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ORIGINAL ARTICLE



TSLP promotes asthmatic airway remodeling via p38-STAT3 signaling pathway in human lung fibroblast

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ABSTRACT

Purpose: Thymic stromal lymphopoietin (TSLP) acts as a critical cytokine involved in asthmatic airway remodeling. Our study aimed to characterize the crosstalk between airway epithelial cells and fibroblasts regulated by TSLP through the signaling pathways of Mitogen-activated protein kinase (MAPK) and signal transducer and activator of transcription 3 (STAT3). **Materials and Methods:** Human biopsy specimens and lung tissues from mice were stained with hematoxylin and eosin (H&E) and immunohistochemistry. Human lung fibroblasts were stimulated with human recombinant TSLP. The protein expression of phosphorylation of STAT3 (p-STAT3) and phosphorylation of MAPK as well as the expression of collagen I and alpha-smooth muscle actin (α -SMA) were detected by Western blotting and immunofluorescence. Co-culture was performed to detect the influence of TSLP secreted by airway epithelial cells on fibroblasts. An ovalbumin (OVA)-induced asthmatic murine model was established with or without intraperitoneal injection of SB203580 (inhibitor of p-38). Protein expression in lung tissue was detected by immunohistochemistry and western blotting. **Result:** TSLP could activate MAPK in HLF-1. SB203580 could inhibit the activation of p38, attenuate phosphorylation of STAT3, and decrease the expression of collagen I and α -SMA consequently in human fibroblasts. Co-culture demonstrated that TSLP secreted by epithelial cells could promote the expression of collagen I and α -SMA and aggravates airway remodeling in fibroblasts. In vivo, expression of TSLP, collagen I, α -SMA, p-p38 and p-STAT3 was upregulated in airway tissue of OVA-challenged mice and downregulated in mice which were treated by SB203580. The tissue staining showed that airway structure change was attenuated by SB203580 compared with OVA challenged mice as well. **Conclusions:** TSLP might promote asthmatic airway remodeling via p38 MAPK-STAT3 axis activation and the crosstalk between airway epithelial cells and fibroblasts could aggravate remodeling. Blockade of p38 could alleviate airway remodeling which might provide a new therapeutic target for asthma.

ARTICLE HISTORY

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KEYWORDS

TSLP; asthma; airway remodeling; MAPK; STAT3

Introduction

Asthma is a complex multifactorial disease characterized by chronic inflammation and structural alterations in airways¹. Structural alterations termed 'airway remodeling' encompass thickening of lamina reticularis, epithelial shedding, sub-epithelial fibrosis, goblet cell hyperplasia, increased smooth muscle mass and angiogenesis². Airway remodeling, which contributes to airway dysfunction and provokes clinical symptoms is common in severe asthma³.

Thymic stromal lymphopoietin (TSLP) is an IL-7 like cytokine produced mainly by epithelial cells and expressed in various tissues including skin, lung, thymus and intestinal mucosa⁴. TSLP is as well recognized to be a critical mediator involved in Th2 inflammation⁵. Recently, TSLP has emerged as a key regulator for boosting local remodeling in asthmatic airway⁶. Several groups have found the level of TSLP measured in bronchoalveolar lavage fluid (BALF) is shown to be significantly higher in asthmatic patients than

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healthy controls⁷. TSLP protein is also elevated in the peripheral serum and airway epithelium of asthmatic patients and the level is correlated with the severity of asthma^{8,9}.

Mitogen activated protein kinases (MAPK) family comprises proline-directed, protein-serine/threonine kinase, which participates in signal transduction pathways that control cellular programs such as proliferation, differentiation and apoptosis¹⁰. Classical MAPKs include extracellular signal-regulated kinase 1 and 2 (ERK1/2), c-Jun N-terminal kinases (JNK)/p38 and ERK5 (ERK5 is reported to be related to endothelial cells and there is no evidence to demonstrate that ERK5 participates in asthma. Consequently, this paper does not involve with ERK5). Bronchial epithelial cells, airway smooth muscle cells and lung fibroblasts are recognized to be the critical cells in pathogenesis and persistence of asthma^{11,12} and the dysfunction of these cells may be associated with crosstalk of TSLP, MAPK and STAT3¹³.

Fibroblasts reside close to the basal epithelium and the activation, differentiation and secretion of fibroblasts contribute vitally to airway remodeling². Despite fibroblast plays such a crucial role in development of asthma, data of the mechanism involved in fibroblast activation by TSLP is limited¹⁴. STAT3 belongs to the family of STAT transcription factors participating in cellular responses to a variety of cytokines and growth factors¹⁵. Several groups have proved that in regulation of airway remodeling, STAT3 signaling pathway is involved in various processes, such as smooth muscle cell proliferation, repair of epithelium and fibroblast migration^{16,17}.

Considering the above evidences, we hypothesized that correlation between TSLP, MAPK, and STAT3 in lung fibroblasts might play a critical role in airway remodeling and MAPKs and STAT3 signaling can be integrated into a network. Besides, we think that TSLP secreted by airway epithelial cells may promote airway remodeling by binding to its receptors in fibroblasts. Therefore, the present study aimed to better understand the essential role of TSLP in airway remodeling and the regulation of MAPKs and STAT3 signaling pathways in lung fibroblasts and in mice models.

Table 1. Clinic characteristics of normal subjects and asthma patients.

	Healthy subjects	Asthma patients
Sex (M/F)	5/5	7/3
Age (y)	47.90 ± 10.66	41.70 ± 6.40
FEV1 (%predicted)	97.91 ± 7.03	79.80 ± 5.31
FEV1/FVC (%)	92.37 ± 4.961	69.81 ± 8.46

Values are presented as means ± SD. FEV1, forced expiratory volume in one second; FVC, forced vital capacity; SD, standard deviation.

Methods and materials

Patients

The study was approved by the Ethics Review Committee for Human Studies at Qilu Hospital of Shandong University. 10 patients with asthma were enrolled into the study. They were all outpatients or inpatients of Qilu Hospital of Shandong University and underwent a bronchoscopic investigation for differential diagnosis between asthma and COPD or other diseases. Before bronchoscopy, all patients signed the informed consent. A definite diagnosis of asthma was established in the 10 patients. Healthy control specimens were obtained from Qilu Hospital of Shandong University via pulmonary lobectomy or body donation. The information of patients was shown in Table 1.

Reagents and mice

Human recombinant TSLP was purchased from PeproTech (USA). Antibodies (Ab) against c-Jun N-terminal kinase (JNK), phospho-extracellular signal regulated kinase (p-ERK), phospho-p38 (p-p38) and phospho-c-Jun N-terminal kinase (p-JNK) were purchased from Santa Cruz Biotechnology (USA). Ab against STAT3, p38, TSLP, ERK, α -SMA, p-STAT3 and collagen I were from ABCam (USA). The MAPK (ERK1/2, JNK, p38) inhibitor U0126, SP600125 and SB203580 were purchased from Selleckchem (USA). OVA were purchased from Sigma, ST. and aluminum hydroxide was from ThermoScientific (USA).

Thirty 6-week-old female BALB/c mice were obtained from Shandong University Laboratory Animal Centre, separated into control, OVA and OVA + SB203580 groups, ten in each group, and housed under specific pathogen-free conditions. The animals were maintained in a 12-/12-h light-

dark cycle and free access to food and water. All experimental procedures involving animals were approved by the laboratory animal ethics committee of Qilu Hospital of Shandong University. Mice were sensitized by intraperitoneal injection with 100 µg ovalbumin (OVA) and 2 mg of Al(OH)₃ in a total volume of 0.2 ml phosphate buffered saline (PBS) on days 0, 7, and 14. From day 21, sensitized animals were challenged with aerosolized 5% OVA for 30 min per two days until two months, while the control group were challenged with aerosolized PBS. SB203580 (3 mg/kg) were injected intraperitoneally on sensitized mice 1 h before challenge.

Cell culture, inhibitor treatment, co-culture and transfection

Human lung fibroblast cell line (HLF-1) was purchased from cell bank (Kunming cell bank, Kunming, China). Human airway epithelial cells (BEAS-2B) and asthmatic primary bronchial/tracheal epithelial cells (PCS-300-011) were purchased from ATCC (Manassas, VA). Cultured cells were kept in our laboratory in DMEM (Gibco, USA) supplemented with 10% fetal bovine serum in a humidified 5% CO₂ atmosphere at 37 °C. Once the cells reached 60% confluence, they were treated with recombinant human TSLP. In some experiments, SB203580, SP600125 and U0126 were applied 1 h prior to TSLP stimulation.

HLF-1 cells were separately preincubated with or without SB203580 10 µM, SP600125 10 µM or U0126 10 µM for 1 h, after which the cells were left unstimulated or treated with indicated TSLP stimulants as well. Cells extracts were further analyzed as described below.

Human airway fibroblasts were first seeded in 12-well tissue culture plates at a density of 5×10^3 cells/cm² in complete medium (2 mL/well). After 1 day, a Transwell insert (pore size 0.4 µm; Millipore, Billerica, MA, USA) was placed in each well, and airway epithelial cells were seeded in the inserts at four different densities (2×10^4 , 2.5×10^5 , 5×10^5 and 1×10^6 cells/well) to initiate co-culture. The culture lasted for 2 days and medium was replaced by DMEM which did not contain serum. From this time on, in some coculture experiments, either recombinant human

TSLP and inhibitor of p-38 was supplemented in the medium. The level of TSLP in medium was measured by ELISA and western blot was performed by cultured fibroblasts to detect the influence of TSLP secreted by epithelial cells on fibroblasts.

At 50–70% confluence, epithelia cells were transfected with TSLP-plasmid (Santa Cruz, USA) or TSLP-shRNA (Santa Cruz, USA). Transient transfections were performed using lipofectamine 2000 reagent (Invitrogen Inc., USA) according to the manufacturer's instructions. The transfected cells were then co-cultured with the human airway fibroblasts following the procedure above.

ELISA

The level of TSLP in co-culture medium was measured by ELISA (CUSABIO MD, USA) according to the instructions.

Protein isolation and western blot analysis

Right lung tissues from mice or cell extracts were prepared using radioimmunoprecipitation assay (RIPA) buffer supplemented with 1% protease and phosphatase inhibitor according the manufacturer's instructions. Protein concentration was determined by a BCA Protein Assay Reagent Kit. The primary antibodies included anti-ERK1/2, anti-p-ERK1/2, anti-JNK, anti-p-JNK, anti-p38, anti-p-p38, anti-STAT3, anti-p-STAT3, anti-collagen I, anti-α-SMA and anti-GAPDH. The immunoreactive bands were visualized using an enhanced chemiluminescence (ECL) substrate in a ChemiDoc XRS Imaging System (bio-rad). The intensity of bands was evaluated against the loading control to derive a ratio value using ImageJ.

Immunofluorescence staining

Cells were fixed with paraformaldehyde/PBS for 15 min and were then permeabilized with 0.5% Triton X-100/PBS for 20 min. Cells were blocked with 10% goat serum for 30 min, then incubated with the primary Ab for 12 h at 4 °C, negative group with PBS. After washing with PBS, cells were incubated separately with conjugated anti-

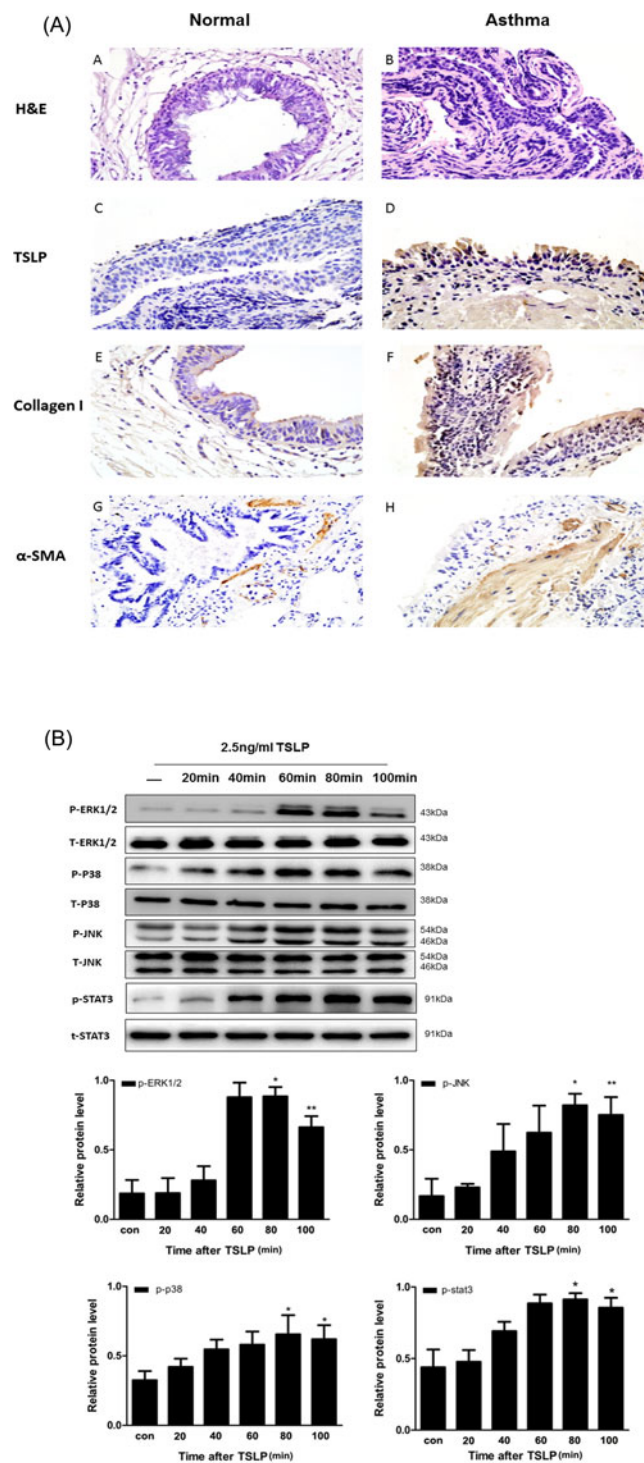


Figure 1. A. Evidently increased thickness of basement and loss of integrity of epithelium was observed compared with the normal. The level of expression of TSLP, collagen I and α -SMA was significantly upregulated in asthmatic epithelium compared with the normal (x400). B. The expression of p-ERK1/2, p-JNK, p-p38 and p-STAT3 in HLF-1 were gradually increased with the increase in time with the stimulus of TSLP in a concentration of 2.5ng/mL, and the expression reached the maximum at the time of 60 min.

rabbit and anti-mouse secondary Ab for 1 h and then with DAPI for 5 min. Cells were washed with PBS and analyzed under a confocal microscopy (Olympus fluoview 500, Tokyo, Japan).

Histology and immunohistochemistry

The specimens from patients and left lobes from mice underwent formalin-fixed and paraffin-embedded and then cut into 5 μ m thick sections.

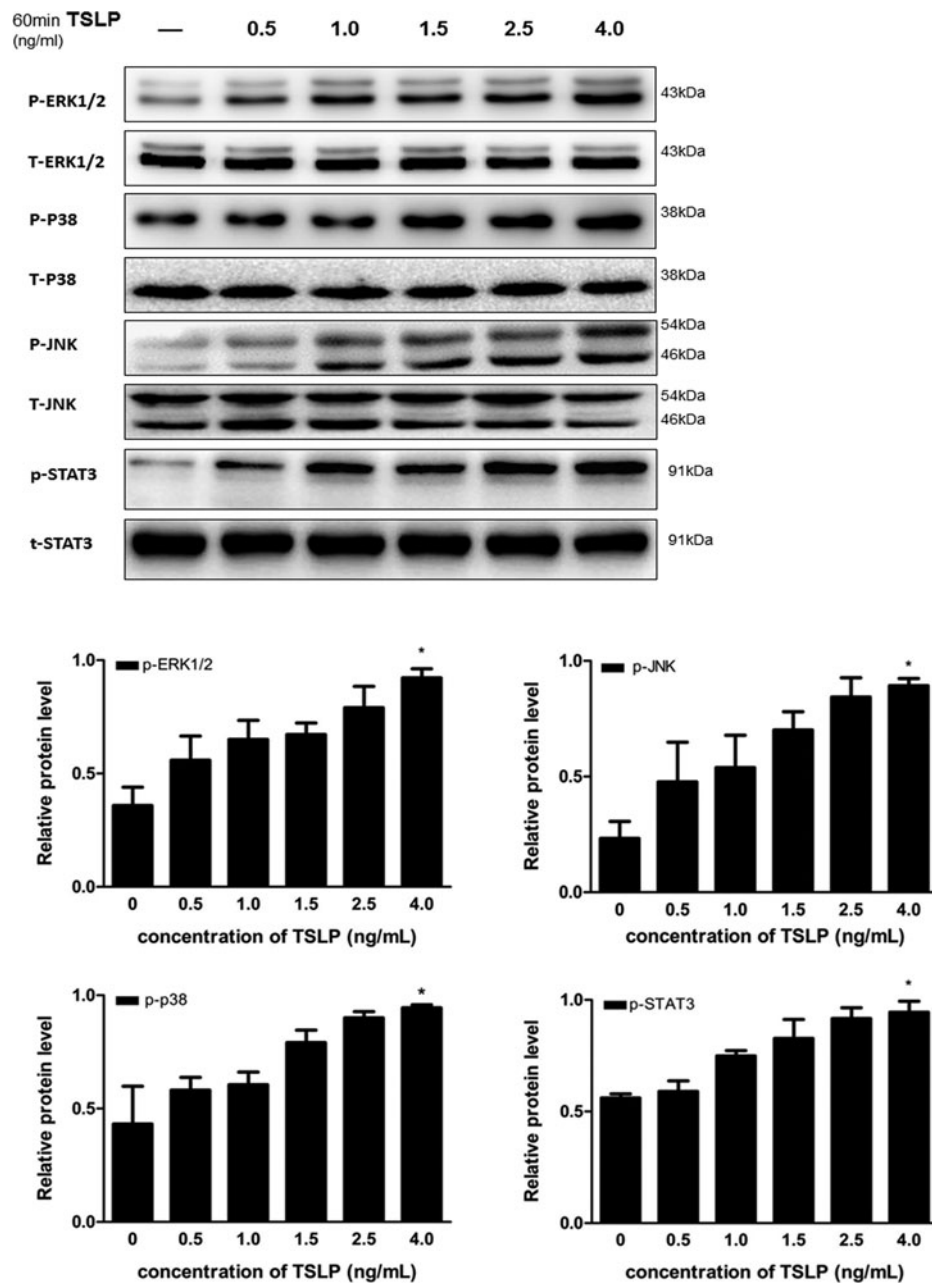


Figure 2. The expression of p-ERK1/2, p-JNK, p-p38 and p-STAT3 in HLF-1 were gradually increased with the increase in concentration of TSLP with the stimulus of TSLP for 60 min, and the expression reached the maximum at the concentration of 2.5 ng/mL.

The sections were then stained with hematoxylin and eosin (H&E) or Masson Trichrome to identify the characteristic structural changes.

The slides were incubated with antibodies against TSLP (1:100), collagen I (1:200) and α -SMA (1:200), respectively, overnight at 4°C. HRP-conjugated secondary antibodies were applied at room temperature for 1 h. The sections were then processed with 3,3-diaminobenzidine for 2 min, counterstained with hematoxylin for 3 min. The positive area of the target protein was measured from the

total 6 randomly fields of each slide by Image-Pro Plus 6.0 software (Media Cybernetics, USA).

Statistical analysis

Data were collected and analyzed with SPSS 16.0 for windows. Quantitative variables were presented as mean $\pm$ standard deviation (mean $\pm$ SD) and analyzed by analysis of variance (ANOVA). A value of $P < 0.05$ was considered statistically significant.

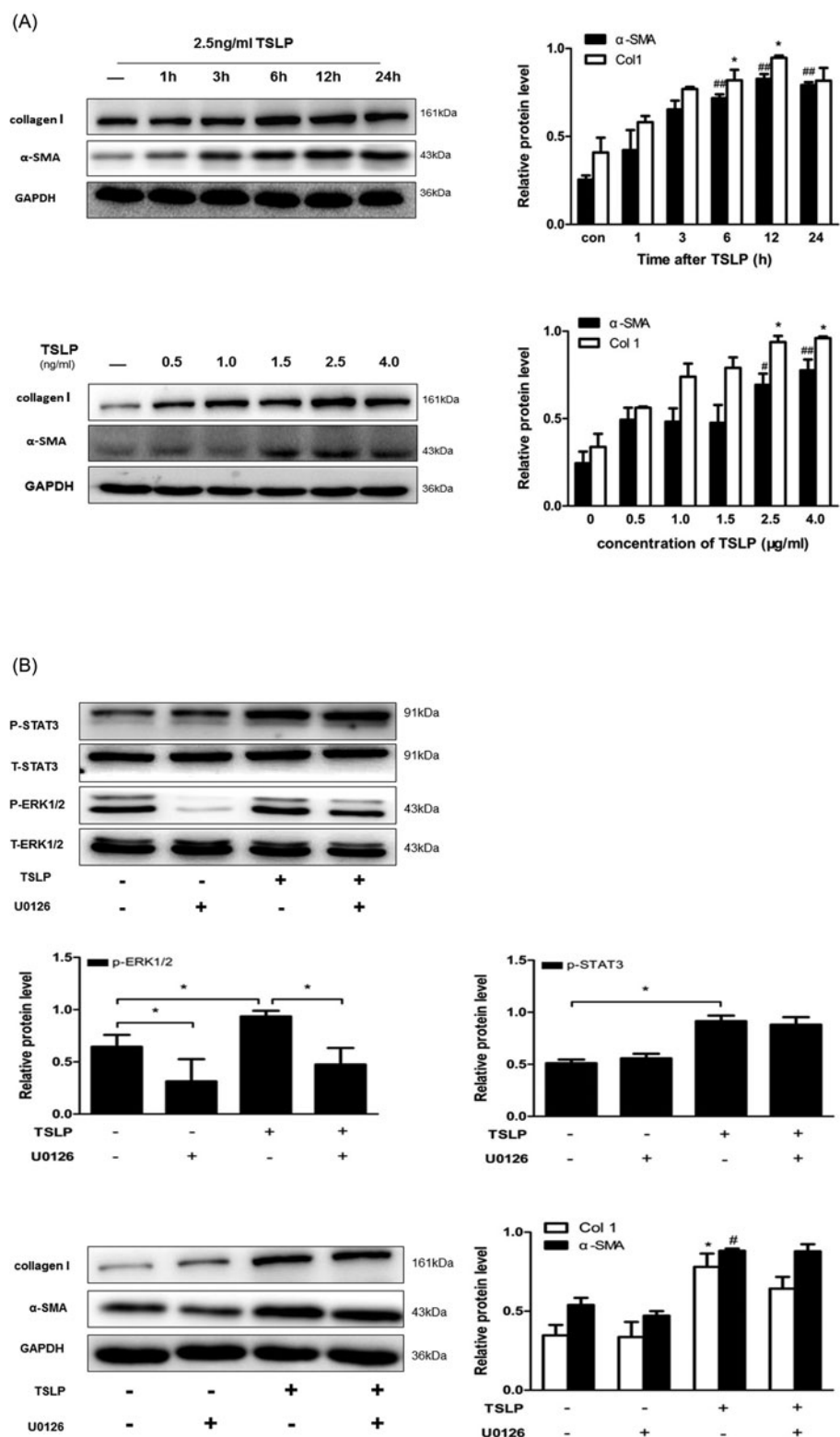


Figure 3. A. The expression of collagen I and α -SMA in HLF-1 were gradually increased with the increase in time with the stimulus of TSLP in a concentration of 2.5ng/mL, and the expression reached the maximum at the time of 12 hours. The expression of collagen I and α -SMA in HLF-1 were gradually increased with the increase in concentration of TSLP with the stimulus of TSLP for 12 hours, and the expression reached the maximum at the concentration of 2.5ng/mL. B. The graph, respectively, represented the bands and density ratio of p-ERK1/2 to total ERK1/2 ($n = 3$, $*p < 0.05$), p-STAT3 to t-STAT3 ($n = 3$, $*p < 0.05$), collagen I and α -SMA to GAPDH ($n = 3$, $*p < 0.05$; $n = 3$, $*p < 0.05$). These results showed that TSLP could promote the phosphorylation of STAT3 and increase the expression of collagen I and α -SMA. U0126 had no influence on the phosphorylation of STAT3 and the expression of collagen I and α -SMA.

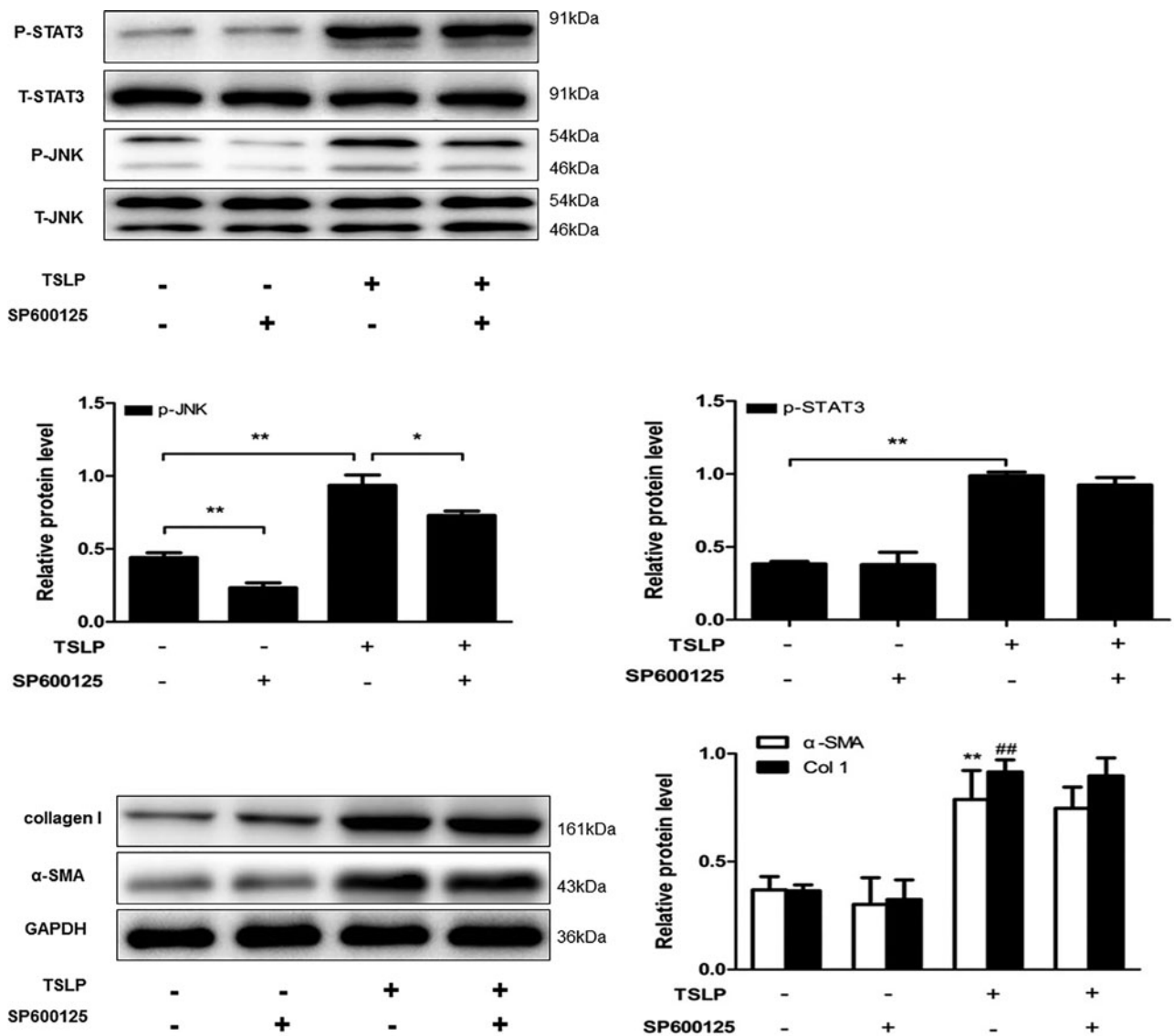


Figure 4. The graph, respectively, represented the bands and density ratio of p-JNK to t-JNK ($n=3$, $*p<0.05$, $**P<0.05$), p-STAT3 to t-STAT3 ($n=3$, $*p<0.05$, $**P<0.01$), collagen I and α -SMA to GAPDH ($n=3$, $*p<0.05$, $**P<0.01$; $n=3$, $^{\#}p<0.05$, $^{\#\#}p<0.01$). These results showed that TSLP could promote the phosphorylation of STAT3 and increase the expression of collagen I and α -SMA. SP600125 had no influence on the phosphorylation of STAT3 and the expression of collagen I and α -SMA.

Results

TSLP highly expressed in human asthmatic epithelium and correlated with airway remodeling

More thickening basement membrane and less integrity of epithelium were found in bronchial specimens from patients with asthma by H&E staining while healthy subjects had no evident structure changes (Figure 1A).

As shown in the representative immunohistochemical images of biopsy specimens or lung tissue, TSLP was observed to be mainly expressed in epithelium and little in the mesenchyme. α -SMA, collagen I and TSLP were expressed

within airway tissues and the levels of expression were upregulated in the asthmatic airway compared with the normal (Figure 1A).

TSLP induced expression of collagen I and α -SMA in human lung fibroblast as well as phosphorylation of STAT3 and MAPK

HLF-1 cells were stimulated with 2.5ng/mL TSLP for different time periods (20, 40, 60, 80, 100 min) and with different concentration (0, 0.5, 1.0, 1.5, 2.5, 4.0 ng/mL) of TSLP for 60 min. MAPK (ERK1/2, p38 and JNK) was activated and the phosphorylation of MAPK (p-ERK1/2, p-p38

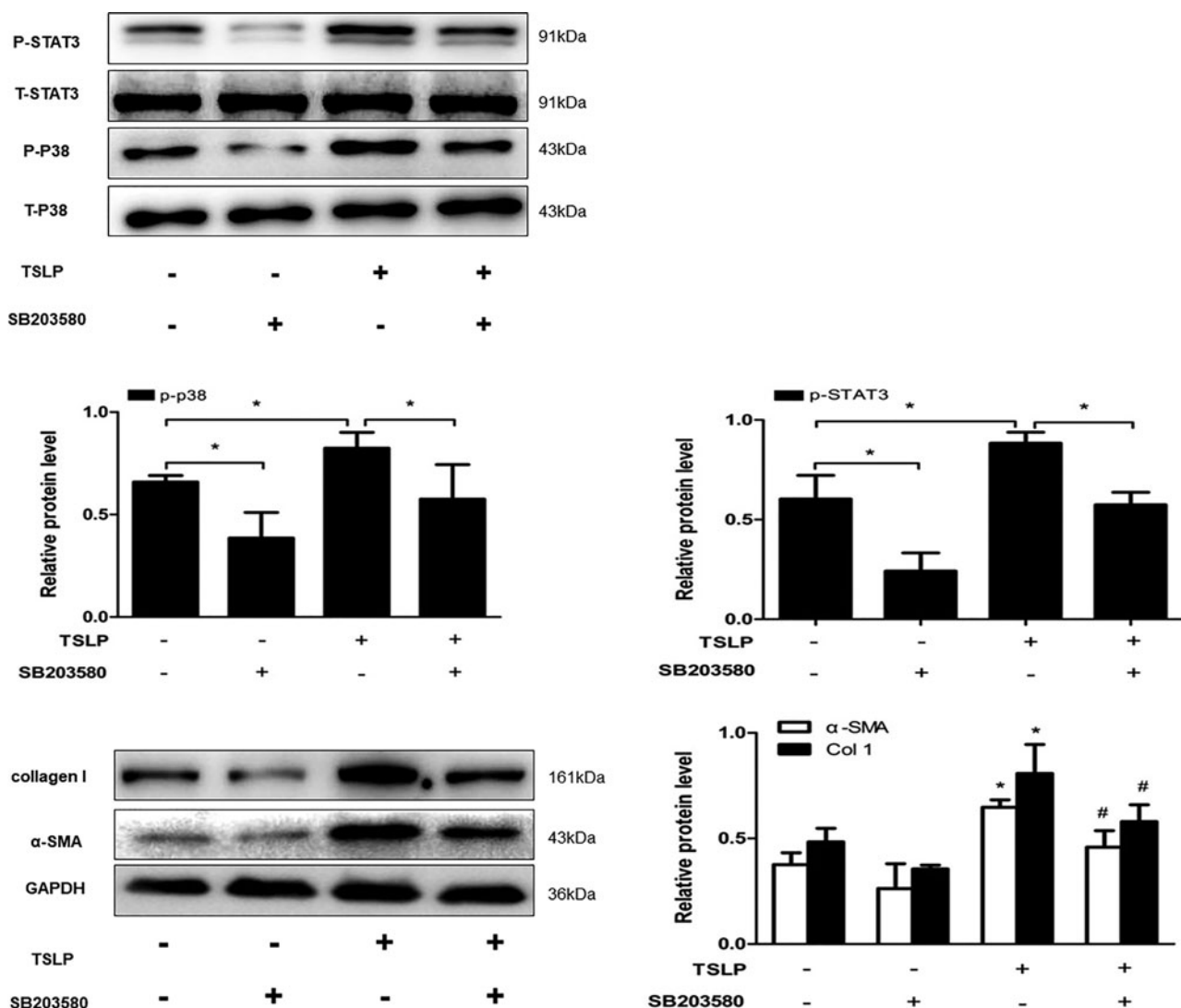


Figure 5. The graph, respectively, represented the bands and density ratio of p-p38 to t-p38 ($n = 3$, $*p < 0.05$), p-STAT3 to t-STAT3 ($n = 3$, $*p < 0.05$), collagen I and α -SMA to GAPDH ($n = 3$, $*p < 0.05$; $n = 3$, $*p < 0.05$). These results showed that TSLP could promote the phosphorylation of STAT3 and increase the expression of collagen I and α -SMA. SB203580 could offset partial influence of TSLP on the phosphorylation of STAT3 and the expression of collagen I and α -SMA.

and p-JNK) was gradually increased as the concentration or time increased. The maximum activation of MAPK and STAT3 were found occurred and changed to be steady at 60 min with TSLP 2.5 ng/ml. The semi-quantitative analysis of phosphorylated protein in comparison with total-ERK1/2 (t-ERK1/2), total-p38 (t-p38), total-JNK (t-JNK) and total-STAT3 (t-STST3) indicated that the level of phosphorylation of the three molecules significantly reached the peak at 60 min after 2.5 ng/ml TSLP exposure compared with unstimulated control (Figures 1B and 2).

As expected, the activation of collagen I and α -SMA altered in time-dependent and concentration-dependent manners since TSLP exposure.

The semi-quantitative analysis of collagen I and α -SMA in comparison with GAPDH, respectively, and p-STAT3 in comparison with t-STAT3 indicated that at 12 h with exposure of TSLP 2.5 ng/ml ($P < 0.05$; $n = 3$), the level of p-STAT3, collagen I and α -SMA significantly elevated and reached steady (Figure 3A).

Verification of MAPK-STAT3 signaling pathway

It was found that the pretreated inhibitors specifically reduced the TSLP-induced phosphorylation of ERK1/2, p38 and JNK, respectively. It was also observed that the level of p-STAT3 was reduced since added p38 inhibitor SB203580 compared

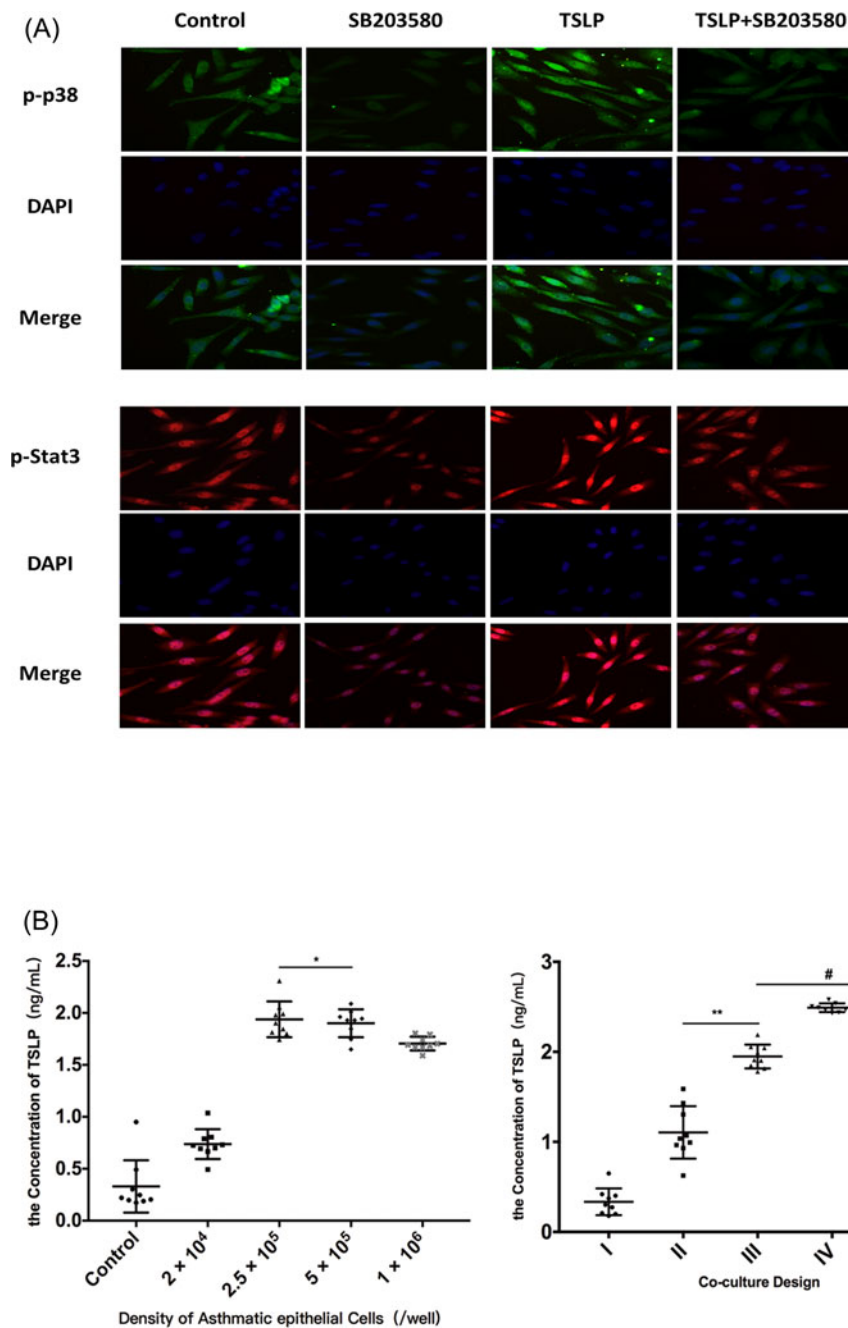


Figure 6. A. P-p38 was stained with green fluorescence, and nucleus was stained with DAPI (blue fluorescence) ($\times 400$). Immunofluorescence staining of p-STAT3 in HLF-1. P-STAT3 was stained with red fluorescence, and nucleus was stained with DAPI ($\times 400$). B. With the increase of densities of asthmatic epithelial cells, the concentration of TSLP increased and it reached peak at the density of 2.5×10^5 cells/well (* $p = 0.633$). Compared with normal airway epithelial cells, asthmatic epithelial cells secreted more TSLP. The inhibitor of p38 had no influence on TSLP secretion. (** $p < 0.05$, # $p > 0.05$) (Co-culture illustration: I: HLF, II: HLF co-cultured with BEAS-2B, III: HLF co-cultured with asthmatic airway epithelial cells, IV: HLF cultured in medium with TSLP, V: HLF co-cultured with asthmatic airway epithelial cell in medium with p38 inhibitor).

with TSLP-induced groups ($p < 0.05$; $n = 3$), while ERK1/2 inhibitor U0126 and JNK inhibitor SP600125 had no evident impact on phosphorylation of STAT3. To determine whether MAPKs inhibitors could block expression of collagen I and α -SMA, we used the HLF-1 cells pretreated with specific MAPKs inhibitors, respectively, and

analyzed the protein expression level with western blotting. Our results demonstrated that TSLP could significantly increase the expression of collagen I and α -SMA, and the increased protein level was attenuated with the addition of p38 inhibitor SB203580 ($P < 0.05$; $n = 3$) as well as p-STAT3, whereas ERK and JNK inhibitors had no

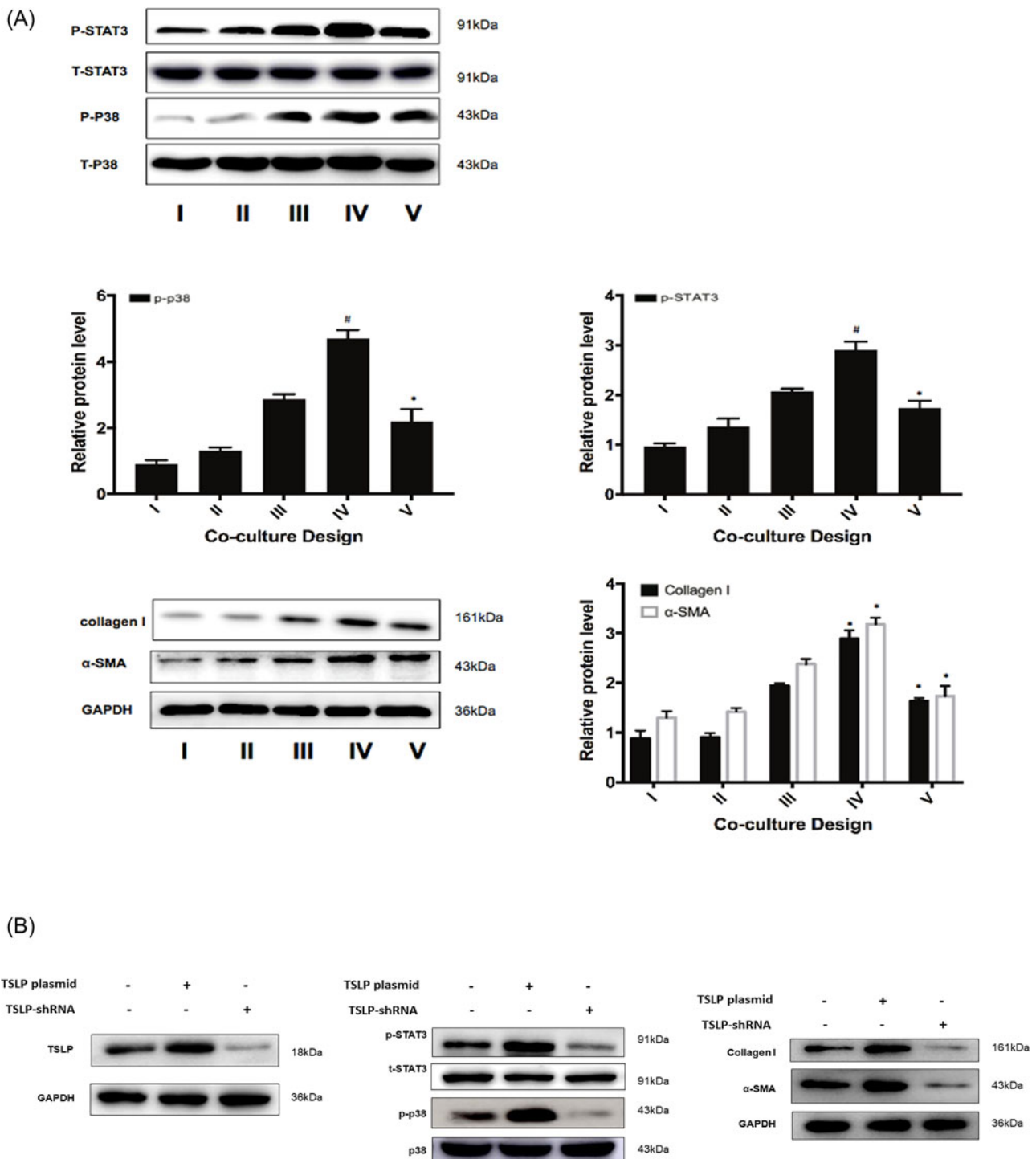


Figure 7. A. The graph, respectively, represented the bands and density ratio of p-p38 to t-p38 ($n = 3$, $*p < 0.05$), p-STAT3 to t-STAT3 ($n = 3$, $*p < 0.05$), collagen I and α -SMA to GAPDH ($n = 3$, $*p < 0.05$; $n = 3$, $\#p < 0.05$). These results showed that TSLP secreted by asthmatic epithelial cells could promote the phosphorylation of STAT3 and increase the expression of collagen I and α -SMA in fibroblasts. SB203580 could offset partial influence of TSLP on the phosphorylation of STAT3 and the expression of collagen I and α -SMA. (Co-culture illustration: I: HLF, II: HLF co-cultured with BEAS-2B, III: HLF co-cultured with primary asthmatic airway epithelial cells, IV: HLF cultured in medium with TSLP, V: HLF co-cultured with primary asthmatic airway epithelial cell in medium with p38 inhibitor). B. These results showed that TSLP plasmid enhanced the expressions of TSLP in airway epithelia cells and TSLP-shRNA suppressed the expression ($p < 0.01$, $p < 0.01$, respectively). P-STAT3 and p-p38 were upregulated in HLF-1 co-cultured with epithelia cells transfected with TSLP-plasmid ($p < 0.01$, $p < 0.01$, respectively), while co-cultured with epithelia cells transfected with TSLP-shRNA depressed the expressions in HLF-1 cells ($p < 0.01$, $p < 0.01$, respectively). Compared with the control groups, collagen I and α -SMA were overexpressed in TSLP plasmid groups ($p < 0.01$, $p < 0.01$, respectively), while expressions of collagen I and α -SMA proteins significantly decreased in HLF-1 cells co-cultured with epithelial cells transfected with TSLP-shRNA ($p < 0.01$, $p < 0.01$, respectively).

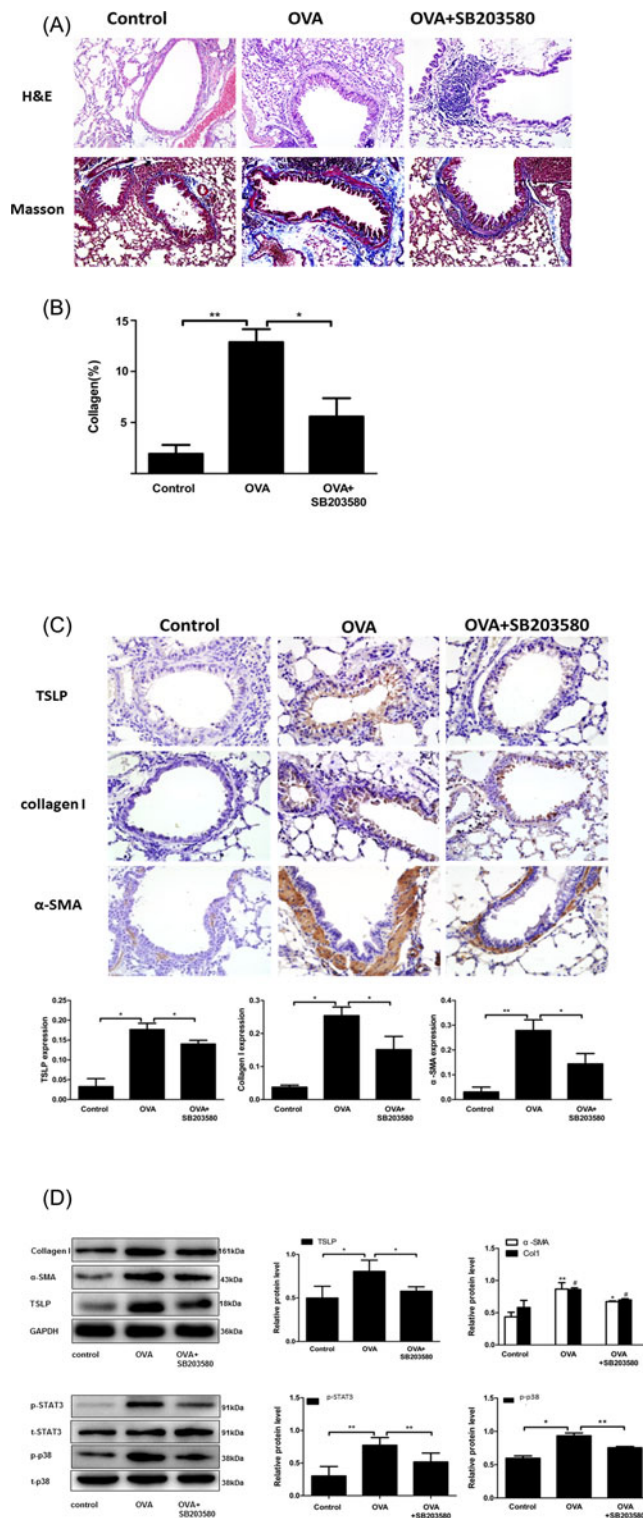


Figure 8. A. Lung tissue from each group (10 mice/group) underwent H&E and Masson staining ($\times 200$). B. statistical analysis of Masson staining revealed collagen deposition increased evidently in airway of OVA challenged mice ($**p < 0.01$, $**$ significantly different from control group) and SB203580 could significantly reduce the OVA induced collagen deposition around airway ($*p < 0.05$, $*$ significantly different from OVA group). C. Expression of TSLP, collagen I and α -SMA was upregulated in OVA challenged mice airway and SB203580 could evidently reduce the expression of TSLP, collagen I and α -SMA ($\times 400$). The statistical analysis of TSLP ($*p < 0.05$), collagen I ($\#p < 0.05$) and α -SMA expressed in epithelium ($*p < 0.05$). D. The expression of TSLP ($*p < 0.05$), collagen I ($\#p < 0.05$), α -SMA ($*p < 0.05$, $**p < 0.01$), p-p38/t-p38 ($*p < 0.05$) and p-STAT3/t-STAT3 ($*p < 0.05$) were upregulated in asthmatic lung tissues and downregulated with the addition of SB 203580.

evident impact (Figures 3B, 4 and 5). The fluorescence intensity of p-p38 as well as p-STAT3 could be attenuated by SB203580 (Figure 6A).

TSLP secreted by epithelial cells in asthma directly promoted p38-STAT3 activation and aggravated airway remodeling

We selected the density of 2.5×10^5 cells/well of epithelial cells to initiate the co-culture, as ELISA demonstrated that levels of TSLP in medium were maximum at this density and cells grew in good condition. The TSLP levels in medium demonstrated that asthmatic epithelial cells secreted more TSLP compared with normal (Figure 6B). Western blot showed that TSLP from asthmatic epithelial cells promoted phosphorylation level of p38 and STAT3 as well as the expression of collagen I and α -SMA (Figure 7A). The expression of TSLP was upregulated in epithelia cells transfected with TSLP plasmid and suppressed in epithelia cells with TSLP-shRNA. P-STAT3 and p-p38 levels were markedly enhanced in HLF-1 cells co-cultured with epithelia cells transfected with TSLP-plasmid. Significantly, the p-STAT3 and p-p38 were decreased in HLF-1 cells co-cultured with epithelia cells transfected with TSLP-shRNA. Meanwhile TSLP-induced α -SMA and collagen I upregulation is in the p38-STAT3 dependent manner. Compared with the control groups and TSLP plasmid groups, collagen I and α -SMA proteins expression significantly decreased in HLF-1 cells co-cultured with epithelia cells transfected with TSLP-shRNA (Figure 7B).

SB203580 attenuated airway remodeling in an asthmatic murine model

H&E and Masson trichromatic staining showed that SB203580 alleviated the thickening of basement membrane, peribronchial collagen deposition and smooth muscle hyperplasia in OVA challenged mice (Figure 8A and B).

In addition, immunohistochemistry results indicated that OVA-challenge induced overexpression of the marker of airway remodeling, collagen I and α -SMA, and expressions of the protein in airway and lung tissues were suppressed by SB203580 (Figure 8C). Western

blotting analysis of lung tissue protein accordingly obtained a result (Figure 8D).

Effects of SB203580 on expression of TSLP in airway epithelium and airway remodeling in murine models

It was observed that compared with control groups, TSLP expressed in airway epithelium was elevated in OVA challenged mice, and lowered in mice pretreated with SB203580 before OVA challenge. Semi-quantitative analysis indicated TSLP expression was remarkably promoted in OVA groups and significantly reduced by SB203580 ($P < 0.05$; $n = 3$, Figure 8D). In coincidence with western blotting analysis results, TSLP extracted from total lung tissue protein increased significantly in OVA group and decreased in OVA + SB203580 group ($P < 0.05$; Figure 8D). TSLP expression in lung tissues was also regulated by p38 signaling pathway.

Discussion

In present study, we observed characteristic loss of epithelium integrity and thickening basement membrane parallel to elevated expression of TSLP in airway epithelium from asthma patients which is consistent to previous studies^{5,6}. However, less attention has been paid to regulation of TSLP on human lung fibroblast, which might be crucial to remodeling as well. Hence in our vitro experiments, the human lung fibroblasts (HLF-1) line was used and stimulated with TSLP. As expected, TSLP functioned as an activator of fibroblast. TSLP induced the upregulation of collagen I and α -SMA, markers of airway remodeling, in time-dependent and concentration-dependent manners. Coincidence with our vivo experimental results, TSLP accompanied with collagen I and α -SMA was overexpressed in airway of an OVA-induced asthmatic murine model. Given the time-dependent and concentration manners of TSLP stimuli, the hypothesis that the level of TSLP was related to the degree of airway remodeling was considered and further studies are needed.

Inhibition of p38 could reduce activation of STAT3. Our results indicated STAT3 might be MAPK-p38 substrate. STAT3 is an important

signaling pathway molecule involved multiple processes in airway remodeling which encompassed epithelial-mesenchymal transition (EMT) and smooth muscle cells proliferation^{13,18}, and suppressing activation of STAT3 in our previous study could reduce the role of fibroblast in promoting airway remodeling¹⁴.

P38 is the member of MAPK family involved in a variety of asthmatic cellular activities, such as goblet cells hyperplasia¹⁹, smooth muscle cells migration and cytoskeleton remodeling and epithelial-mesenchymal transition (EMT)^{20,21}. Consequently, we exhibited and confirmed the role of p38 in the TSLP-stimulated fibroblasts which probably contributed to airway remodeling primarily and reconfirmed in vivo that inhibition of p38 could suppress the airway remodeling. Recent several studies, fibroblast/myofibroblast differentiation was proved regulated by p38 signaling pathway^{22,23}. As we observed, α -SMA, a marker of differentiation of fibroblasts to myofibroblasts, was found decreased since inhibition of p38 both in vivo and vitro. Moreover, p38 not only participated in regulating the fibroblast differentiation, but also the contraction of myofibroblasts, which probably contributed to airway hyperresponsiveness and remodeling²⁴.

Although lung fibroblast functions as a structural cell, its role in inflammation could not be ignored²⁵. Several proinflammatory cytokines released by fibroblast were reported regulated via MAPK signaling pathway²⁶. Despite we proved the effect of TSLP on activating MAPK signaling pathway, the definite role of TSLP-ERK1/2/JNK signaling pathway in asthma haven't been explored. Therefore, we gave a hypothesis that TSLP may induce inflammatory response of human lung fibroblasts through MAPKs signaling pathway as well in asthma and further detection is undergoing. A recent study discovered that ERK1/2-STAT1 axis signaling pathway existed in human lung fibroblasts, and activation of this pathway was involved in regulating airway remodeling²⁷. It might suggest that activation of MAPK might have links with other signaling molecules and further studies are needed. Furthermore, several groups demonstrated that the expression of TSLP in airway could be regulated via p38 and ERK1/2 signaling pathway^{28,29}

while we found the expression of TSLP in epithelium of asthmatic mice could be attenuated by SB203580 but not U0126. The thickened basement membrane and airway wall fibrosis are relative to the resistance to inhaled corticosteroid therapy³⁰. Since our study indicated p38 MAPK inhibitor could attenuate airway remodeling via reducing collagen synthesis, it would provide a possible synergetic therapeutic approach of p38 MAPK inhibitor to enhance the effect of corticosteroid on airway inflammation and remodeling.

Numerous other cytokines are important for the initiation and development of pulmonary immune response to environmental stimuli, such as IL-25, IL-33 and Fizz1²². Here we only discussed the role of TSLP in asthmatic airway remodeling and further detections of other molecule and mechanism are needed.

We reported a novel signaling mechanism, p38-STAT3 axis signaling pathway, activated by TSLP which further the understanding of the effect of TSLP on human lung fibroblast and progress of airway remodeling. P38 might regulate TSLP expression in lung tissue as well. Besides, this may provide a new therapeutic target that contributes to slow down the development of remodeling in asthma.

Declaration of interest

The authors declare that they have no conflict of interest.

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