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RESEARCH

STC-1 alleviates airway inflammation by regulating epithelial cell apoptosis through the 5-LO pathway

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Abstract
Airway inflammation plays a key role in the pathogenesis and development of asthma. Stanniocalcin-1 (STC-1) has powerful antioxidant, anti-inflammatory and anti-apoptotic functions but its impact on the airway inflammation in asthma lacks evidence. Here, we investigated the effect and potential mechanism of STC-1 on airway inflammation through asthmatic mice model and lipopolysaccharide (LPS)-treated BEAS-2B cells. The data showed that STC-1 treatment before the challenge exerted protective effect on ovalbumin (OVA)-induced asthmatic mice, i.e., decreased the inflammatory cell infiltration, mucus secretion, cytokine levels, apoptosis levels, and p38 MAPK signaling. Additionally, STC-1 reduced 5-LO expression. Meanwhile, STC-1 decreased p38 MAPK signaling, cytokine production, mucin MUC5AC production, 5-LO expression and nuclear translocation, and LTB4 production in vitro. Ultimately, transforming growth factor β (TGF-β), as a 5-LO inducer, reversed the anti-inflammatory and anti-apoptotic effects of STC-1 in BEAS-2B cells by up-regulating 5-LO expression. It reveals the potential of STC-1 to act as an additional therapy to mitigate airway inflammation in asthma and inhibit 5-LO expression.

Keywords Asthma · Stanniocalcin-1 · Airway inflammation · Apoptosis

Introduction

Bronchial asthma is a chronic lung disease due to a complex interplay of genetic and environmental factors [1], impacting a global population of 339 million individuals [2]. Airway inflammation is a core of the pathophysiology, clinical characteristics, and susceptibility to risks in asthma [3]. Up-regulating reactive oxygen species (ROS) is a common feature in airway inflammation [4, 5], contributing to oxidative stress in the airways and reducing the ability of airway epithelial cells to repair damage [6]. The epithelium is a coordinator of airway inflammation and a therapeutic target in asthma [7]. Airway epithelial cells in asthma have produced more oxidants than healthy people [8]. Studies have shown that local lipopolysaccharide (LPS) exposure induces apoptosis in the lungs after 2 h and peaks at 24 h [9]. Persistent or recurrent airway inflammation can cause structural changes such as epithelial apoptosis, goblet cell hyperplasia, subepithelial fibrosis, and muscle cell hyperplasia [10]. Investigating the mechanisms underlying airway inflammation is of paramount importance for improving the clinical management of asthma.

Lipoxygenases, a group of redox enzymes, can be simply categorized into 5-lipoxygenase (5-LO), 12-lipoxygenase, and 15-lipoxygenase. These enzymes can catalyze the oxidation of unsaturated fatty acids, generating potent pro-inflammatory mediators such as leukotrienes [11]. Among these, leukotriene B4 (LTB4) plays a pivotal role during asthma attacks [12–14]. LTB4 mainly influences the recruitment, activation, and survival of myeloid leukocytes, including neutrophils and eosinophils [15–17]. 5-LO (gene name ALOX5) is a key enzyme in the synthesis of leukotrienes [11, 18], mediating bronchoconstriction and inflammatory modifications [19]. Besides, 5-LO can mediate the generation of ROS [20].

Stanniocalcin-1 (STC-1) is a glycoprotein hormone synthesized and secreted by bronchial or pulmonary epithelial cells, which plays an important role in the regulation of calcium and phosphate homeostasis in vivo by autocrine or paracrine mode [21]. Extensive evidence has indicated that STC-1 reduces ROS production by up-regulating mitochondrial uncoupling protein-2 [22]. Meanwhile, STC-1 inhibited

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the production of pro-inflammatory mediators by inhibiting the ROS/MAPK pathway [23, 24]. However, the influence of STC-1 on the airway inflammation in asthma remains elusive.

In this study, we applied ovalbumin (OVA)-induced asthmatic mice model and LPS-induced BEAS-2B cells to investigate the potential effect of STC-1 against asthma. Our study aims to explore the intricate mechanisms between STC-1 and airway inflammation in asthma.

Materials and Methods

In Vivo Method

Animals

Specific pathogen-free (SPF) female BALB/c mice (6-8 weeks old, 20 ± 2 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd., Shanghai, China. After a one-week of acclimation period, the mice were utilized for the experiments. Mice were accommodated in an SPF environment, maintaining constant temperature (20-24 °C), with a 12-h light-dark cycle. They had free access to food and water. All experimental procedures involving animals were ethically approved by the Laboratory Animal Center of the First Affiliated Hospital of Wenzhou Medical University, according to the Guide for the Care and Use of Laboratory Animals (No. WYYYIACUCAEC2023038).

Establishment and Treatment of Asthma Model

Four groups of twenty-four female BALB/c mice were randomly assigned as follows: normal control group (PBS, n=6), asthma model group (OVA, $n = 6$), asthma model + STC-1 (Noveprotein, China) 80 μg/kg group (OVA+STC-1, n=6), and STC-1 80 μg/kg group (STC-1, $n = 6$). As previously reported, asthmatic mice were induced through OVA (Sigma-Aldrich, USA) sensitization and challenge [25]. On days 0, 7, and 14, the mice received intraperitoneally injections of 200 μL phosphate buffered saline (PBS) mixed with 20 μg OVA and 2 mg aluminum hydroxide (Sigma-Aldrich, USA). On days 21 to 23, mice were anesthetized every day with a 1% sodium pentobarbital solution and intranasal administration was used with 50 μL OVA solution (5% OVA dissolved in PBS). Simultaneously, mice in PBS and STC-1 groups underwent a sensitization period with intraperitoneal injections of 200 μL PBS. From day 21, mice in the STC-1 treated groups received daily treatment with 20 μL STC-1 solution (80 μg STC-1 dissolved in 20 μL sterile distilled water), while mice in PBS and OVA groups received an equal volume of PBS daily via nasal administration. Mice were sacrificed one day after the last challenge with OVA and their body weights were recorded. Bronchoalveolar lavage fluid (BALF), serum and lung tissue were collected and stored at -80 °C.

Bronchoalveolar Lavage Fluid (BALF) Analysis

To collect BALF, a total of 500 μL of 4 °C PBS was injected into the trachea of mice to flush the lung tissue for three times. Subsequently, the BALF collected was centrifuged at 1000 rpm, at 4 °C for 15 min. The supernatant was carefully preserved at -80 °C for later cytokine analysis, while the cell precipitation was resuspended in 50 μL sterile PBS to count and sort inflammatory cells (Hemavet950 instrument, UK).

Detection of Cytokines

C-X-C motif chemokine ligand 1 (CXCL-1) is a homologue of human interleukin-8 (IL-8). Levels of tumor necrosis factor-α (TNF-α) and CXCL-1 in BLAF and serum, as well as TNF-α, IL-8, and LTB4 in cell supernatant were measured using ELISA kits (Neobioscience, China), following the instructions provided.

Lung Histopathology

The bronchial plane section of the middle lobe was precisely sliced into 4 μm sections and then embedded in paraffin. These samples were stained by hematoxylin and eosin (H&E) and periodic acid-schiff (PAS) staining techniques. These stained slides were viewed and the image was captured under an upright fluorescence microscope (Leica, Germany). H&E staining was used to assess the inflammation of peri-bronchial and perivascular infiltrating cells and airway structure. Pulmonary mucus secretion was evaluated by PAS staining.

In Vitro Method

Cellular Culture and Treatment

The human bronchial epithelial cell line BEAS-2B cells were obtained from the Shanghai Culture Collection of the Chinese Academy of Sciences (Shanghai, China). BEAS-2B cells were cultured in Dulbecco Modified Eagle Medium (DMEM, Gibco, USA), supplemented with 10% fetal bovine serum (FBS, Gibco, USA) and maintained at 37 °C in a humidified atmosphere of 5% CO_2. Four groups participated in this experiment. Control group: BEAS-2B cells were treated with PBS (Biosharp, China); LPS group: BEAS-2B cells were treated with 1000 ng/mL LPS (Sigma-Aldrich, USA) for 3 h; LPS+STC-1 group: BEAS-2B cells were treated with 100 ng/mL STC-1 half an hour before LPS stimulation and then 1000 ng/mL LPS for 3 h [26, 27]; LPS+STC-1+TGF-β group: BEAS-2B cells were treated with 10 ng/mL trans-

forming growth factor-β (TGF-β, GLPBIO, China) 30 min, STC-1 and LPS followed.

Cell Viability Assay

Cell viability was evaluated using the cell counting kit-8 (CCK8, Elabscience, China). Cells were inoculated into 96-well plates at a density of 2×10^3 cells per well and treated with different concentrations and different time of LPS, respectively. Following treatment, 10 μL of CCK8 solution was added to each well and incubated for 2 h. Optical density measurements at 450 nm were conducted by using a Multiskan Spectrum (SpectraMax 190, Molecular Devices, USA).

Apoptosis Assay

According to the instructions provided, the Annexin V-APC/7-ADD apoptosis kit (MultiSciences, China) was used to detect apoptosis. Apoptosis cells were detected using flow cytometry (Thermo Fisher Scientific, USA) and subsequent analysis was performed using FlowJo software.

ROS Measurement in BEAS-2B Cells

ROS levels were determined using a ROS assay kit (Beyotime, China). The treated cells were harvested and resuspended in FBS-free DMEM medium. Images were observed and recorded using a fluorescence optical microscopy system (Leica, Germany).

Protein Extraction and Western Blotting

Total protein extraction was performed using cold RIPA lysis solution (Beyotime, China), following the instructions provided. Additionally, cytoplasmic and nuclear proteins were extracted using a nuclear and cytoplasmic protein extraction kit (Beyotime, China) according to the specified protocol. Protein concentration was determined using the BCA protein assay kit (Meilunbio, China), and a sample loading buffer was added, accordingly. Proteins were separated using 10% to 12% SDS-PAGE gels and subsequently transferred to 0.45 μm onto polyvinylidene fluoride (PVDF) membranes. Subsequently, the membranes were blocked with 5% skim milk for 1 h at room temperature and then incubated with primary antibodies (1:1000) overnight at 4 °C. The secondary antibodies (1:10000) were then applied for 1 h at room temperature. Protein bands were visualized using a Bio-Rad ChemiXRS imaging system (Bio-Rad, USA) and analyzed using ImageJ software. The relative values of the target protein to the control proteins (GAPDH or β-actin) were calculated. Thereafter, the relative values of other groups in comparison to the control group were computed. The relative values were then plotted in GraphPad Prism to create bar graphs. The antibodies utilized in the experiments included p38, p-p38, 5-LO, BAX, BCL-2, caspase-3, GAPDH, β-actin, and PCNA. The first five were obtained from Cell Signaling Technology (Danvers, USA), and the last four were obtained from Affinity (Shanghai, China).

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR)

Total RNA extraction from BEAS-2B cells was carried out using a RNAiso Plus reagent (TaKaRa, Japan). Subsequently, the purity of cDNA was evaluated the A260/280 ratio, total RNA was reversed in cDNA according to the instructions provided (Takara, Japan). RT-qPCR was performed to detect expression levels of IL-8, TNF-α, IFN-γ, IL-1β, MUC5AC, 5-LO, and GAPDH. The primer sequences are detailed in Table 1. Relative expression of target genes was normalized to the levels of GAPDH within each experimental group.

Statistical Analysis

Data and images were analyzed using GraphPad Prism 9.0 (Graph-Pad, San Diego, CA, USA). The results are presented as mean $\pm$ standard deviation (SD). Quantitative analysis between two groups was conducted utilizing an unpaired Student's t-test, whereas the assessment of differences among three or more groups was performed through an analysis of variance (ANOVA) followed by the Tukey's post hoc multiple comparisons test. Statistical significance was established at $p < 0.05$. * represents versus the Control/PBS group; # represents versus the LPS/OVA group; & represents versus the LPS+STC-1 group.

Results

Effects of STC-1 on Inflammation in OVA-Induced Asthmatic Mice

We used OVA to induce asthmatic model in mice. To verify the effects of STC-1 in asthmatic mice, we administered the STC-1 solution via nasal drops half an hour before the OVA challenge phase (Fig. 1a). To evaluate pathological changes in lung tissue, inflammation and mucus secretion of lung tissue was then observed by H&E and PAS staining. We found that STC-1 inhibited the tissue inflammation, as manifested by a significant reduction in the inflammatory score (Figs. 1b and S1a). As seen in Figs. 1b and S1b, the OVA stimulation caused goblet cell hyperplasia, as assessed by periodic acid-Schiff (PAS) staining, which relieved by STC-1. In BALF, total cells, eosinophils and neutrophils counts, and proteins increased in the OVA group, while total cells, eosinophils, neutrophils and proteins decreased after STC-1 treatment

Table 1 Real-time quantitative polymerase chain reaction primer sequences

Gene	Primer sequences (5' to 3')
GAPDH (human)	CGG AGT CAA CGG ATT TGG TCG TAT
	AGC CTT CTC CAT GGT GGT GAA GAC
5-LO (human)	CCG CTT CAT GCA CAT GTT CCA G
	AAC CTC ACA TGG GCT ACC AG
IL-8 (human)	AAC TGA GAG TGA TTG AGA GTG G
	ATG AAT TCT CAG CCC TCT TCA A
TNF-α (human)	AGC TGG TGG TGC CAT CAG AGG
	TGG TAG GAG ACG ATG CG
IL-1β (human)	GCG GCA TCC AGC TAC GAA TCTC
	AAC CAG CAT CTT CCT CAG CTT GTC
INF-γ (human)	AGT GAT GGC TGA ACT GTC GC
	ACT GGG ATG CTC TTC GAC CT
MUC5AC (human)	CAG CAC AAC CCC TGT TTC AAA
	GAG CAC AGA GGA TGA CAG T

(Fig. 1d). The levels of TNF-α and CXCL-1 in BALF and serum increased in the OVA group (Fig. 1e). P38 is a member of the MAPK family, involving in various cellular processes, including apoptosis, inflammation, cell differentiation, and cell growth [28], and responsible for the production of inflammatory cytokines [29]. Western blotting demonstrated that OVA stimulation increased the phosphorylation levels of P38 (Fig. 1g). Nevertheless, when mice pretreated with STC-1, these OVA-induced inflammatory changes were mitigated (Fig. 1b-f).

Effect of LPS on BEAS-2B Cells

To evaluate the effect of LPS on BEAS-2B cells, we used PBS to dissolve the LPS powder and BEAS-2B cells were treated with LPS of different concentrations and treatment time. Western blotting demonstrated that LPS increased p-p38 expression in a dose-dependent and time-dependent manner (Fig. 2a-b). Cell viability was then measured using the CCK8 assay. Consequently, cell viability was reduced at a dose of 0-1000 ng/mL, while treatment with LPS at more than 1000 ng/mL had no evident effect on cell viability (Fig. 2c). Therefore, we chose 1000 ng/mL LPS to excite cells for 1/3/6 h. Within 6 hours, LPS inhibited cell activity (Fig. 2d). Accordingly, 1000 ng/mL and 3 h were selected to be used as the working concentration and treatment time in subsequent experiments. Western blotting revealed increased levels of p-p38 in the LPS group compared to those of the control (Fig. 2e). An ELISA assay was performed to measure the levels of TNF-α and IL-8 to analyze the inflammatory effect of LPS on BEAS-2B cells. Increased TNF-α and IL-8 were observed in the LPS group compared to the control group (Fig. 2f-g).

Role of STC-1 in Inflammation in LPS-stimulated BEAS-2B Cells

The molecular weight of stanniocalcin-1 is 26.9 kDa. The effect of STC-1 on inflammation was assessed in LPS-stimulated BEAS-2B cells. STC-1 (100 ng/mL) was added to treat cells half an hour before LPS stimulation. Western blotting results depicted that p-p38 expression in the LPS group increased, while p-p38 in the LPS+STC-1 group decreased (Fig. 3a). Excess pro-inflammatory cytokines and abnormal mucus secretion are the main features of asthma. The inflammatory cytokines IL-8 and TNF-α in cell supernatants were detected using ELISA. The results demonstrated that IL-8 and TNF-α levels increased in the LPS group, whereas IL-8 and TNF-α decreased in the LPS+STC-1 group (Fig. 3b). Furthermore, inflammatory cytokines, including IL-8, TNF-α, INF-γ and IL-1β, and mucin MUC5AC, were detected using RT-qPCR. Under LPS stimulation, the mRNA expressions of the inflammatory factors IL-8, TNF-α, INF-γ, and IL-1β increased and the mRNA expression of MUC5AC increased, while the mRNA expressions of IL-8, TNF-α, INF-γ, IL-1β, and MUC5AC decreased after STC-1 treatment (Fig. 3c-d). Taken together, STC-1 alleviated LPS-stimulated inflammation and mucin production in BEAS-2B cells.

Effects of STC-1 on Apoptosis in OVA-induced Asthmatic Mice and LPS-stimulated BEAS-2B Cells

To evaluate the effect of STC-1 on epithelial apoptosis in LPS-stimulated BEAS-2B and OVA-induced asthmatic mice, western blotting were used to detect apoptosis-related proteins Bcl-2, Bax, and caspase-3. As depicted in Fig. 4a-b, BCL-2 decreased, BAX and caspase-3 increased in the

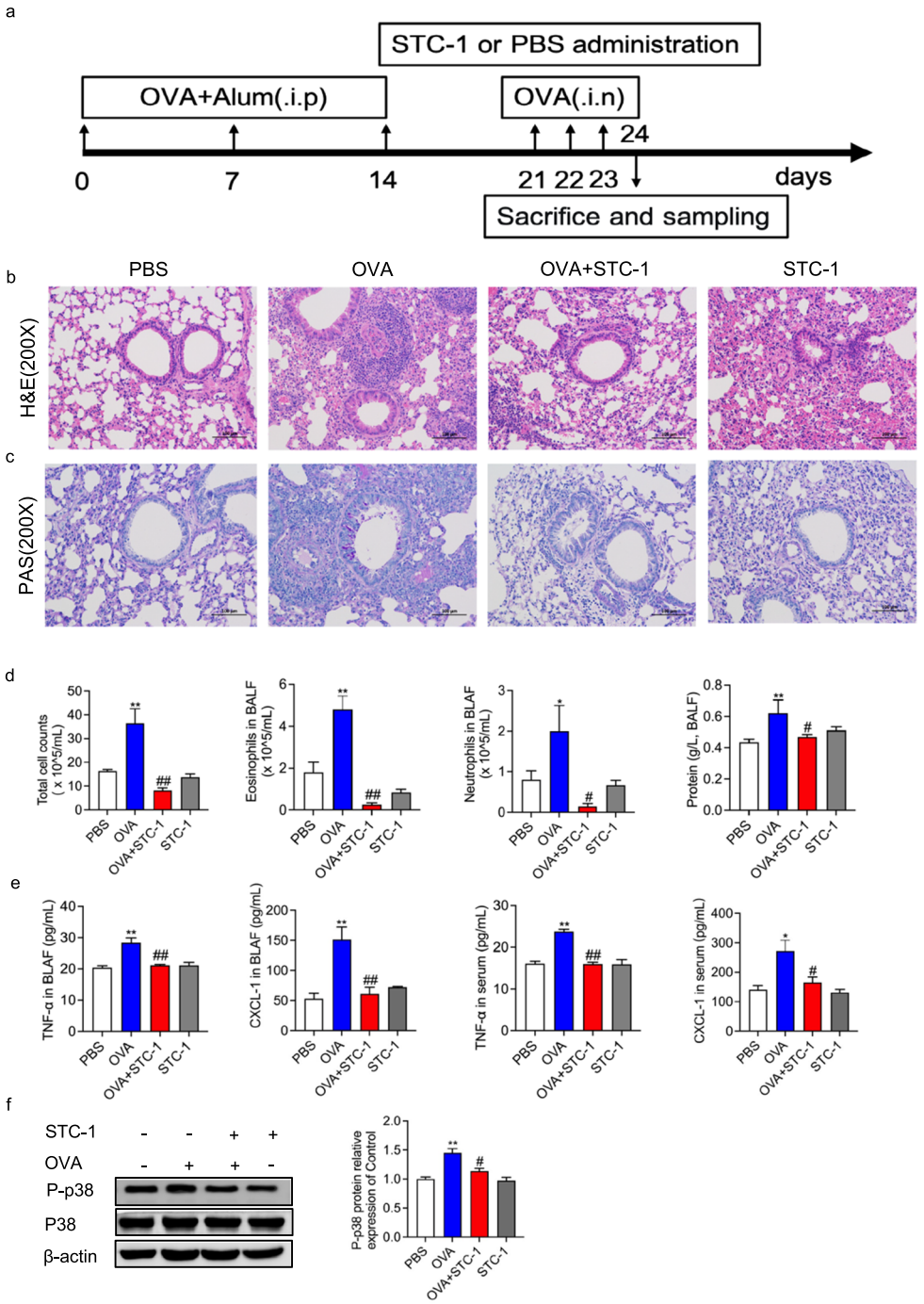

Fig. 1 STC-1 inhibits airway inflammation in OVA-induced asthmatic mice. **a** Specific protocols and materials for the establishment and treatment of acute asthma models. **b** Hematoxylin and eosin (H&E) stained images of lung tissue from the PBS, OVA, OVA+STC-1 and STC-1 groups were shown (200×). **c** Periodic acid-schiff (PAS) stained images of lung tissue of mice were shown (200×). **d** the content of total cells, eosinophils, neutrophils, and proteins in the BALF. **e** the levels of CXCL-1 and TNF-α in the BALF and serum. **f** Western Blotting of p-p38 and p38 in lung tissue from different groups. Data are shown as mean ± SD by one-way analysis of variance (ANOVA). (N = 5-6 mice per group). $*p < 0.05$, $**p < 0.01$ compared to the PBS group. $\#p < 0.05$, $\#\#p < 0.01$ compared to the OVA group

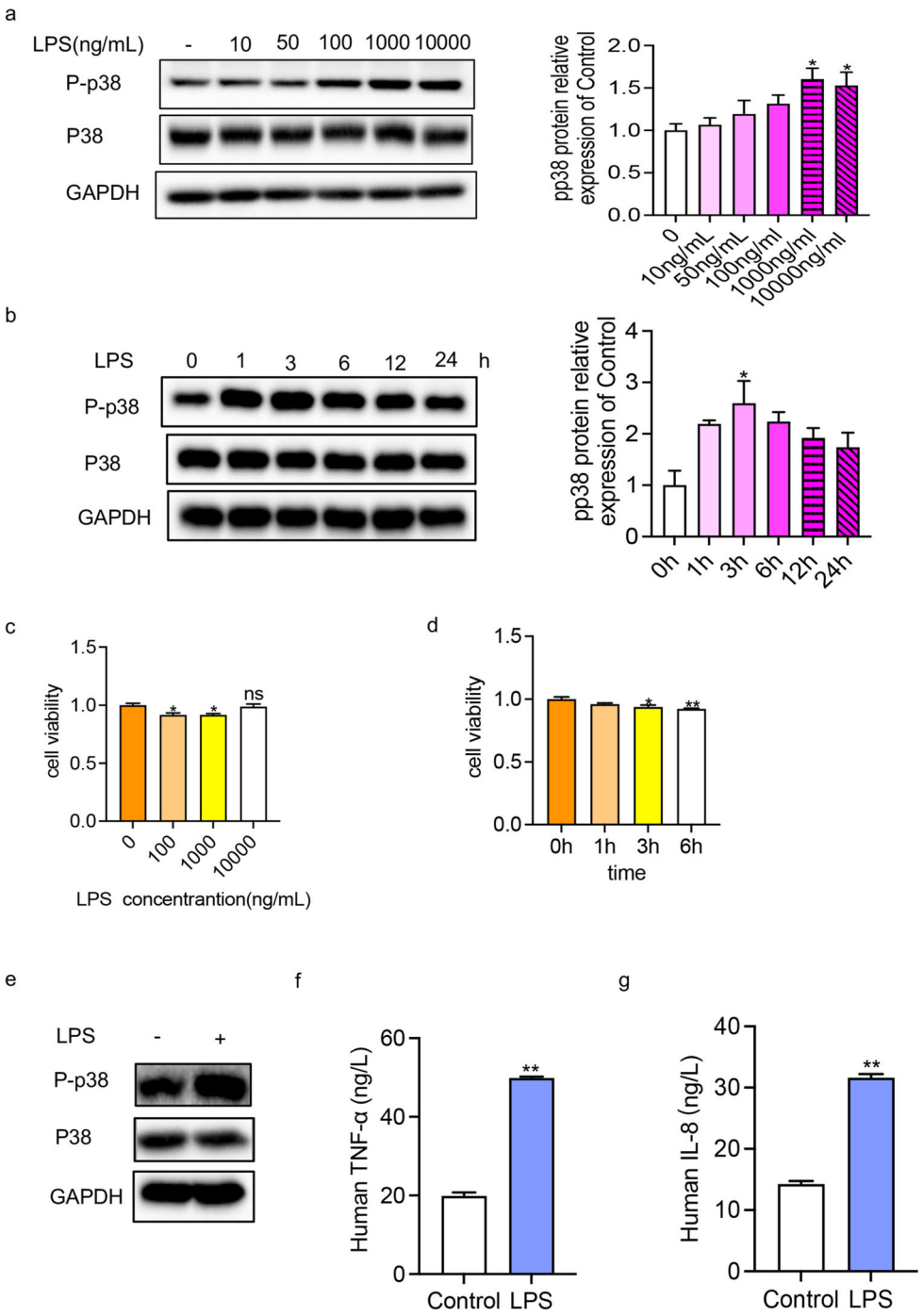

Fig. 2 LPS induced BEAS-2B cells to produce an inflammatory injury model. **a** Western Blotting of p-p38 and p38 after different concentration LPS stimulation. **b** Western Blotting of p-p38 and p38 after LPS stimulation for different treatment time. **c** and **d** The CCK8 assay was used to detect the viability of BEAS-2B cells after stimulation with different concentrations and treatment times of LPS. **e** Western Blotting of p-p38 and p38 after 1000 ng/mL LPS stimulation for 3 h. **f** and **g** the expression of pro-inflammatory factors TNF-α and IL-8 in the cell supernatant was measured by ELISA. All data were shown as mean ± SD. The student's t test was used for 2 group comparisons and one-way analysis of variance (ANOVA) was used for more than 2 groups. *p < 0.05 versus the control group, **$p < 0.01$ versus the control group

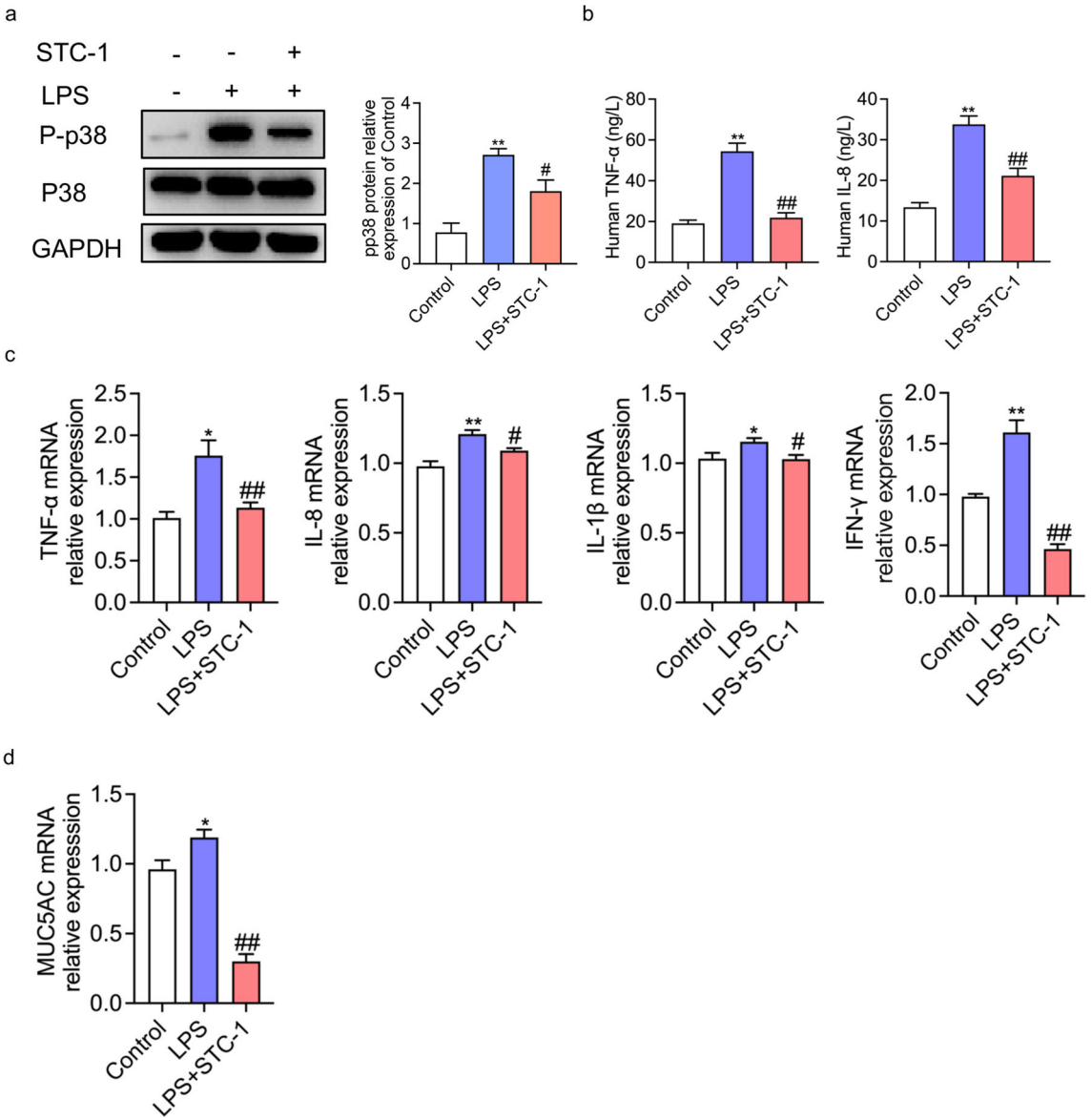

Fig. 3 STC-1 inhibits LPS-induced airway epithelial inflammation in vitro. **a** Results of Western Blotting of p-p38 and p38 protein were shown. **b** ELISA detected TNF-α and IL-8. **c** RT-qPCR was used to determine the changes in the expression of pro-inflammatory factors TNF-α, IL-8, IL-1β and INF-γ. **d** RT-qPCR detected MUC5AC mRNA expression. All data were shown as mean $\pm$ SD. $*p < 0.05$, $**p < 0.01$ versus the control group; $\#p < 0.05$, $\#\#p < 0.01$ versus the LPS group determined by one-way analysis of variance (ANOVA)

OVA group, while BCL-2 increased, and BAX and caspase-3 decreased in the OVA+STC-1 group. In BEAS-2B cells, BCL-2 decreased, BAX and caspase-3 increased in the LPS group, while BCL-2 increased, and BAX and caspase-3 decreased following the administration of LPS combined with STC-1 (Fig. 4c-d). Immunofluorescence results showed that ROS levels in the LPS group enhanced, and ROS levels reduced when STC-1 treatment (Fig. 4e). Apoptotic cells increased in the LPS group and decreased in the LPS+STC-1 group (Fig. 4f), indicating that STC-1 inhibits epithelial apoptosis in OVA-induced asthmatic mice and LPS-stimulated BEAS-2B cells.

STC-1 Down-regulates the 5-LO Pathway in OVA-induced Asthmatic Mice and LPS-stimulated BEAS-2B Cells

5-Lipoxygenase (5-LO) is an enzyme that catalyzes the peroxidation of arachidonic acid, leading to the production of

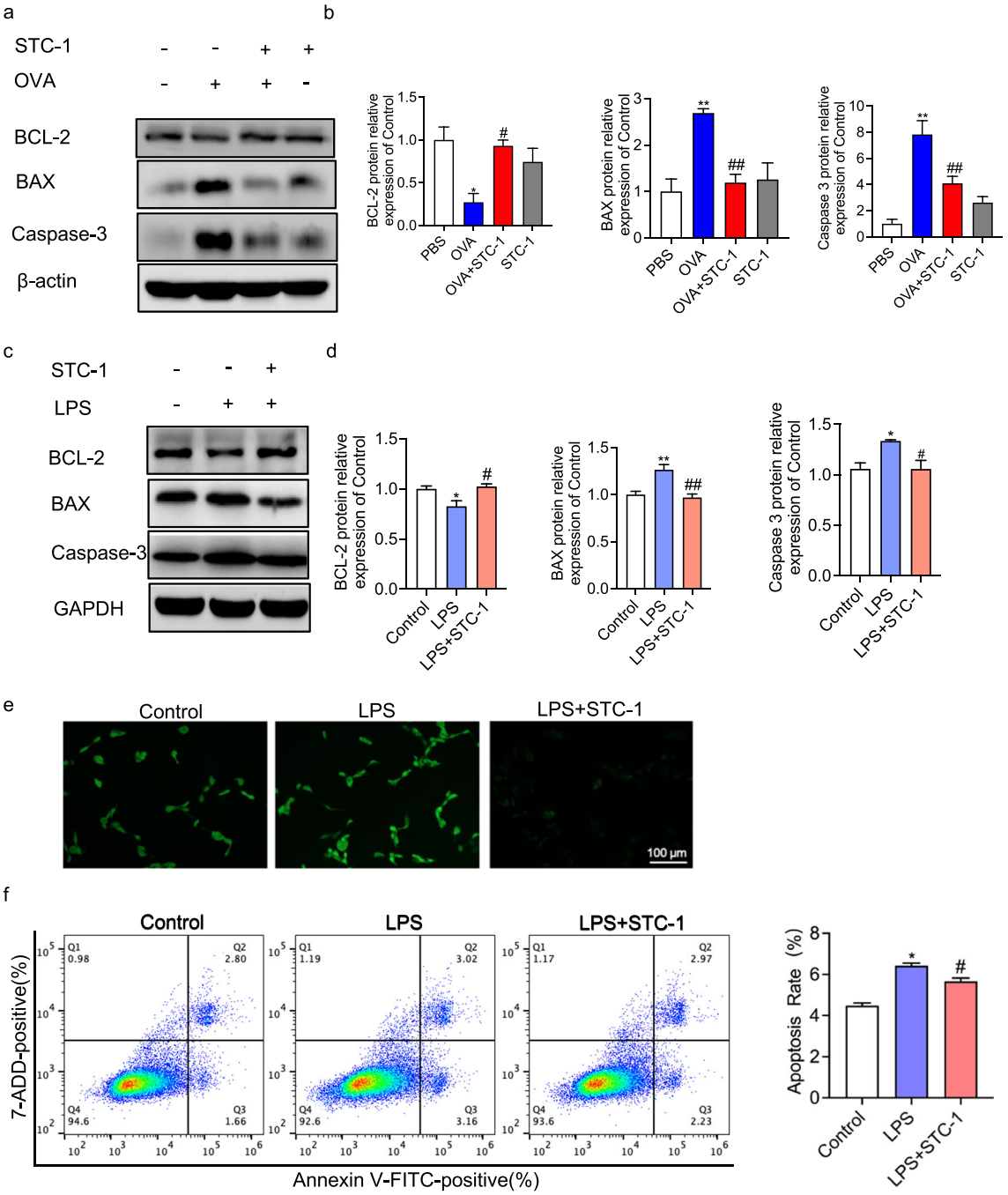

Fig. 4 STC-1 inhibits epithelial apoptosis in OVA-induced asthmatic mice and LPS-induced BEAS-2B cells. **a** and **b** Western blotting of BCL-2, BAX and Capsese-3 in lung tissue in mice. **c** and **d** Western blotting of BCL-2, BAX and Capsese-3 in BEAS-2B cells. **e** Representative images of ROS in BEAS-2B cells. (Magnification: 100×). **f** Apoptotic ratios were determined by flow cytometry analysis. All data were shown as mean ± SD. $*p < 0.05$, $**p < 0.01$ compared to PBS group or Control group; $\#p < 0.05$, $\#\#p < 0.01$ compared to LPS group or OVA group determined by one-way analysis of variance (ANOVA)

leukotrienes, such as leukotriene B4 (LTB4). In order to elucidate the influence of STC-1 on the expression levels of 5-LO, Western blotting assay was utilized to quantify the protein expression of 5-LO. The results demonstrated 5-LO expression increased in lung tissue in OVA-induced asthmatic mice, while STC-1 decreased 5-LO expression (Fig. 5a). In addition, STC-1 inhibited the nuclear translocation of 5-LO protein in LPS-stimulated BEAS-2B cells (Fig. 5b). To detect the activity of 5-LO, the level of LTB4 in cell supernatant was detected by ELISA. The results depicted that LTB4 in the LPS group increased and LTB4 in the LPS+STC-1 group decreased (Fig. 5c). Moreover, 5-LO mRNA increased under LPS stimulation and decreased after STC-1 treatment (Fig. 5d). Collectively, these results suggested that STC-1 down-regulates the 5-LO pathway.

STC-1 Inhibits Epithelial Cell Apoptosis Through The 5-LO Pathway

To explore the role of STC-1 in modulating airway inflammation via the 5-LO pathway, BEAS-2B cells were treated with TGF-β (10 ng/mL) and STC-1, followed by LPS as previously described. TGF-β is an inducer, which induced 5-LO protein expression [30]. The expressions of 5-LO, apoptosis and inflammation markers were then evaluated. The LPS+STC-1+TGF-β group exhibited heightened 5-LO protein as well as mRNA expression, increased cell apoptosis, and elevated BAX/Caspase-3 with decreased BCL-2, alongside upregulated TNF-α and IL-8 mRNA expressions (Fig. 6a-g).

Discussion

Asthma is a major noncommunicable disease that affects people of all ages [31]. In 2019, there were estimated 455000 deaths due to asthma and its comorbidities, which imposes a severe economic and health burden on both patients and society [32–34]. Currently, controlling asthma exacerbations with conventional glucocorticosteroid-based therapies remains a challenge [35, 36], making novel approaches to therapy urgently necessary. In our study, based on OVA-induced asthmatic mice or LPS-stimulated epithelial inflammatory injury, STC-1 has been shown to have anti-inflammatory and anti-apoptosis effects in asthma.

STC-1 was first found in the corpuscles of stannius, the endocrine glands in the kidneys of bony fish [37]. In mammals, the STC-1 gene is expressed in most tissues, including

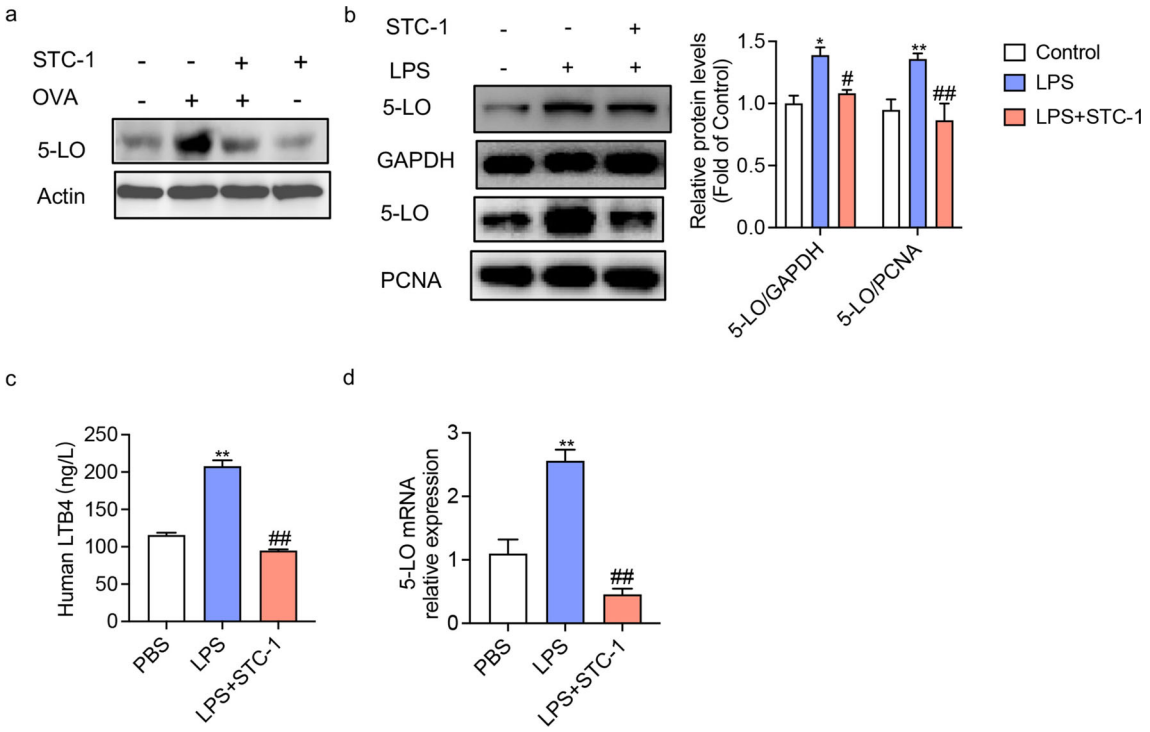

Fig. 5 STC-1 inhibits 5-LO pathway. **a** Western blotting of 5-LO protein in mice from different groups. **b** Western blotting of the expression of 5-LO protein in the cytoplasm and nucleus of BEAS-2B cells were shown. **c** the expression of LTB4 in cell supernatant was measured by ELISA. **d** RT-qPCR detected 5-LO mRNA expression. $*p < 0.05$, $**p < 0.01$ versus the PBS group; $\#p < 0.05$, $\#\#p < 0.01$ versus the LPS group determined by one-way analysis of variance (ANOVA)

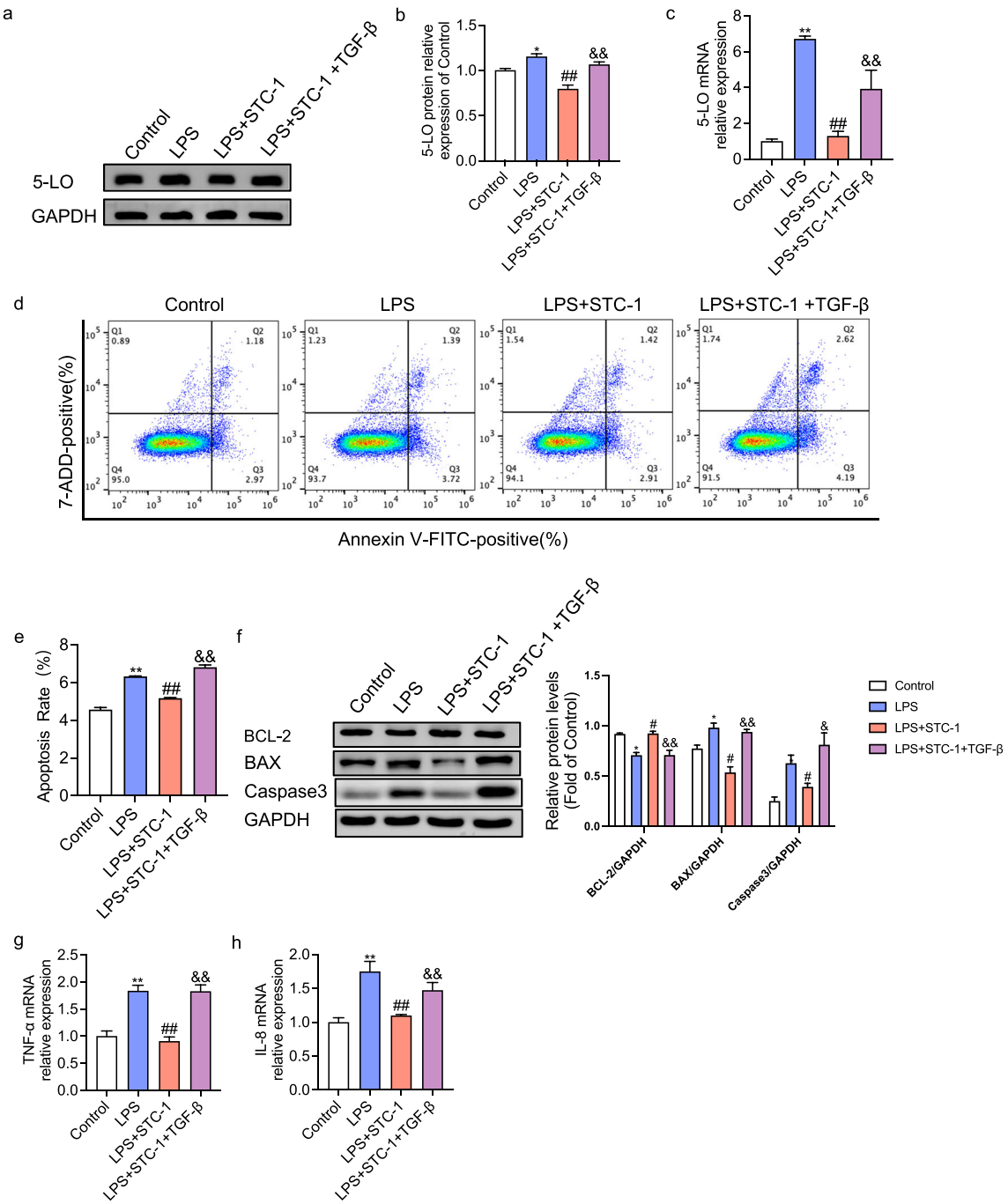

Fig. 6 TGF-β reverses STC-1-inhibited 5-LO pathway in BEAS-2B cells. **a** and **b** Western blotting of 5-LO protein in different groups. **c** RT-qPCR detected 5-LO mRNA expression. **c** and **d** Apoptotic ratios in BEAS-2B cells were determined by flow cytometry analysis. **e** Western blotting of BCL-2, BAX and Capsese-3 in BEAS-2B cells. **f** and **g** RT-qPCR detected TNF-α and IL-8 mRNA expression. Data are shown as mean ± SD by one-way analysis of variance (ANOVA). $*p < 0.05$, $**p < 0.01$ compared to the Control group; $\#p < 0.05$, $\#\#p < 0.01$ compared to the LPS group; &p < 0.05, &&p < 0.01 compared to the LPS+STC-1 group

the ovary, prostate, heart, kidney, thyroid, and lung [38]. It has various biological functions of STC-1, such as antioxidative stress activity, anti-endoplasmic reticulum stress activity, macrophage regulation, and chimeric antigen receptor modified T cell immunotherapy [39, 40]. Recently, Ito et al. identified that hypoxia and hypoxia-inducible factor in human lung epithelial cells induced STC-1 expression [41]. Tang et al. demonstrated that intratracheal STC-1 treatment attenuated LPS-induced acute lung injury in mice by reducing ROS generation [42]. Xu et al. showed that STC-1 levels decreased in serum of the asthmatic population, nasal drip of STC-1 inhibited release of IL-13 and MCP-1, regulated airway smooth muscle contraction and airway hyperresponsiveness by suppressing myosin light chain phosphorylation and blocking store-operated Ca2+ entry [27]. Here, we found that the increased number of inflammatory cells in BALF, the increased levels of CXCL-1 and TNF-α, and the activated p38 MAPK pathway induced by OVA were reduced by STC-1. We also found that STC-1 notably inhibited the activated p38 MAPK pathway and the production of IL-8 and TNF-α in LPS-induced epithelial inflammatory injury. In brief, these results reveal that STC-1 inhibited airway inflammation.

Airway inflammation in asthma can induce goblet cells metaplasia and lead to excessive mucus hypersecretion [43]. These goblet cells are responsible for the secretion of mucin MUC5AC, and an overabundance of MUC5AC can further aggravate airway hyperresponsiveness and result in mucus obstruction [44]. PAS staining results have indicated an elevated number of goblet cells in OVA-induced asthmatic mice [45]. Consistent with these findings, an increase in PAS-positive cells in the OVA group indicated an increase in goblet cell numbers, and goblet metaplasia occurs in asthma. STC-1 treatment reduced the number of goblet cells. Additionally, this study conducted experiments in vitro that substantiated STC-1's ability to suppress MUC5AC mRNA expression, thereby confirming its inhibitory effect on mucus secretion.

A growing body of evidence suggests that loss of epithelial integrity caused by apoptosis in asthma allows exposure of the airway and lung to excess pathogens or environmental allergens [46], which further exacerbates airway epithelium, ultimately leading to an inflammatory loop of the airway. Epithelial apoptosis is due to ROS accumulation, which is derived from increased rates of oxidative phosphorylation in the mitochondria and metabolic reactions in peroxisomes [5, 47]. ROS activates the MAPK pathways in a wide variety of positive circuits to mediate inflammation and induce further apoptosis [4, 48]. In the present study, we found that the decreased BCL-2 and the increased BAX and caspase-3 developed in OVA-induced asthmatic mice, and STC-1 alleviated the change of apoptosis-related proteins, while STC-1 reduced BAX and caspase-3 expression, ROS production and apoptosis cells, suggesting that STC-1 ameliorated epithelial apoptosis.

This study also analyzed the detailed mechanisms underlying the effects of STC-1 on LPS-stimulated epithelial inflammatory injury. Leukotrienes have pathogenic effects on various diseases, such as allergic diseases, inflammatory diseases, neurodegenerative diseases, and cancers [49–52]. LTB4 is the most important leukotriene in airway inflammation, initiating and amplifying neutrophil chemotaxis, and formatting neutrophils extracellular trap [53]. Elevated levels of LTB4 were detected in blood, BALF, and exhaled breath condensates of asthmatic subjects [54–56]. 5-LO is a key enzyme in leukotrienes biosynthesis and involved in the pathophysiology of asthma [13]. Zileuton, a 5-lipoxygenase inhibitor, modulates host immune responses and improves lung function in severe acute respiratory syndrome [57]. Catechols 6b and 6d play an anti-inflammatory role by inhibiting 5-LO [58]. Samuel J Poirier et al. proved that TGF-β in combination with LPS could increase the expression of 5-LO mRNA and protein more than LPS or TGF-β alone in MM1 and THP-1 cells [59]. Thea K Wöbke et al. found that the TGF-β I receptor kinase antagonist SB431542 inhibited 5-LO mRNA induction by 78% [60]. In the current study, we found that increased 5-LO protein expression in OVA-induced asthmatic mice model, 5-LO protein nuclear translocation, LTB4 levels and 5-LO mRNA expression in the LPS group, which reduced by STC-1. We further found that STC-1's inhibition of upregulating 5-LO expression, and elevating apoptotic levels and inflammatory cytokines was reversed by TGF-β, suggesting that STC-1 alleviated apoptosis and airway inflammation by inhibiting the 5-LO pathways. Therefore, the current study provides strong support that STC-1 may be a promising candidate for exerting anti-inflammatory roles in asthma.

One of the limitations of this study is the lack of further in vivo studies to better reveal the regulatory effect of STC-1 on 5-LO expression. In addition, the current OVA stimulated mouse model is an allergic disease model, and it is necessary to further explore the regulatory effects of STC-1 on airway inflammation and 5-LO in a non-allergic mouse model of asthma.

Conclusion

STC-1, as a new protein therapy, mitigated histopathological changes, excessive mucus production, inflammatory cell infiltration, and apoptosis, thereby protecting from asthma. Furthermore, STC-1 alleviated ROS generation, cell apoptosis and inflammatory cytokines production through the downregulation of the 5-LO pathway. This discovery enriches

our knowledge of the therapeutic effect of STC-1 on airway inflammation, which provides a new theoretical basis for the future clinical application of STC-1.

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Author Contributions Shijia Wang conducted the study and wrote the manuscript. Chang Cai designed the study and supervised the process. Zhijian Tu, Chenping Li, and Xiao Jin collected tissue samples and performed other animal experiments. Zehong Chen, Xiaofei Ye, Shuyao Xu, and Jihao Cai gathered all data and performed data analysis. All authors approved the final manuscript; consequently, they have full access to all data in the study and take responsibility for the integrity and security of the data.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Ethical approval The animal study protocol was reviewed and approved by the Laboratory Animal Center of the First Affiliated Hospital of Wenzhou Medical University (WYYYIACUCAEC2023038).

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