

SOCS3 Is Upregulated and Targeted by miR30a-5p in Allergic Rhinitis

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Keywords

Allergic rhinitis · SOCS3 · SOCS1 · miR30a

Abstract

Background: Suppressor of cytokine signaling 1 (SOCS1) and SOCS3 play important roles in T helper cell differentiation, which is involved with the pathologic mechanisms of allergic rhinitis (AR). The aim of this study was to evaluate the expression of SOCS1 and SOCS3 in AR and find their regulatory microRNAs (miRNAs) to provide a basis for the treatment of AR. **Methods:** The expression of SOCS1 and SOCS3 were analyzed by real-time PCR, immunohistochemistry, and Western blot. The correlative regulatory miRNAs were detected by real-time PCR. Luciferase assays and AR mouse model experiments were applied to identify correlative miRNAs that target SOCS3. **Results:** SOCS1 and SOCS3 mRNA were upregulated in the nasal mucosa and peripheral blood mononuclear cells of AR compared with controls. The expression of SOCS3 protein was significantly increased in the nasal mucosa of AR. The immunohistochemical staining results showed that SOCS3 was similarly localized in the superficial epithelium, submucosal glands, and vascular endothelium in the nasal mucosa of AR subjects and controls. However, SOCS3 protein was especially localized in the inflammatory cells, such as eosinophils, monocytes, and lym-

phocytes. **Conclusions:** SOCS3 was targeted by miR30a-5p in AR. Further study should be performed to identify the regulatory effect of miR30a-5p in AR, which may provide insights into a new therapeutic strategy.

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Introduction

Allergic rhinitis (AR) is the allergen-specific IgE-mediated inflammation which is characterized by eosinophil infiltration, T helper (Th)2 cytokine responses, and mucus secretion [1]. AR is a common worldwide disease with an increasing prevalence. Moreover, AR has close relations with chronic rhinosinusitis and asthma due to the “one airway” viewpoint [2]. AR is primarily caused by the imbalance of Th1/Th2 cells. Th1 cells are characterized by the production of cytokines, such as interleukin (IL)-2, interferon (IFN)- γ , and tumor necrosis factor- α . Th2 cells secrete cytokines such as IL-4, IL-5, IL-6, IL-9, IL-10, and IL-13. IL-4 is important for the initiation of allergic immune responses by inducing the Th2 subset of T cells. IL-4 and IL-13 are potent activators for the production of IgE. IL-5 is essential for eosinophil differentiation and maturation. IL-13 regulates many of the effector responses that occur during allergic immune responses [3]. The

deregulated Th2 responses can cause allergy and asthma [4].

Cytokines control and regulate fundamental biological processes and bind to their specific receptors on the surface of cells to transduce biological information to target cells by the signaling pathway. The elaborate extracellular and intracellular control mechanisms have evolved to prevent excessive cytokine signaling that may dysregulate the normal functions of cytokines [5]. In order to maintain the balance between Th1 and Th2, cytokines secreted by Th1 and Th2 cells can be cross-regulated, respectively. Th1 cytokines inhibit Th2 responses, whereas Th2 cytokines inhibit Th1 responses. Recent research has shown that the magnitude and duration of cytokine action are limited by several mechanisms. The suppressor of cytokine signaling (SOCS) proteins are a new family of molecules. They act as negative regulators of cytokine signaling [3] and are known to be involved in the pathogenesis of many inflammatory diseases, including Th2-mediated allergic responses. Furthermore, the SOCS family plays an important role in regulating Th1/Th2 balance and in the crosstalk of complicated cytokine signal networks. The SOCS family is composed of 8 members, namely CIS and SOCS1–7. They are characterized by the presence of a Sec-homology-2 domain and a C-terminal conserved domain called the SOCS box [4]. Recent studies have demonstrated that SOCS1 and SOCS3 are important regulators of inflammation and Th cell differentiation.

MicroRNA (miRNA) is a small noncoding RNA molecule and a novel regulator of gene expression. MiRNAs are involved in the pathogenesis of many inflammatory diseases, including allergic inflammation [6, 7]. Few studies have investigated the miRNAs that may potentially participate in the pathogenesis of AR [8–11]. An increased expression of miR155 was detected in the nasal mucosa of AR patients [12]. Moreover, SOCS1 has been shown to be a target of miRNA-155 in Tregs and Th17 [13, 14]. However, studies involving correlative miRNA regulating SOCS3 in AR have not been published until now.

Here, the expression of SOCS1 and SOCS3 are evaluated in order to elucidate the role of SOCS1 and SOCS3 in the pathogenesis of AR. Furthermore, we detected the expression of correlative miRNA regulating SOCS1 and SOCS3, respectively. We hope to determine the regulatory miRNA by targeting SOCS1 and SOCS3 in AR, which may not only contribute to an understanding of the pathogenesis of AR, but also provide potential pharmaceutical targets for anti-inflammatory treatment.

Materials and Methods

Subjects

Twenty patients with AR (13 men and 7 women; age range 33–63 years) and 20 healthy control subjects (12 men and 8 women; age range 19–52 years) were enrolled in the current study. Patients undergoing septoplasty because of anatomic variations were considered healthy controls and were excluded if they had a history of rhinosinusitis or allergy, smoking, or ongoing drug treatment. All patients with AR had typical symptoms of nasal allergy and a positive skin test. Allergic patients with exacerbation of upper respiratory tract infection during the 4 weeks preceding the study were not included in the study. Other exclusion criteria were current smoking, a medical history of asthma, recurrent infections of the respiratory tract, and use of topical or oral corticosteroids, leukotriene modifiers, and antihistamines during the 4 weeks that preceded the study. This study was approved by the ethics committee of our institution and written informed consent was obtained from all patients.

The samples of healthy nasal mucosa were obtained from the inferior turbinate mucosa by the nasal endoscopy from 20 healthy control subjects and 20 patients with AR. All tissue samples were divided into 3 portions. The first and second portions were frozen in liquid nitrogen and stored at -70°C for RNA and protein isolation. For immunohistochemistry, the third sample was fixed in 4% paraformaldehyde and then dehydrated in a graded series of ethanol to xylene and embedded in paraffin wax.

Whole blood samples were collected from 20 patients with AR and 20 healthy control subjects, and then Ficoll-Hypaque density centrifugation was immediately performed to isolate peripheral blood mononuclear cells (PBMCs). Total RNA and protein were extracted from PBMCs for real-time PCR and Western blot analysis.

TargetScan Human Prediction

TargetScan Human 7.1, an online bioinformatics program, was used to predict the consequential miRNAs targeted as SOCS3 (Fig. 1a). We identified the most correlative miRNA by real-time PCR in AR.

Real-Time PCR Analysis

The tissue samples were extracted by Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Total RNA was incubated with oligodT and reverse transcriptase for 1 h at 42°C . The enzyme was inactivated for 5 min at 95°C . The cDNA was stored at -20°C . Real-time PCR amplification was carried out to detect mRNA. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was used as an internal control. Each sample was performed in duplicate. The reverse and forward primers are listed in Table 1. Each PCR included 1 μL of cDNA, 2 $\times$ TransScript Tip Green qRT-PCR SuperMix 10 μL , and 1 μL of primers. The PCR conditions for 45 cycles were 94°C denaturation for 5 s, 55°C annealing for 15 s, and 72°C extension for 10 s. The relative mRNA expression of the target gene was determined as $2^{-\Delta\Delta\text{Ct}}$. All experiments were performed in triplicate at 3 independent time points.

HEK293 Cells Transfection and Luciferase Assays

To evaluate whether miR30a-5p targets SOCS3 or SOCS1, HEK293 cells were cultured and then transfected with miR30a-5p mimic (Genepharma, Shanghai, China) or control and FAM (car-

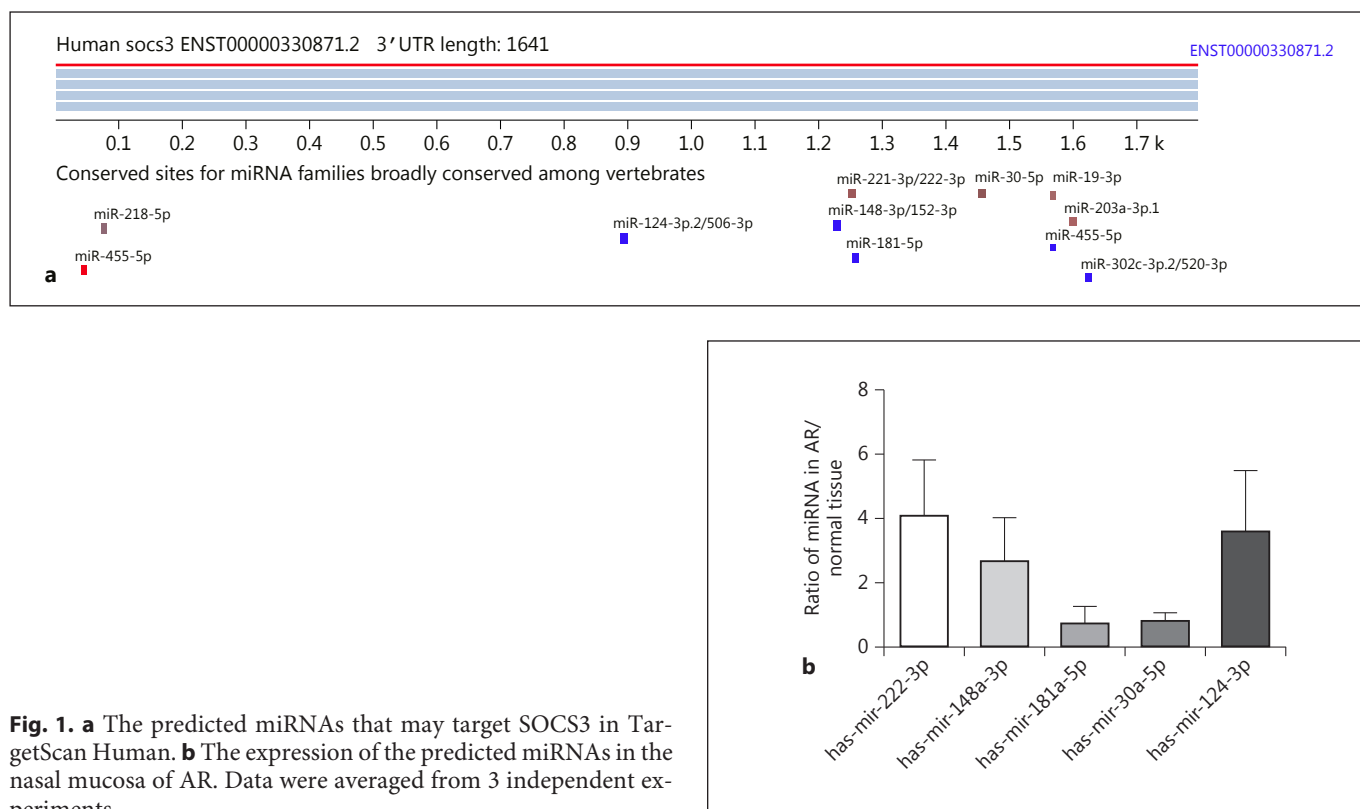


Fig. 1. a The predicted miRNAs that may target SOCS3 in TargetScan Human. **b** The expression of the predicted miRNAs in the nasal mucosa of AR. Data were averaged from 3 independent experiments.

Table 1. Sequences of PCR primers

Primer		Sequences
SOCS1	Forward:	5'TTTTCGCCCTTAGCGTGAAGA3'
	Reverse:	5'GAGGCAGTCGAAGCTCTCG3'
SOCS3	Forward:	5'CCTGCGCCTCAAGACCTTC3'
	Reverse:	5'GTCAGTGCCTCCAGTAGAA3'
Mir155-5p	Forward:	5'TTAATGCTAATCGTGATA3'
	Reverse:	5'GTGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCCT3'
Mir124-3p	Forward:	5'TTAAGGCACGCGGTG3'
	Reverse:	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGCATTTC3'
miR218-5p	Forward:	5'TTGTGCTTGATCTAAC3'
	Reverse:	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACATGG3'
miR181a-5p	Forward:	5'AACATTCAACGCTGTCGG3'
	Reverse:	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACTCAC3'
miR222-3p	Forward:	5'AGCTACATCTGGCTAC3'
	Reverse:	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCAG3'
miR30a-5p	Forward:	5'TGTAACATCCTCGACT3'
	Reverse:	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCTTCCA3'
miR455	Forward:	5'TATGTGCCTTTGGACTA3'
	Reverse:	5'GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGATGT3'

boxyfluorescein). After 36 h, the positive fluorescence was detected. Protein was extracted, then SOCS1 and SOCS3 proteins were assessed by Western blot.

In order to confirm that SOCS3 was targeted specifically by miR30a-5p, HEK293T cells were transfected with 3'UTR-SOCS3 plasmid (0.1 µg), mutated 3'UTR-SOCS3 plasmid, or empty plasmid, and Renilla luciferase plasmid (0.02 µg; Promega) and miRNA (0.4 µg; Genepharma) or miRNA negative control. X-tremeGENE HP (Roche) was used to transfect and lysates were prepared 48 h after transduction. Luciferase activity was measured by the Dual Luciferase Reporter Assay system (Promega). All experiments were performed in triplicate at least 3 independent times.

Immunohistochemical Staining

Paraffin-embedded tissue blocks were cut into 5-µm-thick sections, mounted on the poly-L-lysine-coated glass slides and pretreated with citrate (pH 6.0) buffer for 3 min at 120°C in a pressure cooker. Serial sections from each block were deparaffinized, hydrated, and treated with 0.03% hydrogen peroxide for 10 min. BSA was used to block the sections, and then incubated with 1:50 dilution of anti-SOCS1 (Santa Cruz) overnight at 4°C. In the meantime, 10% goat serum was used to block the sections and then incubated with 1:150 dilution of anti-SOCS3 (Santa Cruz) overnight at 4°C. Negative controls consisted of PBS instead of primary antibodies. Species-matched secondary antibodies were incubated for 30 min at room temperature. The slides were washed 3 times with PBS, and then stained by diaminobenzidine. Following a final wash, the slides were mounted, cover slipped, and sealed. Ten fields per sample were randomly selected and scored by 2 different investigators under the microscope.

Western Blot Analysis

Frozen tissue sections were crushed and vigorously vortexed in a buffer solution containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, and a proteinase inhibitor cocktail. The proteins (50 µg/well) were fractioned by SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and transferred onto a PVDF membrane. After blocking with 5% skimmed milk for 30 min, the membrane was incubated with the primary antibodies (1:1,000 dilution of anti-SOCS3 antibody) for 1 h at room temperature. After being washed with PBS-T, the membrane was incubated with peroxidase-conjugated rabbit anti-human IgG (1:2,000) for 1.5 h at 37°C. The relative bands were detected by the ECL detection kit and photographed with chemiluminescence on X-ray film. As an internal control, GAPDH expression was analyzed in parallel blots (Sigma-Aldrich, St. Louis, MO, USA). The intensities of the detected bands were quantified.

Inhibition of miRNA on SOCS3 in a Nasal Allergic Mouse Model

Specific pathogen-free male BALB/c mice (6–8 weeks old, 18–22 g) were purchased from the Experimental Animal Center, Second Hospital Affiliated to Harbin Medical University (Harbin, China). The animal experiments were approved by the institutional Animal Ethics Committee. Fifty mice were fed to ensure there were 10 mice in each group for the experiment. The mice were intraperitoneally administered 300 µL of saline containing 25 µg of OVA and 2 mg of aluminum hydroxide (Sigma-Aldrich) on days 0, 7, and 14 to promote primary sensitization. On days 21–28, the mice were intranasally sensitized with 20 µL of saline contain-

ing 500 µg of OVA (10 µL per nostril) for secondary immunization. The normal group was intraperitoneally injected with 300 µL of saline on days 0, 7, and 14, and then treated intranasally with 20 µL of saline on days 21–28 after the initial treatment.

The agomiRNA group and miRNA mimic control mice were intraperitoneally sensitized and intranasally challenged with OVA, similar to the AR group. On days 22, 24, and 26, the agomiRNA mice and controls were treated intranasally with 20 µL of saline containing 50 µg agomiRNA and miRNA mimic control (Genepharma), respectively.

Blood was extracted from the orbital venous plexus of the anesthetized mice 24 h after the last nasal cavity challenge. The samples were centrifuged for 5 min at 2,500 rpm to isolate the serum and stored at –20°C. The serum concentration of OVA-sIgE was detected by a mouse ELISA kit (Elabscience, Wuhan, China). The nasal mucous membrane was excised, sectioned, and stored in liquid nitrogen for the detection of SOCS3 and miRNA.

Statistical Analysis

Data are presented as the mean ± standard deviation of the mean. The data were analyzed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA). A statistical analysis of the differences in the expression of miRNA and mRNA was assessed using the Student *t* test. One-way ANOVA was used to evaluate the protein expression in the immunohistochemical staining and Western blot analysis. Significance was defined as $p < 0.05$.

Results

Expression Levels and Distribution Pattern of SOCS1 and SOCS3 in AR

The expression of SOCS1 and SOCS3 was detected in the nasal mucosa and PBMCs of AR patients and control subjects. The quantitative gene expression of SOCS1 and SOCS3 was determined by real-time PCR. Both SOCS1 and SOCS3 mRNA were detectable in all tissue mucosa and PBMCs. The levels of expression of SOCS1 and SOCS3 mRNA were significantly increased in the nasal mucosa (Fig. 2a, b) and PBMCs of AR patients contrasted with controls (Fig. 3a, b). Western blot analysis also detected SOCS3 protein in the tissue mucosa of AR patients and control subjects. The relative abundance of SOCS3 relative to GAPDH was significantly increased in AR patients compared with controls (Fig. 2c, d). However, the abundance of SOCS1 relative to GAPDH was relatively low in AR patients compared with controls (Fig. 2c, d).

The distribution pattern of SOCS1 and SOCS3 in the nasal mucosa of control subjects and patients with AR was analyzed by immunohistochemistry. SOCS3 was similarly localized in the superficial epithelium, submucosal glands, and vascular endothelium in the nasal mucosa of AR subjects and controls (Fig. 2e, 1, 2). The expression of SOCS3 protein was displayed strongly in the

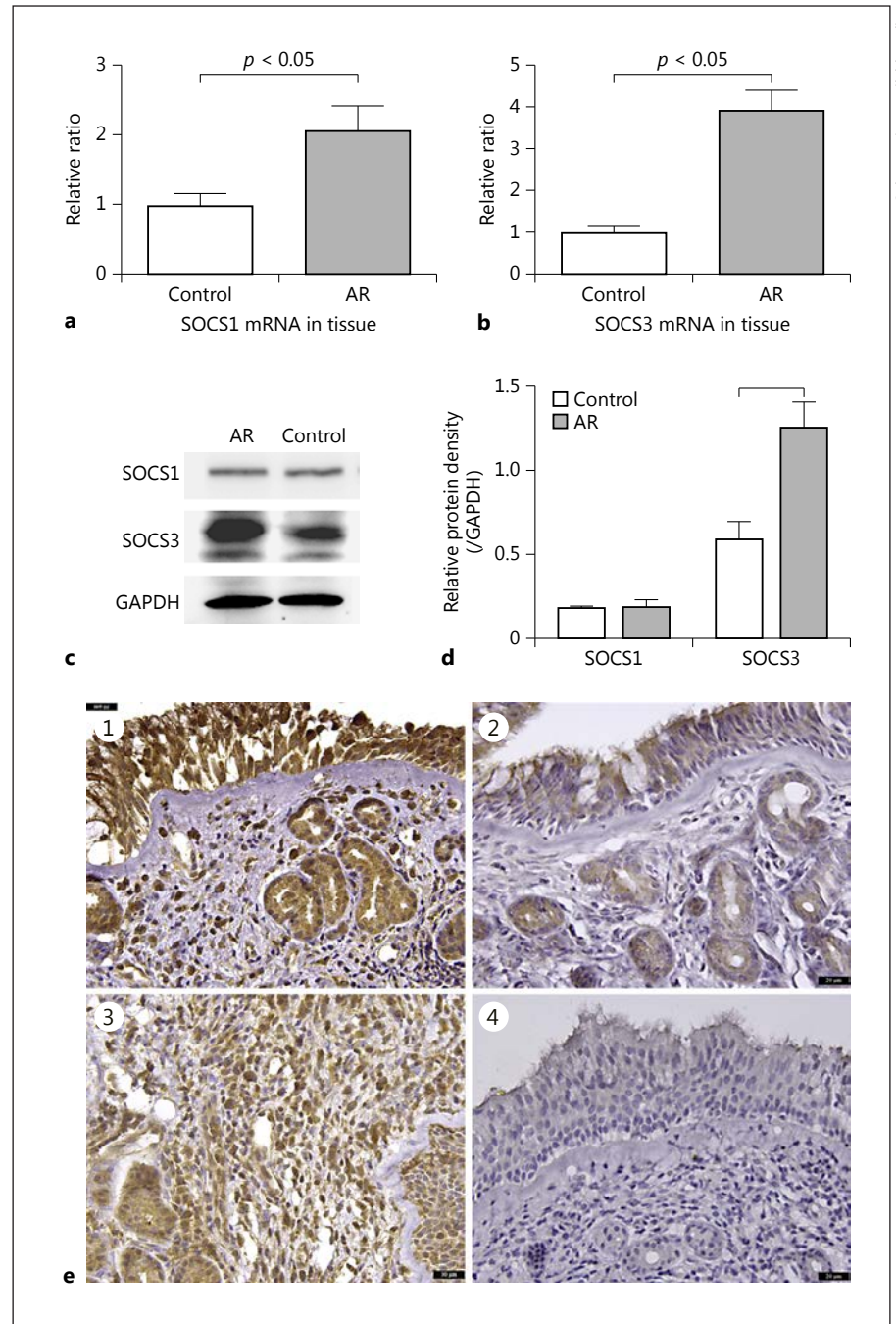


Fig. 2. The mRNA expression levels of SOCS1 (**a**) and SOCS3 (**b**) were detected in the nasal mucosa of AR by real-time PCR. **c, d** The protein levels of SOCS1 and SOCS3 were detected in the nasal mucosa of AR by Western blot. **e** Immunohistochemical staining of SOCS3 in the nasal mucosa of AR subjects (1) and controls (2); the expression of SOCS3 protein was localized in the large number of inflammatory cells (3); the expression of SOCS1 protein was not specially expressed in the nasal mucosa of AR (4).

cytoplasm of the serous glands but not in the mucous glands. There was no significant difference in the superficial epithelium, submucosal glands, and vascular endothelium between AR patients and control subjects. However, in allergic mucosa, the expression of SOCS3 protein was exclusively localized in the inflammatory cells, such as eosinophils, monocytes, and lymphocytes (Fig. 2e, 3). The expression of SOCS1 had no specific staining in the

nasal epithelium, glands, and vascular and inflammatory cells of the AR patients and control subjects (Fig. 2e, 4).

Expression Levels of miRNA in AR

By real-time PCR, miRNA155 was not detectable in the nasal mucosa of AR patients or the controls. However, the expression of miRNA155 was significantly higher in PBMCs of AR patients than the control patients. On the

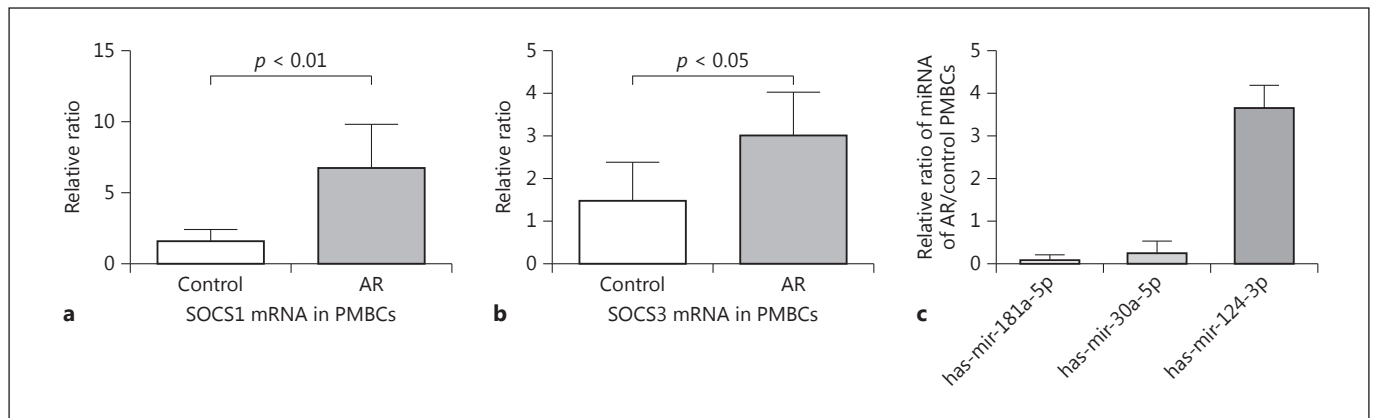


Fig. 3. The expression of SOCS1 (a) and SOCS3 (b) mRNA in PMBCs of AR patients and healthy controls, and the expression of miRNA in correlation with SOCS3 in PMBCs of AR patients (c). Data were averaged from 3 independent experiments.

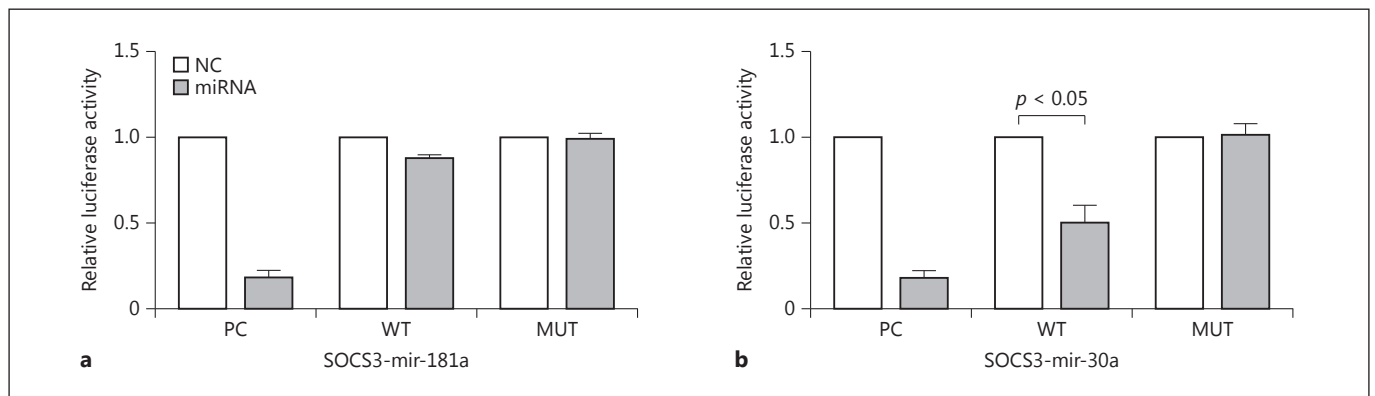


Fig. 4. Luciferase analysis in HEK293 cells. The luciferase activity of HEK293 cells was minimally decreased after cotransduction of the miR3181a-5p (a) expression vector and a 3'UTR vector containing the SOCS3/miR181a-5p target sequence, but was significantly decreased after cotransduction of the miR30a-5p (b) expression vector and a 3'UTR vector containing the SOCS3/miR30a-5p target sequence. Data were averaged from 3 independent experiments. NC, negative control; PC, positive control; WT, wild-type; MUT, mutant type.

basis of the predicted results of TargetScan Human, we detected the expression of miR455, miR124-3p, miR218-5p, miR181a-5p, miR222-3p, and miR30a-5p in the nasal mucosa of AR patients and the controls. The expression of miR455 and miR218-5p was not detected. The expression levels of miR124-3p, miR181a-5p, miR222-3p, and miR30a-5p are demonstrated in Figure 1b. MiR-124-3p and miR222-3p were upregulated in AR, while miR181a-5p and miR30a-5p were downregulated in AR (Fig. 1b). The expression of miR-124-3p was higher in PMBCs of AR subjects in comparison with controls. The expression of miR181a-5p and miR30a-5p were lower in PMBCs of the AR patients when contrasted with the controls (Fig. 3c).

SOCS3 Is Targeted by miRNA30a-5p

According to the higher expression of SOCS3 and lower expression of correlative miRNAs, we applied luciferase assays to confirm miRNA that targets SOCS3 in AR. We found that luciferase activity of HEK293 cells was significantly decreased after cotransduction of the miR30a-5p expression vector and a 3'UTR vector containing the SOCS3/miR30a-5p target sequence (Fig. 4b). Although miR181a-5p had the same effect of combination with SOCS3, the inhibition of luciferase activity was not statistically different (Fig. 4a).

We detected the expression of protein SOCS1 and SOCS3 after transfecting HEK293 cells with miR30a-5p mimic. SOCS3 was inhibited significantly by miR30a-5p

Fig. 5. a, b The expression of SOCS1 and SOCS3 were detected by Western blot in HEK293 cells transfected by miR30a-5p mimic.

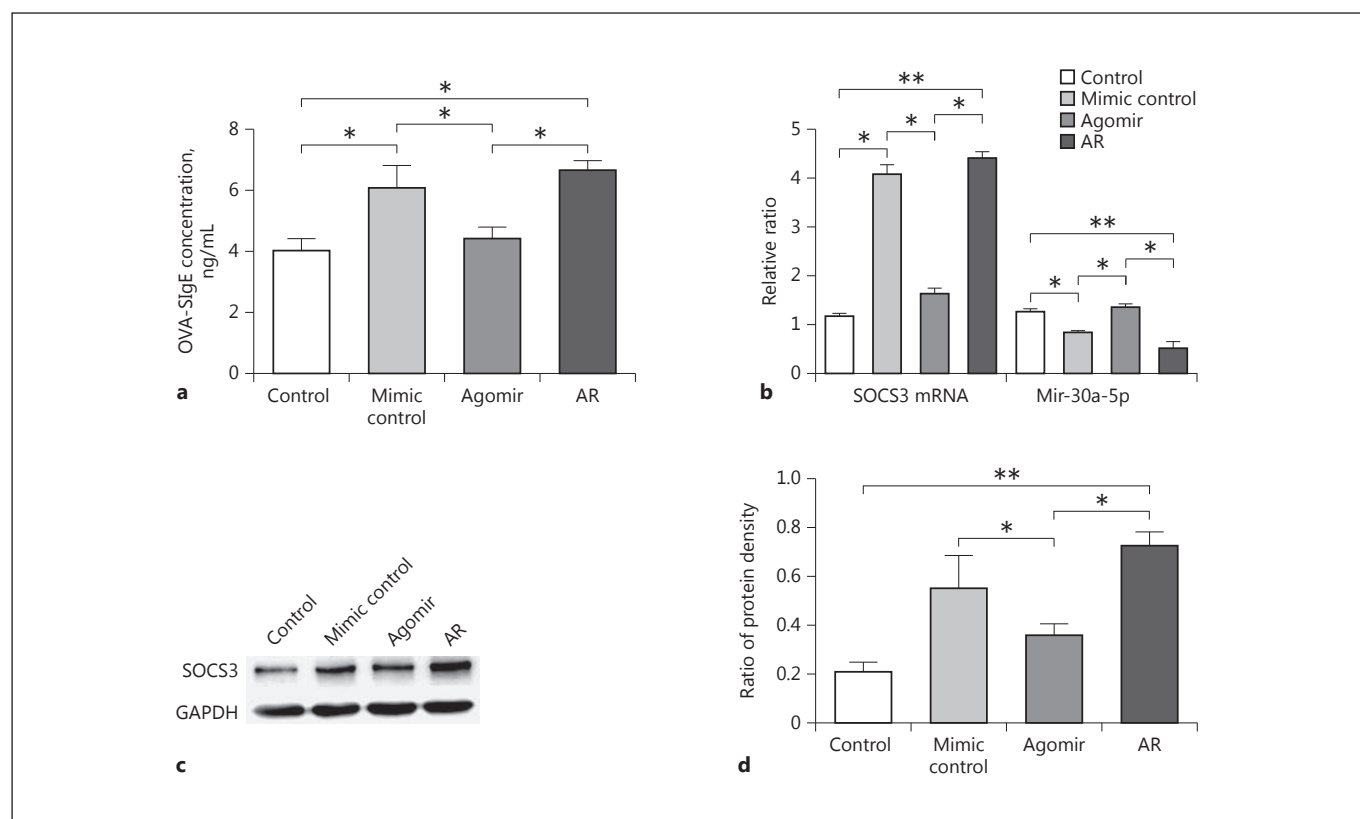
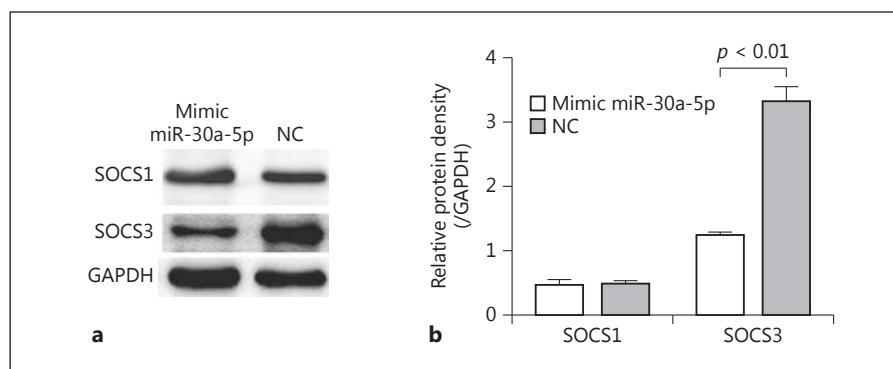


Fig. 6. The inhibition of miR30a-5p on SOCS3 in an AR mouse model. **a** The serum OVA-sIgE concentrations were detected by ELISA in the control, mimic control, agomir, and AR groups 24 h after the last nasal cavity challenge. **b** The relative expression of SOCS3 mRNA and miR30a-5p was detected by real-time PCR. **c, d** Western blot analysis was used to detect the relative protein expression levels of SOCS3 ($n = 10$ mice/group). * $p < 0.05$, ** $p < 0.01$. Data were averaged from 3 independent experiments. OVA, ovalbumin; AR, allergic rhinitis.

in contrast to negative controls (Fig. 5a, b). However, the expression of SOCS1 was not significantly different between miR30a-5p mimic transfection and the negative controls (Fig. 5a, b). Thus, we inferred that SOCS1 was not targeted by miR30a-5p and SOCS3 was targeted specifically by miR30a-5p.

Furthermore, we performed animal experiments in order to validate that SOCS3 was specifically targeted by miR30a-5p in AR. The AR mice were successfully induced by OVA with such symptoms as nose scratching, sneezing, and watery nasal discharge. The level of total OVA-sIgE in the serum was significantly upregulated in the AR mice

compared to the control mice (Fig. 6a). Following the intervention with agomiR30a-5p and miR30a-5p mimic control, it was demonstrated that the serum OVA-sIgE concentration in the agomir group was significantly lower than that in the mimic control and AR groups.

In the AR mouse model experiment, the expression of SOCS3 mRNA and protein was significantly higher in the AR group than the control group (Fig. 6b–d). The expression of miR30-5p was significantly lower in the AR group than the control group (Fig. 6b). These results were consistent with the results in AR patients.

Following the intervention of agomiR30a-5p, the expression levels of miR30a-5p were significantly higher in the agomir group than those of the mimic control and AR groups (Fig. 6b). The expression of SOCS3 was significantly lower in the agomir group than in the control and AR groups (Fig. 6b–d).

Discussion

AR is initiated by the inhalation of specific allergens and is the inflammation resulting from a cascade of reactions by the inflammatory cells, including eosinophils, lymphocytes, mast cells, basophils, and macrophages. The pathophysiologic mechanism of AR is a combination of early- and late-phase inflammatory reactions. The vast array of inflammatory mediators and cytokines are released, which lead to the symptoms of AR. Th2 cytokines, including IL-4, IL-5, IL-9, and IL-13, are fundamental to the pathogenesis of AR. Recent studies have demonstrated that upregulated Th17 cytokines are also involved with the pathogenesis of AR [15–17]. Moreover, epithelial cell-derived cytokines like TSLP, IL-33, IL-25, and innate lymphoid cells play a crucial role in the regulation of AR [18]. The cytokine network coordinates the inflammatory processes underlying AR. Modulating this network with biological therapy will provide a new method for the treatment of AR.

The SOCS protein family represents a novel group of cytoplasmic negative feedback regulators. The SOCS proteins have similar structures and negatively regulate cytokine signaling by at least 3 distinct mechanisms [3]. First, SOCS proteins can directly bind to and inactivate tyrosine kinases. Second, they bind to cytokine receptors and can occupy docking sites otherwise available for signal transduction mediators such as STATs. Third, they function as components of E3 ligases that regulate the ubiquitination and subsequent proteasomal degradation of target substrates [3].

Recent studies have predominantly focused on SOCS1 and SOCS3. It has been shown that SOCS1 can act in an autotfeedback loop to regulate IL-4 or IFN- γ signaling and is critical for regulating cytokines that balance Th1 and Th2 development [19]. SOCS1 deficiency can suppress Th17 differentiation, and also plays an important role in the regulation of Tregs [14, 20]. The research has indicated that transgenic SOCS3 expression in T cells inhibits Th1 development and promotes Th2 development [21]. Several studies have also demonstrated that the absence of SOCS3 has dramatic proinflammatory effects by promoting Th17 development [22–24].

It has been indicated that SOCS1 and SOCS3 are associated with the chronic inflammatory response in various diseases. The levels of SOCS1 and SOCS3 are significantly increased in PBMCs from patients with rheumatoid arthritis compared with healthy volunteers [25]. The methylation of SOCS1 can be detected in the serum of HLA-B27+ ankylosing spondylitis patients [26]. Studies have shown the high levels of SOCS3 in colon epithelial cells in a mouse model of intestinal bowel disease as well as in patients with ulcerative colitis and Crohn disease [27, 28]. The expression levels of SOCS1 and SOCS3 were increased in chronic rhinosinusitis [29], which may be involved with the pathogenesis of the inflammation in the sinus mucosa.

SOCS1 and SOCS3 have also gained much attention in allergic disorders, such as asthma, atopic dermatitis, AR, and food allergy. SOCS1 and SOCS3 were highly expressed in patients with psoriasis or allergic dermatitis [30, 31]. The functional genetic polymorphism of SOCS1 is involved with the development of adult asthma [32]. The overexpression of SOCS3 has been detected in the mouse allergic conjunctivitis and asthma models [33, 34]. SOCS3 plays an important role in regulating the onset and maintenance of Th2-mediated allergic diseases [35].

The expression of SOCS1 and SOCS3 has been demonstrated in the upregulation in AR. SOCS1 and SOCS3 mRNA and protein expression was significantly increased in the nasal mucosa and PBMCs of patients with mild and moderate/severe persistent AR compared with healthy controls [36]. In our study, SOCS1 and SOCS3 mRNA were also highly expressed in the nasal mucosa and PBMCs in AR patients. However, only SOCS3 showed higher protein expression in AR, which is not consistent with the previous study. By immunohistochemical detection, we found that SOCS1 showed nonspecific expression and SOCS3 was similarly localized in the epithelium, submucosal glands, and vascular endothelium in the nasal mucosa of AR and controls. Furthermore, in AR patients, the expression of SOCS3 protein was intensively localized in

the inflammatory cells below the epithelium, such as eosinophils, monocytes, and lymphocytes, which may result in a higher protein expression compared with healthy controls. These inflammatory cells play an important role in cytokine release in the pathophysiology of AR.

Cytokines, including ILs, IFNs, and hematopoietic growth factors activate the Janus kinase (JAK)-signal transducer and activator of transcription (STAT) pathway. The discovery of the SOCS proteins appeared to have identified an important mechanism in the negative regulation of the cytokine-JAK-STAT pathway [37]. SOCS3 is a strong inhibitor of JAKs, and also an important regulator of Th cell differentiation and functions. SOCS3 is selectively expressed in Th2 cells and tightly correlates with the severity of the allergic diseases. The extent of IgE, Th2 cytokines, and infiltration of eosinophils into bronchoalveolar lavage fluid was enhanced in SOCS3-transgenic mice, which indicated that high SOCS3 expression skews Th cell differentiation toward Th2 cells [35]. In asthma and nonasthmatic eosinophilic bronchitis, the expression of SOCS3 in eosinophils and CD4 T cells from patients was higher than in healthy subjects [38]. It has been shown that treatment with anti-SOCS3 antibody significantly decreased ovalbumin-specific antibodies and neutrophil infiltration in ovalbumin-induced AR mice [39]. SOCS3 can also regulate macrophages and dendritic cells, which play an important role in the innate and adaptive immunity [40, 41]. AR is antigen-induced and IgE-mediated inflammation, which is involved with the vast majority of inflammatory cells and cytokines. In our results, SOCS3 showed a high expression in the epithelium, vascular endothelium, and inflammatory cells, which confirmed its important role in allergic inflammation.

In our results, SOCS3 was inclined to be expressed in the cytoplasm of the mucous glands rather than the serous glands, which may indicate that SOCS3 is related with mucus secretion in AR. Although SOCS1 had a high expression in the mRNA level in AR, it showed no specific expression in the protein level in AR. Therefore, we deduce that SOCS3 is the main cytokine regulator in the development of AR.

Recent studies have shown that dysregulation of miRNAs is associated with inflammatory diseases, including allergy [12, 42, 43]. Some key miRNAs play an important role in the pathogenesis of AR by influencing Th differentiation, the cytokine pathways, and mucin secretion [8–11]. SOCS1 and SOCS3 are ideal therapeutic targets for inflammatory diseases, including allergy. Identification of miRNAs targeting SOCS1 and SOCS3 may

provide a new strategy for the treatment of AR. Several studies have shown that SOCS1 is the target of miR155 in allergic disease [13, 14, 44, 45]. In order to elucidate the relationship of miR155 and SOCS1 in AR, we detected the expression of miR155 and SOCS1. In the mRNA expression level, both miR155 and SOCS1 are overexpressed in PBMCs of AR patients. MiR155 was not detected in the nasal mucosa of AR, and SOCS1 was still overexpressed in the nasal mucosa of AR. The expression of miR155 and SOCS1 has no negative relationship, which is not coincident with the expression tendency of miRNA and target genes. Furthermore, in the protein level, the expression of SOCS1 is nonspecific and no significant difference was found in the nasal mucosa of AR subjects compared with controls. Thus, SOCS1 may not play an important role in the pathogenesis of AR.

In order to identify miRNA targeting SOCS3, we used TargetScan Human to predict the upstream regulatory miRNAs. By quantitative real-time PCR, we found that the expression of miR-124-3p and miR222-3p were higher in AR, while the expression of miR181a-5p and miR30a-5p were lower. The luciferase assay results revealed a strong repression of luciferase activity after transfection with miR30a-5p and wild-type 3'UTR of SOCS3. In the AR mouse model experiment, agomiR30a-5p reduced the serum OVA-sIgE concentration, and downregulated the mRNA and protein levels of SOCS3. Recent studies have shown that miR30a is a potent inhibitor of autophagy [46] and related to the pathogenesis of several tumors. Overexpression of miR30a-5p suppressed the proliferation, migration, and invasion of breast cancer cells [47]. miR30a was also found to be a significantly downregulated miRNA and may function as a novel tumor suppressor in castration-resistant prostate cancer [48]. Our study demonstrated that miR30a-5p may regulate inflammation by targeting SOCS3 in AR, which could provide a new therapy for AR. A related study has found that SOCS3 overexpression enhanced the expression of miR124-3p and vice versa in chronic myeloid leukemia [49]. Our study has shown that miR124-3p was significantly upregulated in the nasal mucosa and PBMCs of AR, while SOCS3 mRNA and protein were highly expressed in AR. A subsequent study will be performed to confirm the regulation of miR30a-5p by targeting SOCS3 for the allergic inflammation and the relationship between miR124-3p and SOCS3 in AR. The identification of a regulatory role for miRNAs in the inflammation of AR may not only illuminate the pathogenesis of AR, but also provide new insights into the diagnosis and development of a new therapeutic strategy.

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Disclosure Statement

All authors contributed in a substantive and intellectual manner to the work described and declare no conflicting financial interests.

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