

Salidroside Attenuates Airway Inflammation and Remodeling via the miR-323-3p/SOCS5 Axis in Asthmatic Mice

Xi Ming^{a, b} Xingzhu Yu^b Jijun Li^c Junyu Wang^d Jialin Zheng^e Lei Xiong^b

^aDepartment of Pediatrics, Department of Integrative Medicine on Pediatrics, No. 1 Hospital Affiliated Yunnan University of Chinese Medicine, Kunming, China; ^bFirst Clinical Medical College, Yunnan University of Chinese Medicine, Kunming, China; ^cDepartment of Integrative Medicine on Pediatrics, Shanghai Children's Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China; ^dDepartment of Anesthesiology, Yan'an Hospital Affiliated to Kunming Medical University, Kunming, China; ^eFirst Clinical Medical College, Shandong University of Traditional Chinese Medicine, Jinan, China

Keywords

Asthma · Salidroside · Airway inflammation · Airway remodeling · miR-323-3p/SOCS5

Abstract

Introduction: Salidroside (Sal) a bioactive component extracted from *Rhodiola rosea* is remarkable for its anti-asthmatic effects. The study aimed to explore the molecular mechanism of Sal in airway inflammation and remodeling in asthmatic mice and provide a novel theoretical basis for asthma treatment. **Methods:** An asthmatic mouse model was established via ovalbumin (OVA) treatment, followed by injection of Sal and transfection of miR-323-3p-mimic and sh-suppressor of cytokine signaling 5 (SOCS5). Expressions of miR-323-3p, SOCS5 mRNA, collagen (COL)-I, and COL-III were detected via reverse transcription quantitative polymerase chain reaction. SOCS5 protein level was detected via Western blot. Levels of IgE, IL-13, IL-4, and IL-5 were detected via enzyme-linked immunosorbent assay. Inflammatory cell infiltration was observed via hematoxylin-eosin staining. Collagen disposition was observed via Masson staining. Resistance index (RI) of airway hyperresponsiveness, and the number of total cells, inflammatory cells (eosinophil, macro-

phage, neutrophil, and lymphocyte) in bronchoalveolar lavage fluid (BALF) were observed. The binding relationship between miR-323-3p and SOCS5 was predicted through the RNA22 website and verified via dual-luciferase reporter assay. **Results:** miR-323-3p was highly expressed in OVA-treated mice. Sal treatment reduced inflammatory cell infiltration, COL disposition, miR-323-3p expression, and IgE, IL-13, IL-4, IL-5, COL-I, and COL-III levels, RI value, and the number of total cells and inflammatory cells in BALF. miR-323-3p inhibited SOCS5 transcription. miR-323-3p overexpression or SOCS5 downregulation reversed the protecting role of Sal in asthmatic mice. **Conclusion:** Sal inhibited miR-323-3p expression to promote SOCS5 transcription, thereby attenuating airway inflammation and remodeling in asthmatic mice.

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Introduction

Asthma is a chronic respiratory disease, prevailing in 334 million populations globally [1]. It is characterized by intermittent and reversible airway compression and in-

Edited by: A. Haczku, Sacramento, CA.

flammation in response to infection, environmental allergens, pollutants, and drug toxicity [2, 3]. The clinical management of asthma is challengeable due to the heterogeneity in clinical presentations, including episodic wheezing, breathing difficulty, chest tightness, cough, and diaphoresis [4]. Despite nonspecific asthma symptoms, airway inflammation, and remodeling are major domains in the pathogenesis of asthma [5]. Airway inflammation is presented with pro-inflammatory cytokines secreted by T helper cells, eosinophilic infiltration, and immunoglobulin (Ig) E synthesis [6, 7]. Airway remodeling refers to changes in composition, content, structure, and thickness of the airway wall, which relates to inflammatory cell infiltration and deposition of collagen (COL) [8, 9]. Emerging evidence has shed light on the role of therapeutic targets for airway inflammation and remodeling in asthma treatment [10].

Salidroside (Sal) is a bioactive tyrosol glucoside extracted from *Rhodiola rosea*, which has been reported to display pharmacological activities, including anti-inflammation, antitumor, and neuroprotection [11]. Intriguingly, Sal prevents airway inflammation and remodeling via modulating inflammatory signaling pathways, proliferation, migration, and extracellular matrix deposition in airway smooth muscle cells [11, 12]. However, the mechanism of Sal in asthmatic mice remains unclear.

microRNAs (miRNAs) are generally a class of small noncoding RNAs and play a potentially significant role in the pathology of asthma [13]. Sal could regulate expression patterns of miRNAs in various diseases, such as miR-138 and miR-133a [14, 15]. miR-323-3p emerges as a crucial gene in response to inflammation and immune systems [16]. Moreover, miR-323-3p is specifically expressed in peripheral blood mononuclear cells of asthmatic patients and impairs T-cell function, thus driving asthma progression [17]. Additionally, miR-323-3p is evidenced to have a binding relationship with the suppressor of cytokine signaling 5 (SOCS5) via dual-luciferase reporter assay in the present research. The SOCS family, consisting of 8 proteins functions as cytokine signaling inhibitors and affects the functions of Th1 and Th2 cells [18]. Notably, SOCS5 abundant in Th1 cells, and limiting Th2 cell differentiation is involved in the initiation of allergic immune diseases, including asthma [19]. SOCS5 expression is diminished via the rs6737848 polymorphism and further inhibited the interleukin (IL)-4-mediated inflammatory response, consequently alleviating asthma [20]. Nevertheless, the regulatory relationship between Sal and miR-323-3p/SOCS5 in asthma has not been reported at home and abroad.

Based on the above information, we hypothesize that Sal could alleviate airway inflammation and remodeling via regulating the miR-323-3p/SOCS5 axis. Therefore, this study set out to confirm the role of Sal as a novel therapeutic target in asthma management.

Materials and Methods

Ethics Statement

The animal experiment followed the Guidelines for the Use and Management of Laboratory Animals [21] and was approved by the Laboratory Animal Ethics Committee of Yunnan University of Chinese Medicine (YN202002028).

Asthmatic Mouse Model Establishment

Female BALB/c mice (4–6 weeks, 18–22 g) (SYXK [Shanghai] 2017-0008) purchased from Shanghai SLAC Experimental Animal Co., Ltd, were raised in separate cages in a specific pathogen-free room at 22°C–25°C. Mice were randomly allocated into 8 groups: sham group, ovalbumin (OVA) group, saline group, Sal group, Sal + mimic-NC group, Sal + miR-323-3p-mimic group, Sal + sh-NC group, and Sal + sh-SOCS5 group. The animal treatment was performed in accordance with previous research [22]. Mice were sensitized and challenged using an injection of OVA (Sigma, St. Louis, MO, USA). At 0 day and 14th day, each mouse was injected with 0.2 mL phosphate buffer saline (PBS) containing 20 µg OVA and 20 mg Al (OH)₃ (Thermo, Waltham, MA, USA). On the 27th, 28th, 29th, and 30th days, each mouse inhaled 1% aerosolized OVA/PBS for 20 min. Mice in the sham group were subjected to the same treatments, except the same volume of PBS rather than 1% OVA/PBS. In the Sal group, 1 h before each stimulation, each mouse was intraperitoneally injected with Sal (50 mg/kg, Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) dissolved in 0.9% saline every 24 h, [23]. Mice in the saline group were injected with the same volume of saline. On the 27th day, on the basis of Sal treatment, mice in the Sal + mimic-NC group were injected with lentiviral vectors containing mimic-NC; mice in the Sal + miR-323-3p-mimic group were injected with lentiviral vectors containing miR-323-3p-mimic; mice in the Sal + sh-NC group were injected with lentiviral vectors containing sh-NC; and mice in the Sal + sh-SOCS5 group were injected with lentiviral vectors containing sh-SOCS5. Lentiviral vectors were purchased from Shanghai GenePharma Co., Ltd (Shanghai, China) and injected via caudal vein at the titer of 5×10^7 TU/mL and volume of 2×10^7 TU [24].

AHR Determination

As described in prior research [25], upon the last time of OVA stimulation, mice were anesthetized with 1% pentobarbital sodium (40 mg/kg) and ventilated using a machine. The AHR was determined using the animal lung function instrument (Buxco Electronics, Troy, NY, USA). Simply put, methacholine was delivered at increasing concentrations (ranging from 3.125 to 50 mg/mL) by connection to a nebulizer. Baseline airway resistance was assessed using aerosolized PBS. Then, the total lung and airway resistance index (RI) was calculated by using an instrument.

Detection of Inflammatory Cells in BALF

At 24 h after AHR determination, mice were euthanatized using pentobarbital sodium (intraperitoneal injection, 100 mg/kg) and perfused with 0.5 mL PBS through endotracheal intubation 3 times (1.5 mL in total), followed by collection of bronchoalveolar lavage fluid (BALF). The number of total cells in BALF was measured using a hemocytometer. The cell smears were prepared using centrifugation (Cytospin 3; Thermo Scientific, Pittsburgh, PA, USA) and visualized using the Diff-Quik staining assay kit (Sysmex Co., Kobe, Japan). At least, 200 cells per slide were counted under a light microscope. Inflammatory cells, including macrophage (mac), eosinophil (eos), neutrophil (neu), and lymphocyte (lym) were characterized and numbered according to the morphological criteria and staining features.

H&E Staining

After acquisition of BALF, lung tissues were perfused with 0.1 M PBS (Solarbio) containing 4% paraformaldehyde, formalin (10%, Sigma)-fixed, and paraffin-embedded. Paraffined sections were cut into slices of 5 μ m in thickness. Sections were stained with hematoxylin (Solarbio, Beijing, China) and eosin (Sangon, Shanghai, China), and observed under Leica DM 2500 fluorescence microscope (Buffalo Grove, IL, USA) to evaluate histological changes and cellular infiltration.

Masson Staining

The accumulation of COL protein was determined via Masson trichrome staining. Briefly, sections of lung tissues were cultured in hematoxylin solution (Solarbio) for 6 min, followed by 1 min incubation with ponceau (Solarbio) and acid fuchsin solution (Solarbio) and then 5 min incubation with phosphomolybdic acid (Solarbio). After re-staining with aniline blue solution (Solarbio) for 5 min, sections were photographed using an Olympus microscope.

Enzyme-Linked Immunosorbent Assay

According to the manufacturer's protocols, levels of IL-13 (SEA060Mu), IL-4 (SEA077Mu), and IL-5 (SEA078Mu) in BALF were determined using enzyme-linked immunosorbent assay (ELISA) kits (USCN, Hubei, China). Similarly, OVA (CSB-E08914m)-specific IgE in serum samples was assessed using an ELISA kit (CUSABIO, Hubei, China).

Reverse Transcription Quantitative Polymerase Chain Reaction

Total RNAs were extracted from lung tissues using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The total RNA was reverse transcribed into complementary DNA for detection of miR-323-3p expression, followed by analysis through Applied Biosystems 7500 Fast Real-Time PCR system (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and TaqMan MicroRNA (Applied Biosystems; Thermo Fisher Scientific, Inc). complementary DNA was synthesized from total RNA using M-MLV reverse transcriptase (Takata, Dalian, Liaoning, China) followed by qPCR using SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) in the same PCR system to determine expressions of SOCS5, COL- β , and COL- α 1. Primers of PCR were synthesized by Sangon Biotech (Shanghai) Co., Ltd (shown in Table 1). The relative gene expression was measured using the $2^{-\Delta\Delta Ct}$ method. $\Delta\Delta Ct$ = (mean Ct value of target genes from the experiment group – mean Ct

value of housekeeping genes from the experiment group) – (mean Ct value of target genes from the control group – mean Ct value from housekeeping genes of the control group).

Western Blotting

Proteins were extracted using RIPA lysis buffer (Beyotime, Shanghai, China), incubated with protease inhibitor (Thermo Fisher Scientific), and quantified using the Bicinchoninic Acid Protein Assay kits (Beyotime). Then, proteins were separated using sodium dodecyl sulfate-polyacrylamide gel and transferred to the polyvinylidene fluoride membranes (Millipore, Billerica, MA, USA). After blockage with 5% skim milk, the membranes were incubated with the primary antibodies SOCS5 (1:1,000, LS-C409498, LifeSpan Biosciences, Seattle, WA, USA) and GAPDH (1:1,000, ab8245, Abcam) at 4°C overnight, washed 5–6 times with PBS containing Tween-20 and then re-cultured with horseradish peroxidase-conjugated secondary antibody IgG (1:2,500, ab6728, Abcam). Ultimately, proteins on membranes were visualized using Pierce™ ECL Western blotting substrate (Thermo Fisher Scientific) and quantified using Image Lab software (Bio-Rad, Hercules, CA, USA).

Dual-Luciferase Reporter Assay

The binding sites between miR-323-3p and SOCS5 were predicted through the RNA22 (<https://cm.jefferson.edu/rna22/Interactive/>). Wild type 3'-UTR sequence of SOCS5 containing miR-323-3p binding sites and mutant type SOCS5 3'UTR were cloned into psiCHECK-2 luciferase reporter vectors (Promega, Madison, WI, USA). The above vectors and miR-323-3p-mimic/mimic-NC were co-transfected into 293T cells. At 48 h after transfection, cells were collected, followed by detection of the luciferase activity using dual-luciferase report detection system (Promega).

Statistical Analysis

GraphPad Prism 8.0 software (GraphPad Software Inc., San Diego, CA, USA) was used for data analysis. Measurement data were represented as mean $\pm$ standard deviation. Pairwise comparisons were analyzed using one-way or two-way analysis of variance followed by Tukey's post hoc test. A value $p < 0.05$ was considered statistical significance.

Results

Sal Attenuated Airway Inflammation and Remodeling in Asthmatic Mice

To analyze the therapeutic mechanism of Sal in asthma, mice were treated with OVA for asthmatic induction, followed by injection of Sal. The results of hematoxylin and eosin staining showed that compared with the sham group, there were inflammatory cell infiltration and fluid extravasation around blood vessels and pulmonary alveoli, leading to impaired alveolar wall and congestion in lung tissues in OVA-treated mice; compared with the saline group, lung tissue structure was restored, inflammatory cell infiltration was reduced and inflammatory exudate was vanished in the

Sal group ($p < 0.05$, Fig. 1a). The results of Masson staining showed that compared with the sham group, COL deposition was increased in the OVA group and decreased in the Sal group ($p < 0.05$, Fig. 1b). The results of reverse transcription quantitative polymerase chain reaction (RT-qPCR) showed that compared with the sham group, the mRNA levels of COL-I and COL-III were elevated in the OVA group; compared with the saline group, the mRNA levels of COL-I and COL-III were diminished in the Sal group ($p < 0.05$, Fig. 1c). The results of ELISA showed that compared with the sham group, levels of IL-13, IL-4, IL-5, and IgE were elevated in the OVA group and reduced after Sal treatment ($p < 0.05$, Fig. 1d). Besides, compared with the sham group, the numbers of total cells and inflammatory cells (eos, mac, neu, and lym) were decreased in the Sal group ($p < 0.05$, Fig. 1e). At the higher concentration of methacholine, RI value was markedly elevated upon OVA treatment and significantly diminished after Sal treatment ($p < 0.05$, Fig. 1f). The above results indicated that Sal could attenuate airway inflammation and remodeling in asthmatic mice.

miR-323-3p Was Highly Expressed in Asthma and Inhibited by Sal

miR-323-3p was documented to be highly expressed in asthma [17]. Hence, we postulated that miR-323-3p was linked to the role of Sal in asthma. To confirm the expression of miR-323-3p in asthma and its relationship to Sal, miR-323-3p expression in lung tissues of asthmatic mice

was detected using RT-qPCR. The results showed that compared with the sham group, miR-323-3p expression was significantly upregulated in the OVA group and downregulated after Sal treatment ($p < 0.05$, Fig. 2). In brief, miR-323-3p was highly expressed in asthma and Sal inhibited the expression of miR-323-3p.

Sal Attenuated Airway Inflammation and Remodeling in Asthmatic Mice via Inhibiting miR-323-3p Expression

To explore the role of miR-323-3p in asthma, the expression of miR-323-3p was upregulated using the lentiviral vector containing miR-323-3p-mimic in the Sal group. The results of RT-qPCR showed that compared with the Sal + mimic-NC group, the expression of miR-323-3p was significantly increased in the Sal + miR-323-3p-mimic ($p < 0.05$, Fig. 3a). After upregulation of miR-323-3p, inflammatory cells exhibited around blood vessels and pulmonary alveoli with impairment of alveolar wall and congestion of lung tissues ($p < 0.05$, Fig. 3b), COL deposition was increased ($p < 0.05$, Fig. 3c), mRNA levels of COL-I and COL-III were upregulated ($p < 0.05$, Fig. 3d) and levels of IL-13, IL-4, IL-5, and IgE were elevated ($p < 0.05$, Fig. 3e). Moreover, upregulation of miR-323-3p augmented the number of total cells and inflammatory cells (eos, mac, neu, and lym) ($p < 0.05$, Fig. 3f) and increased RI value at the higher concentration of methacholine ($p < 0.05$, Fig. 3g). Taken together, Sal at-

Table 1. qPCR primers

Gene	Forward primer (5'–3')	Reverse primer (5'–3')
miR-323-3p	GCCGAGTTGGTACTTGGA	CTCAACTGGTGTCGTGGA
SOC5	GTCTGAAGCGGGAAGCTGAT	CGGACATCTGTTGGGGTTCA
COL-I	TTCTCTGGCAAAGACGGAC	CGGCCACCATCTTGAGACTT
COL-III	GAGGAATGGGTGGCTATCCG	TCGTCCAGTCTTCTCTGACT
U6	AATCACCCCATGCTGGTG	TTTGTCACTAGTGAGGGA
GAPDH	GTAGACAAAATGGTGAAG	CAAAGTGGAGATTGTTGC

SOC5, suppressor of cytokine signaling 5; COL, collagen.

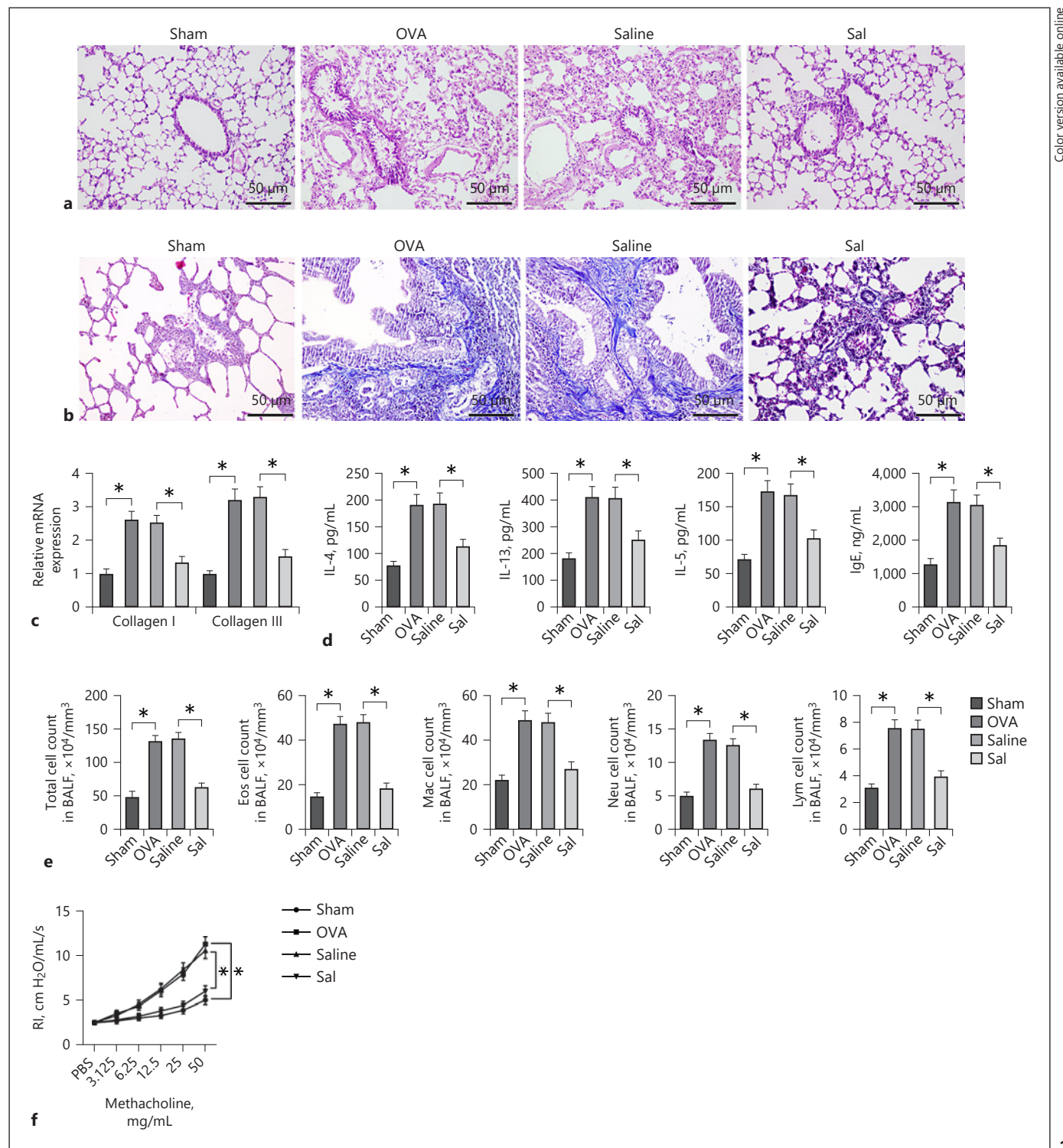
Fig. 1. Sal attenuated airway inflammation and remodeling in asthmatic mice. The asthmatic mouse model was established using OVA, followed by injection of Sal. The sham group was set as the control of the OVA group and the saline group was set as the control of the Sal group. Inflammatory cell infiltration was detected via H&E staining ($N = 6$) (a); COL deposition was detected via Masson staining ($N = 6$) (b); mRNA levels of COL-I and COL-III were detected via RT-qPCR ($N = 6$) (c); Levels of IL-13, IL-4, IL-5, and IgE were detected via ELISA ($N = 12$) (d); The number of total cells and inflammatory cells (eos, mac, neu and lym) in BALF were

numbered ($N = 12$) (e); RI value were detected ($N = 12$). Data were represented as mean $\pm$ SD (f). Data in (c, f) were analyzed using 2-way ANOVA and data in (d, e) were analyzed using 1-way ANOVA, followed by Tukey's post hoc test. * $p < 0.05$. Sal, salidroside; OVA, ovalbumin; H&E, hematoxylin and eosin; COL, collagen; RT-qPCR, reverse transcription quantitative polymerase chain reaction; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; eos, eosinophil; mac, macrophage; neu, neutrophil; lym, lymphocyte; BALF, bronchoalveolar lavage fluid; RI, resistance index; SD, standard deviation; ANOVA, analysis of variance.

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tenuated airway inflammation and remodeling in asthmatic mice via inhibiting the miR-323-3p expression and the therapeutic effects of Sal were associated with miR-323-3p.

SOCS5 Was Directly Targeted by miR-323-3p and Weakly Expressed in Asthmatic Mice
Subsequently, the downstream mechanism of miR-323-3p was analyzed. Previous research demonstrated



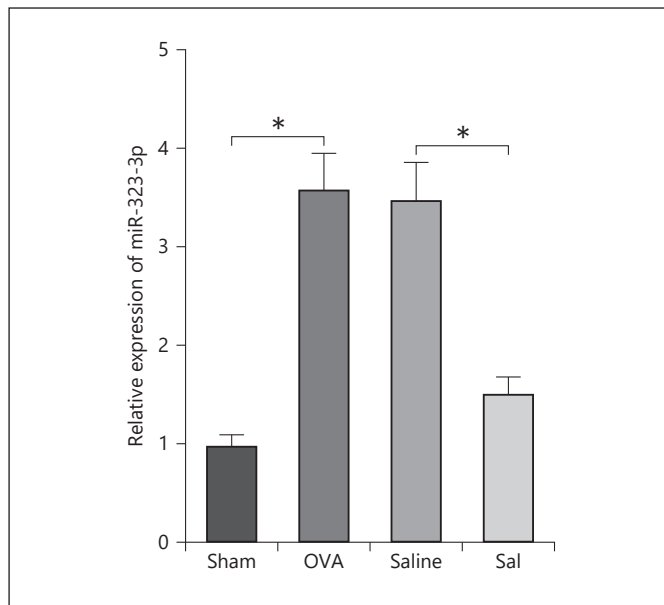


Fig. 2. miR-323-3p was highly expressed in asthma and Sal inhibited miR-323-3p expression. Asthmatic mouse model was established using OVA, followed by injection of Sal. The sham group was set as the control of the OVA group and the saline group was set as the control of the Sal group. miR-323-3p expression was detected via RT-qPCR. $N = 6$, data were represented as mean $\pm$ SD. The data were analyzed using one-way ANOVA and checked by Tukey's post hoc test. * $p < 0.05$. Sal, solidoside; OVA, ovalbumin; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SD, standard deviation; ANOVA, analysis of variance.

that SOCS5 is a target gene of miR-323-3p and is involved in the pathogenesis of asthma [19]. The binding site between miR-323-3p and SOCS5 was predicted through RNA22 (<https://cm.jefferson.edu/rna22/Interactive/>) (Fig. 4a) and verified using dual-luciferase reporter assay (Fig. 4b). The results of RT-qPCR showed that compared with the sham group, the mRNA level of SOCS5 was decreased in the OVA group and improved after Sal treatment ($p < 0.05$, Fig. 4c). Collectively, SOCS5 was directly targeted by miR-323-3p and weakly expressed in asthmatic mice.

Sal Attenuated Airway Inflammation and Remodeling in Asthmatic Mice via Inhibiting miR-323-3p to Promote SOCS5 Transcription

To explore the role of SOCS5 in asthma, the expression of SOCS5 was silenced using the lentivirus vector containing sh-SOCS5 in the Sal group. The results of RT-qPCR and Western blot showed that compared with the Sal + sh-NC group, SOCS5 levels were significantly down-

regulated in the Sal + sh-SOCS5 ($p < 0.05$, Fig. 5a, b). After SOCS5 downregulation, the alveolar wall was destroyed with congestion in the lung tissue ($p < 0.05$, Fig. 5c), COL deposition was enhanced ($p < 0.05$, Fig. 5d), mRNA levels of COL-I and COL-III were increased ($p < 0.05$, Fig. 5e) and levels of IL-13, IL-4, IL-5, and IgE were upregulated ($p < 0.05$, Fig. 5f). Additionally, SOCS5 downregulation augmented the number of total cells and inflammatory cells (eos, mac, neu, and lym) ($p < 0.05$, Fig. 5g) and increased RI value at the higher concentration of methacholine ($p < 0.05$, Fig. 5h). Altogether, SOCS5 inhibition could reverse the therapeutic effect of Sal on asthma and Sal attenuated airway inflammation and remodeling in asthmatic mice via inhibiting miR-323-3p to promote SOCS5 transcription.

Discussion

Asthma is characterized by airway inflammation and remodeling [26]. Sal plays an inhibitory role in airway inflammation and remodeling via targeting relevant factors [11, 12]. miR-323-3p and SOCS5 are also important regulators of inflammatory response in asthma [17, 19]. In this research, we highlighted that Sal could alleviate airway inflammation and remodeling via targeting the miR-323-3p/SOCS5 axis in asthmatic mice.

Initially, we established the asthmatic mouse model via inhalation of OVA. Histopathologically, we observed that OVA-treated mice exhibited inflammatory cell infiltration, fluid extravasation, impaired alveolar wall, congestion, increased number of total cells and inflammatory cells (eos, mac, neu, and lym) in lung tissues and elevated RI value at higher concentration of methacholine. Airway inflammation is associated with elevated cytokines, including IL-13, IL-4, IL-5, and IgE [27]. Additionally, airway remodeling results from COL deposition, manifested by elevated COL-I and COL-III levels [28]. Accordingly, we found the overproduction of IL-13, IL-4, IL-5, IgE, COL-I, and COL-III levels in lung tissues of OVA-treated mice. After injection of Sal, the structure of lung tissues was restored, COL deposition and inflammatory cell infiltration were inhibited, and levels of IL-13, IL-4, IL-5, IgE, COL-I, and COL-III were decreased. Previous works have shed light on the role of Sal in inflammatory responses and extracellular matrix deposition. For instance, Sal overexpression protects human umbilical vein endothelial cells from glycation-induced inflammatory injury and oxidative stress [29] and elevated Sal represses inflammatory injury of alveolar cells via mediating miRNA-

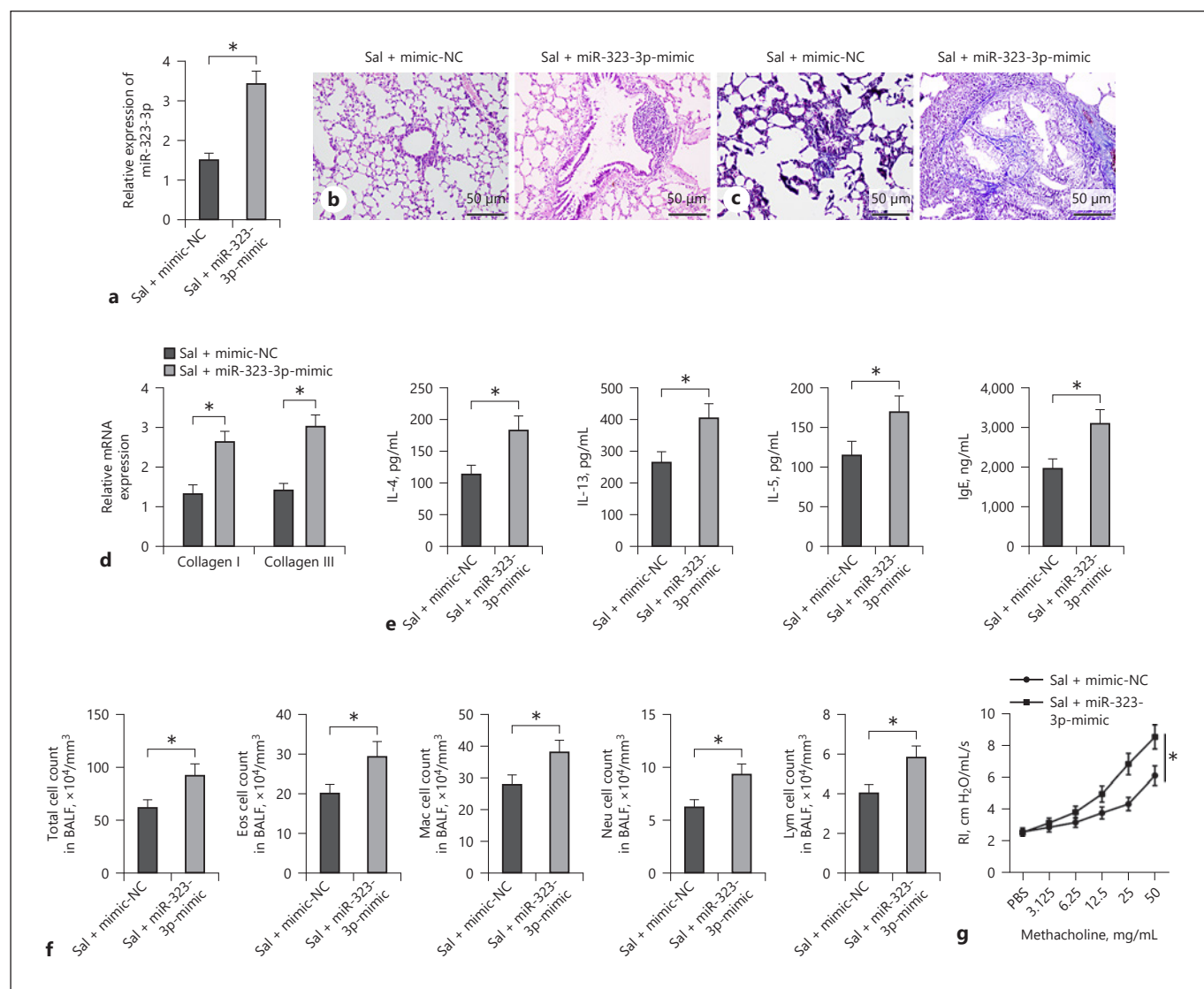


Fig. 3. Sal attenuated airway inflammation and remodeling in asthmatic mice via inhibiting miR-323-3p expression. Sal-treated asthmatic mice were injected with the lentiviral vector containing miR-323-3p-mimic, with the Sal + mimic-NC group as the control. The expression of miR-323-3p in lung tissues was detected via RT-qPCR ($N = 6$) (**a**); Inflammatory cell infiltration was detected via H&E staining ($N = 6$) (**b**); COL deposition was detected via Masson staining ($N = 6$) (**c**); mRNA levels of COL-I and COL-III were detected via RT-qPCR ($N = 6$) (**d**); Levels of IL-13, IL-4, IL-5, and IgE were detected via ELISA ($N = 12$) (**e**); The number of total cells and inflammatory cells (eos, mac, neu, and lym) in BALF were

numbered ($N = 12$) (**f**); RI value were detected ($N = 12$) (**g**). Data were represented as mean $\pm$ SD. Data in (**a**, **e**, **f**) were analyzed using the independent t test and data in (**d**, **g**) were analyzed using 2-way ANOVA, followed by Tukey's post hoc test. * $p < 0.05$. Sal, salidroside; OVA, ovalbumin; H&E, hematoxylin and eosin; COL, collagen; RT-qPCR, reverse transcription quantitative polymerase chain reaction; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; eos, eosinophil; mac, macrophage; neu, neutrophil; lym, lymphocyte; BALF, bronchoalveolar lavage fluid; RI, resistance index; SD, standard deviation; ANOVA, analysis of variance.

146a and toll-like receptor 4 inflammatory signaling pathways in acute respiratory distress syndromes [30]. Moreover, Sal reduces COL deposition via regulating the toll-like receptor 4 /NF- κ B and MAPK signaling pathways to ameliorate renal fibrosis [31]. In asthma, Sal re-

lieved airway inflammation via regulating limiting secretion of IL-33 or restoring the balance between Th1 and Th2 cytokines [12, 32]. Moreover, Sal diminished the accumulation of COL-I to inhibit proliferation and migration of airway smooth muscle cells, thereby alleviating

airway remodeling [11]. In brief, Sal alleviated airway inflammation and remodeling in asthmatic mice.

miR-323-3p is highly expressed in asthma and functions as a negative regulator of the release of IL-22 (the anti-inflammatory signaling in airway inflammation) [17]. Our results showed that the expression of miR-323-3p was upregulated in OVA-treated mice while downregulated after Sal treatment. To analyze the role of miR-323-3p in asthma, we overexpressed miR-323-3p expression in Sal-treated asthmatic mice. After miR-323-3p upregulation, inflammatory cell infiltration and

COL deposition were enhanced, and IL-13, IL-4, IL-5, IgE, COL-I, and COL-III levels were augmented, suggesting that elevated miR-323-3p exacerbated airway inflammation and remodeling. The discussion of miR-323-3p in asthma is largely limited in existing works. However, the pro-inflammatory effect of miR-323-3p is extensively discussed. For example, miR-323-3p promotes inflammation in lipopolysaccharide-induced acute lung injury and connective tissue disorder [33, 34]. Moreover, miR-323-3p overexpression upregulated the mRNA levels of COL-I and COL-III via targeting the tis-

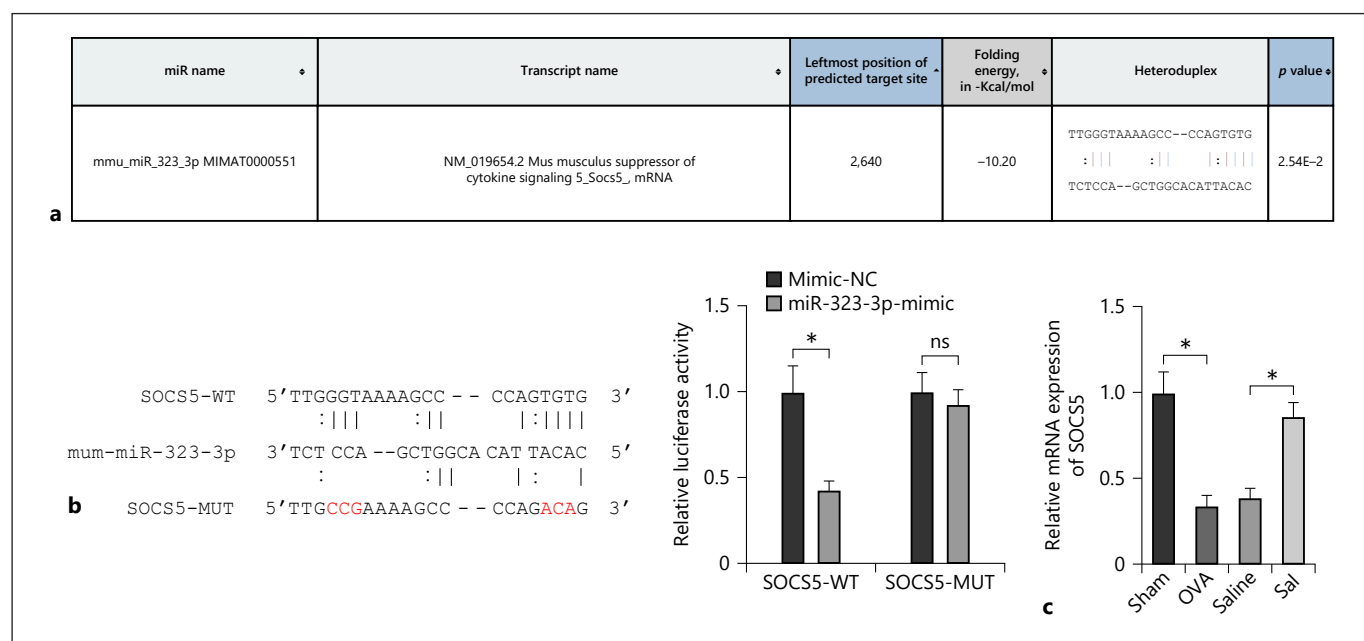


Fig. 4. SOCS5 was directly targeted by miR-323-3p and weakly expressed in asthmatic mice. The binding site between miR-323-3p and SOCS5 was predicted via RNA22 (a); the binding relationship between miR-323-3p and SOCS5 was verified via dual-luciferase reporter assay (b); the mRNA level of SOCS5 was detected via RT-qPCR (c). $N = 6$, data were represented as mean $\pm$ SD. Data in (b)

were analyzed using 2-way ANOVA and data in (c) were analyzed using one-way ANOVA, followed by Tukey's post hoc test. $*p < 0.05$. SOCS5, suppressor of cytokine signaling 5; RT-qPCR, reverse transcription quantitative polymerase chain reaction; SD, standard deviation; ANOVA, analysis of variance.

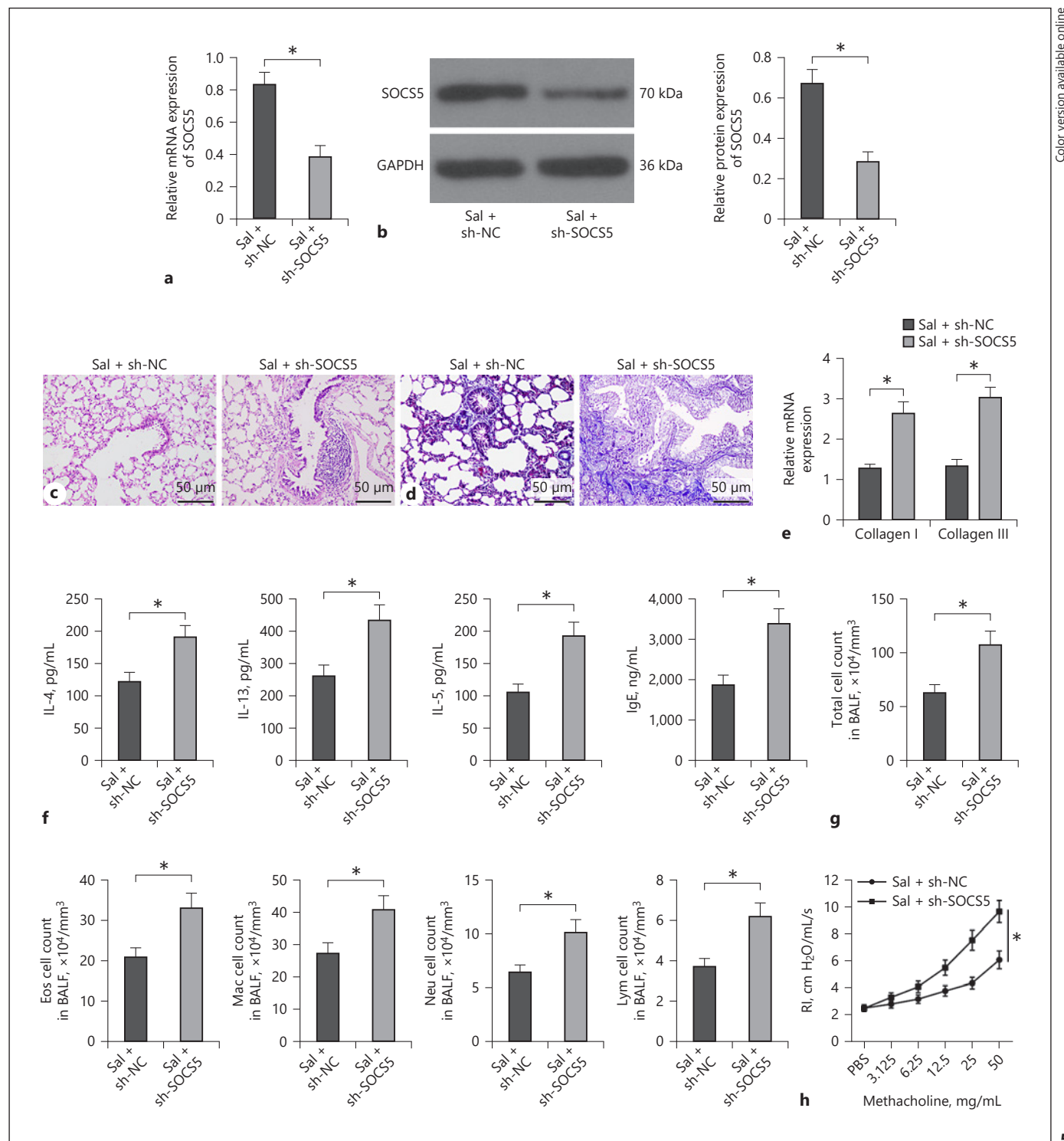
Sal attenuated airway inflammation and remodeling in asthmatic mice via inhibiting miR-323-3p to promote SOCS5 transcription. Sal-treated asthmatic mice were injected with the lentiviral vector containing sh-SOCS5, with the Sal + sh-NC group as the control. The expression of SOCS5 was detected via RT-qPCR and Western blot ($N = 6$) (a, b); Inflammatory cell infiltration was detected via H&E staining ($N = 6$) (c); COL deposition was detected via Masson staining ($N = 6$) (d); mRNA levels of COL-I and COL-III were detected via RT-qPCR ($N = 6$) (e); Levels of IL-13, IL-4 and IgE were detected via ELISA ($N = 12$) (f); The number of total cells and inflammatory cells (eos, mac, neu, and lym) in BALF

were numbered ($N = 12$) (g); RI value were detected ($N = 12$) (h). Data were represented as mean $\pm$ SD. Data in (a, b, f, g) were analyzed using the independent t test and data in (e, h) were analyzed using 2-way ANOVA, followed by Tukey's post hoc test. $*p < 0.05$. Sal, salidroside; H&E, hematoxylin and eosin; COL, collagen; RT-qPCR, reverse transcription quantitative polymerase chain reaction; IL, interleukin; ELISA, enzyme-linked immunosorbent assay; eos, eosinophil; mac, macrophage; neu, neutrophil; lym, lymphocyte; BALF, bronchoalveolar lavage fluid; RI, resistance index; SD, standard deviation; ANOVA, analysis of variance; SOCS5, suppressor of cytokine signaling 5.

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sue inhibitor of metalloproteinase 3 signaling in cardiac fibrosis [35]. Altogether, Sal alleviated airway inflammation and remodeling via inhibiting miR-323-3p expression in asthmatic mice.

Subsequently, we explored the downstream mechanism of miR-323-3p and focused on the role of SOCS5 in asthma. Enhancement of SOCS5 has been confirmed to play a protecting role in OVA-induced asthmatic mice [19]. In this



research, the binding relationship between miR-323-3p and SOCS5 was predicted through the database and confirmed via dual-luciferase reporter assay. The mRNA level of SOCS5 was significantly reduced in OVA-treated mice but upregulated after Sal treatment. Then, to analyze the role of SOCS5 in asthma, SOCS5 was downregulated in Sal-treated asthmatic mice using shRNA. SOCS5 downregulation impaired lung tissue homeostasis and reversed the protecting role of Sal in asthma, manifested by destructed alveolar wall, tissue congestion, elevated RI value, and enhanced inflammatory cell infiltration and COL deposition. SOCS overexpression attenuated vascular and systemic inflammation and COL deposition in atherosclerosis [36]. Moreover, previous research has evidenced that SOCS5 plays an inhibitory role in the release of pro-inflammatory cytokines produced by Th2 cells, consequently attenuating allergic inflammation [19, 37], and the rs6737848 polymorphism of SOCS5 elevates the risk of IL-4-mediated inflammatory response in asthma [20]. Overall, SOCS5 was a target gene of miR-323-3p and relieved airway inflammation and remodeling in asthmatic mice. Nevertheless, the mechanism of Sal targeting miR-323-3p to promote SOCS5 transcription has not been reported before, which potently proves the novelty of our study.

In summary, our results initially revealed that Sal inhibits miR-323-3p expression to promote SOCS5 transcription, thereby attenuating airway inflammation and remodeling in asthmatic mice. However, there are some limitations to this study. For instance, only female BALB/C mice were used to establish asthmatic models because female mice are more susceptible to allergic airway diseases [38]. Besides, we did not investigate the role of other miRNAs affected by Sal and other downstream target genes of miR-323-3p. In the future, we will continue to analyze the therapeutic role of Sal in other asth-

matic models, such as PM2.5 exposure-induced asthma and Sendai virus-induced asthma models, and explore its other targets to improve the integrity of our study.

Statement of Ethics

The animal experiment followed the Guidelines for the Use and Management of Laboratory Animals [21] and was approved by the Laboratory Animal Ethics Committee of Yunnan University of Chinese Medicine (YN202002028).

Conflict of Interest Statement

The authors have no conflicts of interest to declare that are relevant to the content of this article.

Funding Sources

This work was supported by National Natural Science Foundation of China (No. 82060884).

Author Contributions

X.M. and L.X. made substantial contributions to the conception of the present study. X.Y. and J.L. performed the experiments and wrote the manuscript; J.W. and J.Z. contributed to the design of the present study and interpreted the data. All authors read and approved the final version of the manuscript for publication.

Data Availability Statement

The data that support this study are available from the corresponding author upon reasonable request.

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