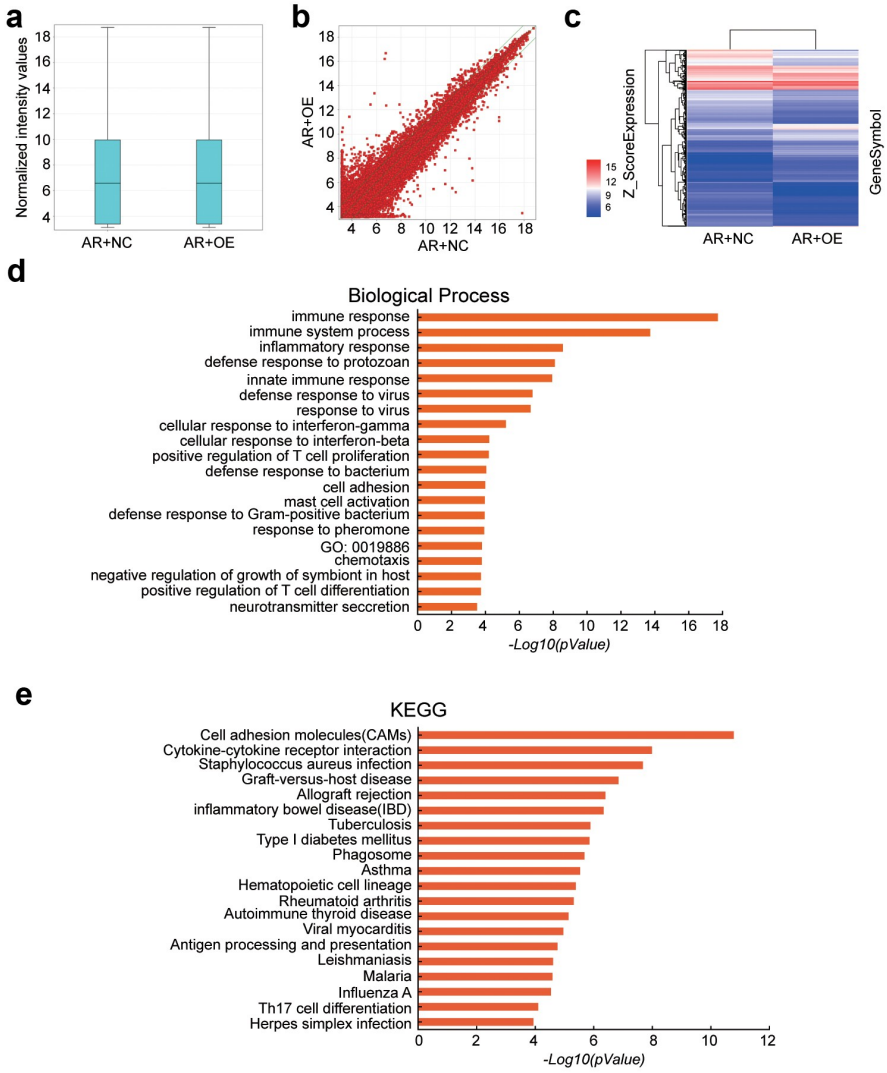

Figure 1. Microarray analysis of AR mice model tissues with negative control vector and forced CC10 overexpression. (a) Box plot showed the normalized intensities of AR mice model with negative control vector and CC10 overexpression vector infection. (b) Scatter plot presented the dysregulated mRNAs in AR model mice with or without forced exogenous CC10 overexpression. (c) Heat map and hierarchical cluster analysis revealed representative DEGs in AR model mice samples. (d) Enriched GO analysis demonstrated the key biological processes for DEGs in AR model mice samples with or without forced CC10 overexpression. (e) KEGG was performed to analyze the key pathways for DEGs in AR model mice samples with or without forced CC10 overexpression. n = 3/group.

suggested that CC10 overexpression could inhibit the inflammatory response by regulating the production of cytokines in AR cell samples.

CC10 overexpression attenuated the injury of nasal mucosa induced by AR

The qRT-PCR results indicated that the transfection of the CC10-overexpression vector into the AR mice increased CC10 expression by 1.5-fold compared with the normal group (Figure 2a). The western blot results showed that after CC10 overexpression, CC10 protein expression increased by about 1.25 times compared with the normal group (Figure 2b). The levels of IgE and IgG1 increased by 2.1-fold and 4.82-fold, respectively, in the AR group, meaning the AR model (C57BL/6 mice) induced by OVA was successfully constructed (Figure 2c). Also, CC10 overexpression reduced the levels of IgE and IgG1 compared

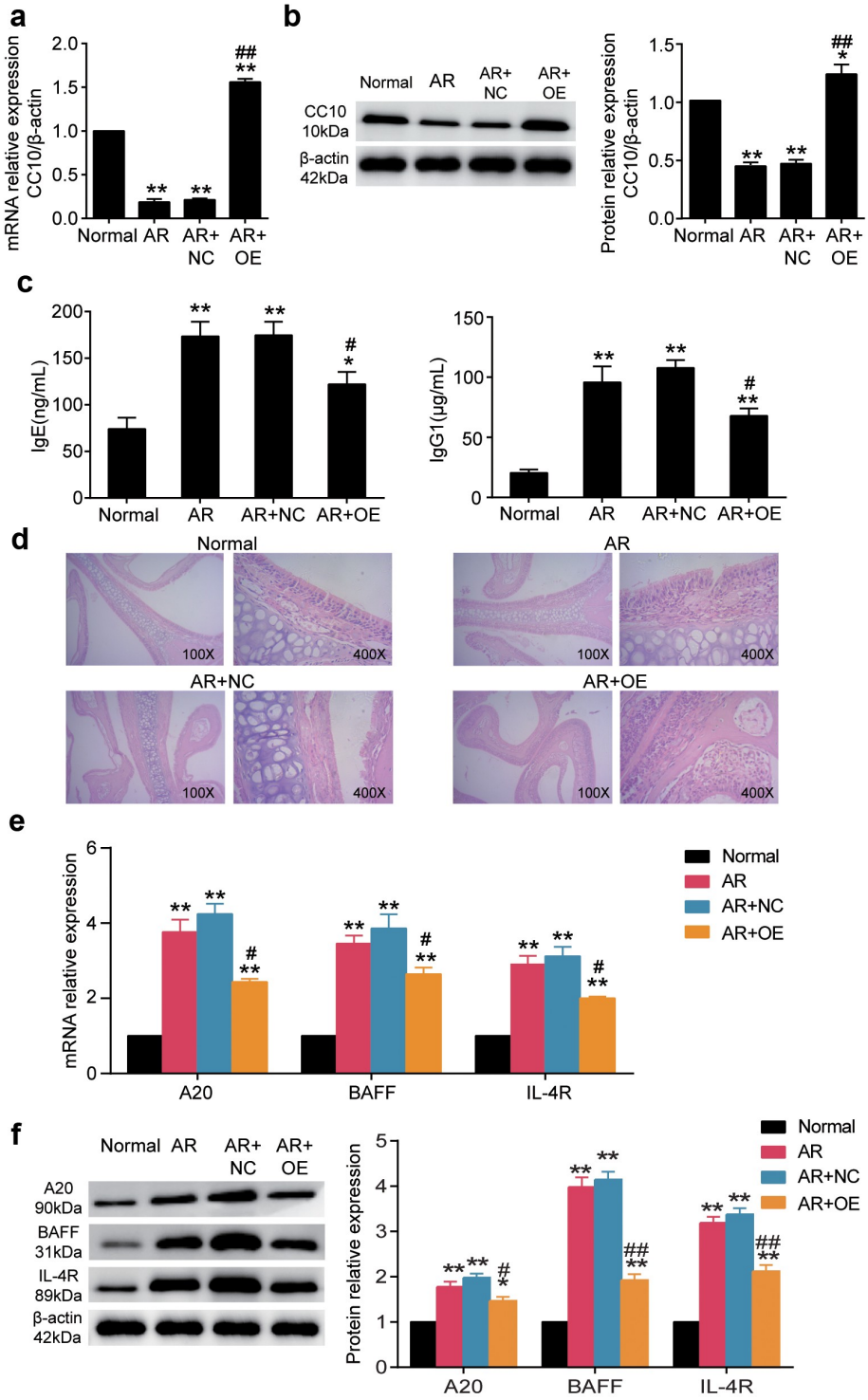

Figure 2. The effect of CC10 overexpression on AR model mice. (a) The mRNA expression of CC10 in normal, AR, AR+NC, and AR+OE tissues. (b) The protein expression of CC10 in normal, AR, AR+NC, and AR+OE tissues. (c) ELISA was performed to detect the levels of IgE and IgG1 in normal, AR, AR+NC, and AR+OE tissues. (d) H&E staining was employed to stain the nasal mucosa tissues. The images were obtained at the 100× and 400× field. (e) The mRNA expressions of A20, BAFF, and IL-4 R were detected by qRT-PCR. (f) The protein expression of A20, BAFF, and IL-4 R in normal, AR, AR+NC, and AR+OE were detected by western blot. The C57BL/6 mice were randomly divided to normal, AR, AR+NC, and AR+OR group. repetition = 3, n = 5/group. Normal: The mice in normal group were fed without any treatment. AR: The mice in AR group were injected with OVA. AR+NC: The mice in AR+NC group were injected with OVA and nasal-dripped with negative control virus particles. AR+OE: The mice in AR+OE group were injected with OVA and nasal-dripped with CC10-expression virus particles. *P< 0.05, **P< 0.001 versus normal group. $^{\#}P$< 0.05, $^{\#\#}P$< 0.001 versus AR group.

with the AR group. The H&E staining results displayed the damage of nasal mucosa tissues in the AR group, such as the thickening of the nasal mucosa, cilia loss, and solid edema (Figure 2d). However, the CC10 overexpression alleviated the injury of nasal mucosa induced by AR. While the mRNA expression levels of A20, BAFF, and IL-4 R with the high level were observed in the AR group compared with the normal group, the mRNA expression levels of A20, BAFF, and IL-4 R with a relatively low level were observed in the CC10 overexpression group compared with the AR group (Figure 2e). Similar to the results of qRT-PCR, western blot data showed an increase in the protein expression levels of A20, BAFF, and IL-4 R in the AR group but a decrease in the protein expression levels of A20, BAFF, and IL-4 R in the AR mice after CC10 overexpression compared with the AR group (Figure 2f). These findings suggested that CC10 overexpression could relieve the injury of nasal mucosa induced by AR.

CC10 overexpression suppressed the activation of ILC2s in AR mice

The activation of ILC2s could produce large quantities of IL-4, IL-5, and IL-13. Because of this, ELISA was employed to detect the levels of IL-4, IL-5, and IL-13. The results showed that the levels of IL-4, IL-5, and IL-13 in the AR group significantly increased (98.71 ± 7.80, 10.14 ± 0.96, 16.72 ± 0.78, respectively) compared with the normal group (Figure 3a). However, the levels of IL-4, IL-5, and IL-13 in the CC10 overexpression group were lower than those in the AR group. Given that c-kit (CD117), CD127, and IL-17RB were the markers of ILC2 maturation, we detected the mRNA expression levels of c-kit, CD127, and IL-17RB. The results revealed an increase in the mRNA expression levels of c-kit, CD127, and IL-17RB in the AR group but a decline in the mRNA expression levels of c-kit, CD127, and IL-17RB in the CC10 overexpression group compared with the AR group (Figure 3b). Western blot results showed that compared with the normal group, c-Kit, CD127, and IL-17RB protein levels in the AR group increased; however, they decreased in the AR+OE group compared with the AR group (Figure 3c). After using immunofluorescence to detect the lineage markers (CD127 and CD117) of ILC2s, we observed that ILC2s were activated in the AR mice and that the number of $CD127^+/CD117^+$ ILC2s increased (Figure 3d). Compared with the AR group, CC10 overexpression significantly reduced the number of $CD127^+/CD117^+$ ILC2s. These findings confirmed that CC10 overexpression could suppress the activation of ILC2s in AR mice.

CD127 was a key marker for the activation of ILC2s in AR patients

The normal human nasal mucosa tissues (n = 3) and AR human nasal mucosa tissues (n = 3) were used to explore the key marker for ILC2 activation. After qRT-PCR was first employed to detect the mRNA expression levels of A20, BAFF, and IL-4 R in normal and AR human tissues, it was found that the mRNA expression levels of A20, BAFF, and IL-4 R increased by 3.49-fold, 2.26-fold, and 2.19-fold, respectively, in AR patients compared with the healthy ones (Figure 4a). Western blot assay was performed to further verify the results of qRT-PCR, and the results showed an increase in the protein expression levels of A20, BAFF and IL-4 R in the nasal mucosa of AR patients (Figure 4b). Because of the response of ILC2s to IL-25 and IL-33 stimulation, the expression levels of IL-25 and IL-33 were detected by immunohistochemistry. The expression levels of IL-25 and IL-33 in AR patients increased compared with normal nasal mucosa tissues; this result indicated that the ILC2s were activated in AR patients (Figure 4c and d). Moreover, the mRNA expression levels of IL-4, IL-5, and IL-13 in AR patients increased, a result that further proved the activation of ILC2s in AR patients (Figure 4e). After staining CD127 and CD117 by immunofluorescence, AR significantly enhanced CD127 expression, but not CD117 (Figure 4f). The results confirmed that the $CD127^+$ could be a marker for ILC activation in AR patients.

Discussion

A large and growing body of research has explored the roles of lymphoid cells in allergic diseases. However, researchers are yet to confirm the interaction between ILC2s and CC10 in AR cell samples. In the present study, the AR model induced

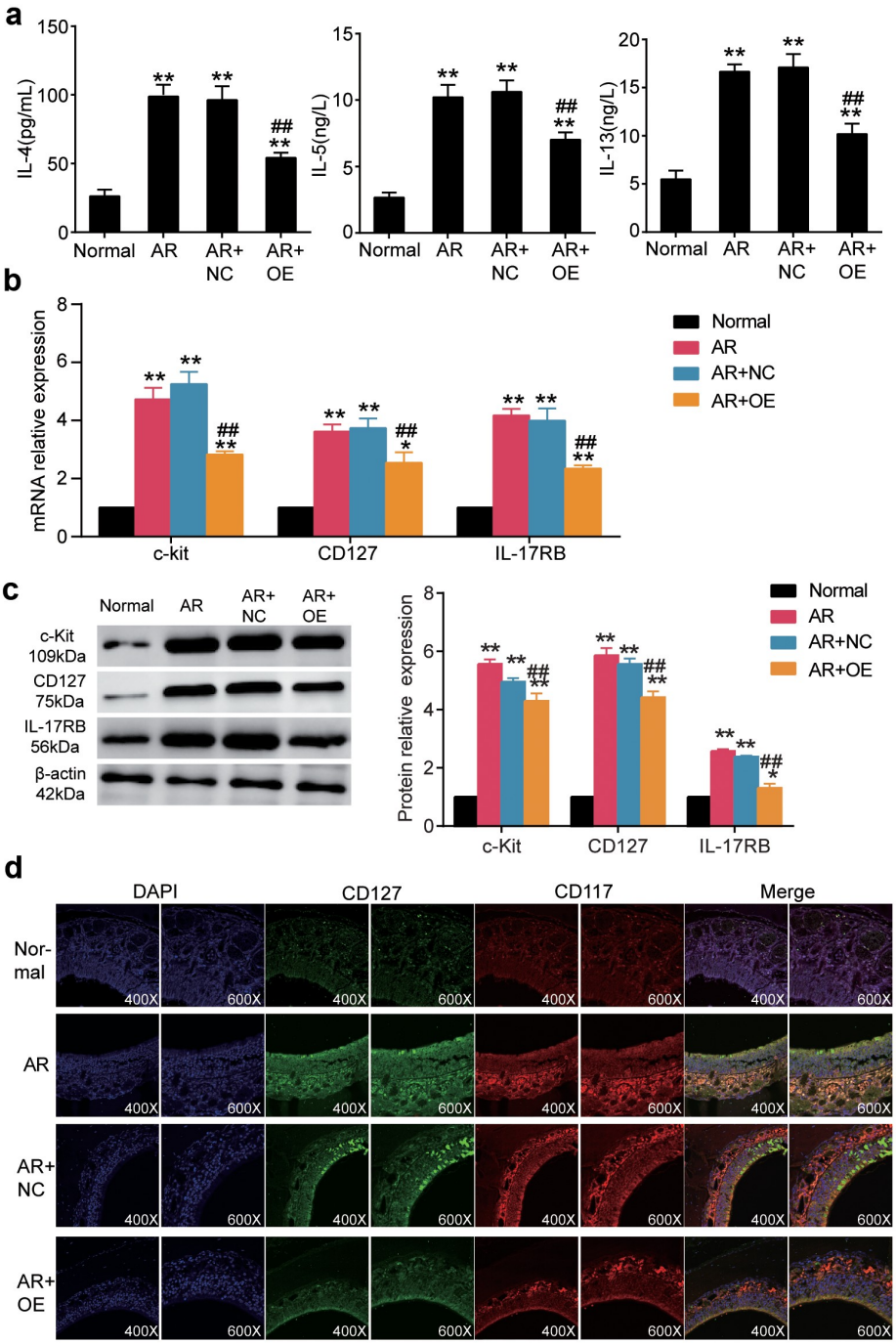

Figure 3. The effect of CC10 overexpression on the activation of ILC2s in normal, AR, AR+NC, and AR+OE groups. (a) The levels of IL-4, IL-5, and IL-13 in the four groups were detected using ELISA. (b) The mRNA expressions of c-kit, CD127, and IL-17RB were measured by qRT-PCR. (c) The protein expressions of c-kit, CD127, and IL-17RB were measured by western blot. (d) The nasal mucosa tissues from normal, AR, AR+NC, and AR+OE mice were stained for CD127 (green), CD117 (red), and DAPI (blue). CD127 and CD117 were the lineage markers of ILC2. The C57BL/6 mice were randomly divided to normal, AR, AR+NC, and AR+OR group. repetition = 3, n = 5/group. Normal: The mice in normal group were fed without any treatment. AR: The mice in AR group were injected with OVA. AR+NC: The mice in AR+NC group were injected with OVA and nasal-dripped with negative control virus particles. AR+OE: The mice in AR+OE group were injected with OVA and nasal-dripped with CC10-expression virus particles. *P< 0.05, **P< 0.001 versus normal group. $^{\#\#}P$< 0.001 versus AR group.

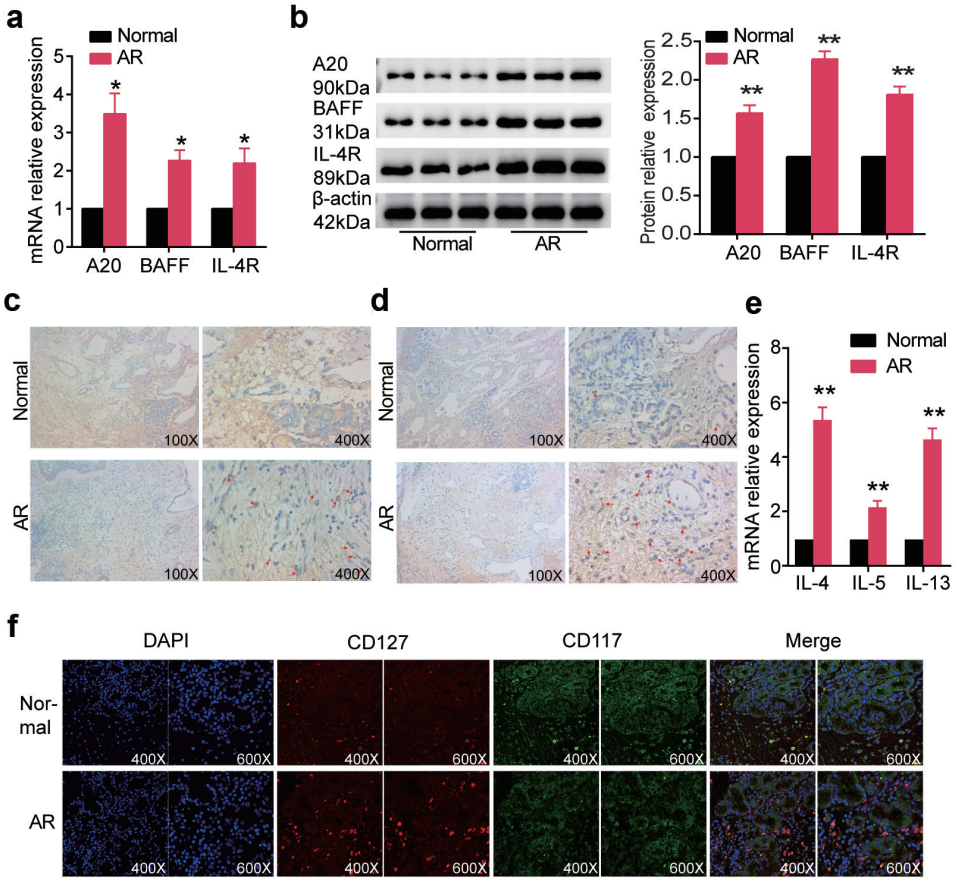

Figure 4. The activation of ILC2s in AR patients. (a) The mRNA expressions of A20, BAFF, and IL-4 R were detected by qRT-PCR. (b) The protein expressions of A20, BAFF, and IL-4 R were detected by western blot. (c) The photomicrographs of IL-25 immunohistochemical staining of human nasal mucosa tissue sections. (d) The photomicrographs of IL-33 immunohistochemical staining of human nasal mucosa tissue sections. (e) The mRNA expressions of IL-4, IL-5, and IL-13 in normal and AR patients. (f) The human nasal mucosa tissue sections were stained for CD127 (red), CD117 (green), and DAPI (blue) detection. CD127 and CD117 were the lineage markers of ILC2. repetition = 3, n = 3. *P< 0.05, ** P< 0.001 versus normal group.

by OVA was successfully constructed in mice. The microarray analysis of mice samples showed that the expression levels of A20, BAFF, and IL-4 R were suppressed in CC10 overexpressed AR mice compared with AR mice. Using ELISA, H&E staining, and qRT-PCR, we found that CC10 relieved the injury of nasal mucosa induced by AR. With immunofluorescence and immunohistochemistry, we discovered that ILC2s were activated in both AR mice and AR patients. However, CC10 overexpression inhibited the activation of ILC2s in AR mice. Our experimental data also showed that CD127+ could be a marker for ILC2 activation. Overall, these findings indicated that by inactivating the ILC2s, CC10 could attenuate the damage of nasal mucosa in AR mice.

AR, an IgE-mediated inflammatory disorder, is characterized by sneezing, nasal itching and rhinorrhea [1]. The OVA-induced AR model in our study not only presented the characteristics of AR but also showed the increased levels of IgE and IgG1. Findings indicated that AR could also increase the level of IgG1, thereby enhancing the immune response. The zinc-finger molecule A20 (A20) induced by NF-κB is also known as tumor necrosis factor-alpha-induced protein 3 (TNF AIP3) [24]. A member of the TNF family, B cell-activating factor (BAFF) was found to be significantly upregulated in children with AR and was documented to be associated with nasal symptoms [25]. Moreover, interleukin 4 receptor α-chain (IL-4 R) could induce isotype class switch from IgG to

IgE production in B cells [26]. In our study, microarray analysis displayed that CC10 overexpression decreased the expression levels of A20, BAFF, and IL-4 R, and this result suggested that CC10 could mitigate the damage of nasal mucosa induced by AR.

Furthermore, evidence suggests that CC10 can perform several anti-inflammatory and immunomodulatory roles. Previous studies reported that CC10 could downregulate Th2 cell differentiation, diminish inflammatory cell chemotaxis, and block prostaglandin D2 receptor-mediated NF-κb activation [16,17,19]. Liu et al. found that CC10 directly decreased osteopontin expression in spleen mononuclear cells stimulated with OVA and inhibited the expression of Th2 cytokines and proinflammatory cytokines [20]. Consistent with the results of Liu et al., we found that CC10 expression was downregulated in AR mice and that CC10 overexpression led to the downregulation of IgE and IgG1 in AR mice serum. Besides, the H&E staining results showed that the injury of nasal mucosa induced by AR was repaired when the AR mice were transfected with the CC10-overexpression vectors. These findings proved that CC10 overexpression could block AR development.

In addition to the results, ILC2s have been proved to secrete large quantities of Th2 cytokines, especially IL-4, IL-5, and IL-13 when they are stimulated with IL-25 and IL-33 [8,27,28]. We used ELISA, immunohistochemistry, and qRT-PCR to detect the levels of IL-4, IL-5, IL-13, IL-25, and IL-33 in the AR mice samples or AR human samples. Because of the high levels of IL-4, IL-5, IL-13, IL-25, and IL-33 in the AR samples, ILC2s were activated in AR. The IL-7 receptor (CD127) binding to IL-7 increased anti-apoptotic molecules, thus priming the newly activated T cell for proliferation [29,30]. Scoville SD et al. found that the ILC1, ILC2, and ILC3 subsets were derived from a common precursor, and they expressed CD127 [31]. CD117, also known as c-kit, is a type III receptor tyrosine kinase, and it participates in cell signal transduction. It was reported that human ILC2s were the only subtype that showed heterogeneous expression of the surface marker CD117 [32]. We detected the expression levels of CD117 and CD127 in AR mice and AR human nasal mucosa by immunofluorescence and found an increase in the levels of CD117 and CD127 in AR mice nasal mucosa; however, only CD127 with the high level was observed in AR human nasal mucosa. Hochdörfer T et al. [32] proved that CD117 was not the biomarker for ILC2 activation. Thus, $CD127^{+}$ might be the biomarker for ILC2 activation. Because of the unclear interaction between CC10 and ILC2s, we investigated the effect of CC10 on ILC2 activation *in vivo*. Our results revealed that the low levels of IL-4, IL-5, and IL-13 were observed in CC10 overexpressed AR mice serum compared with the AR mice serum. Moreover, the expression of CD127 decreased when the AR mice were subjected to forced exogenous CC10 overexpression. These data proved that the activation of ILC2s was negatively correlated with CC10 expression in AR mice.

However, this study has a few limitations. The small sample size used in this study reduces the validity of the results. Besides, our study used female mice to construct AR model, it has been reported that there are gender-related differences in AR among children and adolescents [33]. In future studies, we will expand the clinical samples and study the differential expression of CC10 in humans and mice at different ages and gender.

Conclusion

In sum, this research suggested that CC10 could suppress the activation of ILC2s to attenuate the damage of nasal mucosa and that CD127+ might influence the activation of ILC2s in AR mice and AR patients. Our findings could be instrumental in improving AR treatments. However, future research should further explore the underlying mechanism used by CC10 to suppress the activation of ILC2s.

Authors' contributions

Xiaobo Long performed the experiments and data analysis. Nan Wang conceived and designed the study. Xiaobo Long wrote the paper. Nan Wang reviewed and edited the manuscript. All authors read and approved the manuscript.

Data availability

Differentially expressed genes (DEGs) were then identified with the fold change ≥ 2.0. Afterward, GO analysis and

KEGG analysis were respectively applied to determine the key biological processes and pathways of DEGs by Over Representation Analysis (ORA).

Disclosure statement

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