



Interleukin-37 alleviates airway inflammation and remodeling in asthma via inhibiting the activation of NF- κ B and STAT3 signalings

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ABSTRACT

Asthma is a common respiratory inflammatory disorder disease of childhood, and airway smooth muscle cells (ASMCs) play an important role in this disease. Recently, studies have found that interleukin (IL)-37 inhibits allergic airway inflammation of asthmatic mouse models. The aim of this study was to investigate the exact mechanism of IL-37 in asthma. In this study, we found recombinant human IL-37 protein significantly reduced ovalbumin (OVA)-induced airway hyperresponsiveness, inflammatory cell infiltration, the epithelial-mesenchymal-transition (EMT) process, and levels of IL-4, IL-6 and IL-13, but increased interferon (IFN)- γ expression. Moreover, IL-37 treatment remarkably inhibited transforming growth factor (TGF)- β 1-induced cell proliferation, migration, EMT, and inflammatory response in ASMCs. IL-37 notably upregulated I κ B expression and down-regulated levels of NF- κ B p65, phospho-NF- κ B p65, STAT3 and phospho-STAT3 both in OVA-induced mice and in TGF- β 1-stimulated ASMCs. The effects of IL-37 on TGF- β 1-induced ASMCs were abrogated by STAT3 up-regulation. Additionally, PDTC, a NF- κ B inhibitor, showed the similar effects as IL-37 in ASMCs. In conclusion, IL-37 may alleviate airway inflammation and remodeling in asthma through suppressing the activation of NF- κ B and STAT3.

1. Introduction

Asthma, a respiratory inflammatory disorder disease characterized by goblet cell hyperplasia, mucus hypersecretion, airway inflammation, and airway hyperresponsiveness (AHR) [1,2], affects about 300 million people globally. About 80–90% of adults with chronic asthma experienced symptoms before 5 year-old, and the severity of symptoms in childhood is closely related to the persistence of asthma into adult life [1]. Hence, the proper diagnose and treatment of childhood asthma are extremely urgent [3]. Airway smooth muscle cells (ASMCs) are thought to the main effector cells of airway disorder, narrowing and obstruction via their hyperplastic and hypertrophic qualities [4]. Studies have shown that ASMCs contribute to the pathogenesis of asthma via orchestrating and persistent airway inflammation and remodeling responses [5]. Transforming growth factor (TGF)- β 1 is a growth factor elevated in the airway of asthmatic patients [6]. It is responsible for the phenotypic changes of ASMCs including contractility, proliferation, migration, and secretion of inflammatory mediators [4,7].

Interleukin (IL)-37, a newly identified member of the IL-1 family, plays an important role in suppressing innate and acquired immunity [8]. IL-37 expression is altered and it functions in various diseases including atherosclerosis [9], myocardial ischemia/reperfusion injury [10], calcific aortic valve disease [11], rheumatoid arthritis [12], inflammatory bowel disease [12], and lupus [13]. In recent years, studies have shown that IL-37 expression was significantly lower in the serum and induced sputum of asthmatic patients than that in healthy controls and the expression was correlated with disease severity [14]. IL-37 treatment could abrogate the increased production of pro-inflammatory cytokines from sputum cells induced by asthma [14]. Lunding et al. [15] revealed that intranasal administration of IL-37 reduced allergic airway inflammatory response and alleviated the hallmarks of experimental asthma including mucus hyperproduction and AHR.

Nuclear factor (NF)- κ B (NF- κ B) is regarded as a critical regulator of inflammation and the activation of NF- κ B plays an important role in the airway inflammation in asthma [16]. Signal transducers and activators of transcription (STAT) 3 is an important transcription factor

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that is critical for cytokine signaling. It was initially identified as an acute phase response gene in the liver. It plays a vital role in modulating inflammatory responses via inducing the expression of cytokines, adhesion molecules, and chemokines [17]. Studies have shown that STAT3 was indispensable for allergic inflammatory response in asthma through altering Th2 cell recruitment and effector function [17]. Gavino et al. [18] demonstrated that STAT3 inhibition could prevent lung inflammation, remodeling, and the secretion of Th2 and Th17 cytokines. Studies found that IL-37 suppressed many inflammatory responses that were related to the inhibition of NF- κ B and STAT3 activation [19,20]. However, the relationship between IL-37 and the activation of NF- κ B and STAT3 is still unknown in asthma.

Hence, we speculated that IL-37 may suppress asthma via inhibiting the activation of NF- κ B and STAT3. In this study, we examined the effects of IL-37 in allergic asthma mouse models and TGF- β 1-induced ASMCs. The molecular mechanism was focused on the activation of NF- κ B and STAT3.

2. Materials and methods

2.1. Animal studies

Female BALB/c mice (4–6-week-old) were obtained from the Center of Experimental Animals of Xi'an Jiaotong University College of Medicine (Xi'an, China). All the animals were housed in a specific pathogen-free (SPF) environment with controlled temperature ($23 \pm 1^\circ\text{C}$) and humidity ($60 \pm 5\%$) under a 12-h light/dark cycle. They were allowed free access to food and water. Animals were randomly divided into three groups ($n = 8/\text{group}$): Control group, OVA group, and OVA + IL-37 group. Asthmatic mouse models were performed as previously described [21]. Briefly, mice were firstly intraperitoneally injected with OVA (Sigma, St. Louis, MO, USA; 100 μg OVA were mixed with 1 mg aluminum hydroxide) on days 1 and 14. Then, these animals were challenged with intranasal instillation of 150 μg OVA once per day from day 21 for one week. 1 μg of recombinant human (rh) IL-37 protein (R&D Systems, Minneapolis, MN) dissolved into 50 μL normal saline were intranasally injected into anesthetized mice 24 h before each OVA inhalation challenge [15]. Mice in other groups received the same volume of normal saline as the same methods. All the animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Xi'an Children's Hospital.

2.2. Airway hyper-responsiveness (AHR) measurement

AHR was determined 24 h after the last intranasal OVA challenge by whole-body plethysmography (Buxco Electronics, Troy, NY, USA). In brief, mice were placed in the plethysmograph chamber. Aerosolized normal saline solution or increasing concentrations of methacholine (6.25, 12.5, 25, and 50 mg/mL) were nebulized through the chamber inlet for 90 s. After the inlet was closed, AHR was expressed by enhanced pause (Penh) that was recorded for 3 min and averaged.

2.3. Bronchoalveolar lavage fluid (BALF) cell count

After AHR measurement, mice were sacrificed with 1.5% pentobarbital sodium and BALF was collected to assess differential BALF cell counts. Briefly, lungs were lavaged three times with 750 μL ice-cold PBS and the recovered fluid was called as BALF. The BALF samples were centrifuged at 400g for 5 min to pellet the cells, and the supernatant was collected and stored at -80°C until use. Total cell counts were performed by using hemocytometer (FastRead102, Immune Systems LTD, UK). The cell pellets were re-suspended with PBS and differential cell counts were determined via the Wright's staining (BASO, Zhuhai, China).

2.4. Histological assessment

For lung histology, the left lung lobes of mice were removed and fixed with 10% formalin, dehydrated, embedded in paraffin, and cut into 5 μm sections. Then the sections were performed Hematoxylin and Eosin (H&E) staining.

2.5. Primary ASMC isolation and culture

Mouse ASMCs were isolated as previously described [22]. Trachea of mice were isolated, digested with 0.2% collagenase IV (Sigma-Aldrich, St. Louis, USA) and 0.05% elastase (Sigma-Aldrich) for 30 min at 37°C , and centrifuged at 500g for 5 min. The pellet containing ASMCs was re-suspended with 1:1 DMEM: F12 (Thermo scientific, Waltham, MA, USA) containing 10% fetal bovine serum (FBS) and then ASMCs were seeded into 6-well plates and cultured at 37°C with 5% CO_2 . The cells at passage 3–6 were chosen for the subsequent experiments. ASMCs were treated with 10 ng/mL TGF- β 1 in the presence or absence of rhIL-37 (0.1 ng/mL) for 48 h.

To investigate the molecular mechanism of IL-37 in ASMCs, NF- κ B inhibitor PDTC (100 μM) and constructed pcDNA3.1-STAT3 plasmid were used. Briefly, PDTC (100 μM) was used 2 h prior to treatment with TGF- β 1 stimulation. 1 μg of pcDNA3.1-STAT3 plasmid was transfected into ASMCs that had been cultured overnight. 24 h after transfection, ASMCs were treated with rhIL-37 and TGF- β 1 as described above.

2.6. Cell proliferation assay

ASMC proliferation was determined by the MTT assay. Briefly, ASMCs were seeded into 96-well plates at a density of $1 \times 10^3/\text{well}$ and incubated with designated treatments. 48 h after TGF- β 1 stimulation, the culture medium was changed to fresh medium with 500 $\mu\text{g}/\text{mL}$ MTT (Sigma-Aldrich), and the plates were incubated for an additional 4 h at 37°C . 100 μL DMSO was used to dissolve formazan crystals after removal of the supernatant. The absorbance was recorded at 490 nm by using a microplate reader.

2.7. Transwell analysis

The migration of ASMCs was analyzed by Transwell. ASMCs, which had been stimulated with TGF- β 1 for 48 h, were seeded in the upper chambers with serum-free medium. The lower chambers were filled with medium containing 20% FBS to induce cell migration. After incubation for 24 h, a cotton swab was used to remove the cells attached to the upper surface. The cells attached to the lower surface were fixed with 10% methanol and then stained with 0.1% crystal violet. Lastly, stained cells were counted by using a microscope and six random fields were analyzed for each well.

2.8. Real-time PCR

Total RNA from cells and lung tissues was extracted by Trizol Regent (Invitrogen, Carlsbad, CA, USA) according to the manufactures' instructions. 2 μg of total RNA was reversely transcribed into cDNA by using the Prime-Script RT reagent kit (Takara, Tokyo, Japan). Real-time PCR was performed with SYBR Green (TaKaRa) on the ABI 7500 Fast Real Time PCR system (Applied Biosystems, Foster City, CA, USA). Primers used in this study were as follows: IL-4 (forward: 5'-CACGGA TGCAACGACAATCA-3', reverse: 5'-GAGGACGTTTGGCACATCCA-3'); IL-6 (forward: 5'-GAGGATACCACTCCCAACAGACC-3', reverse: 5'-AAG TGCATCATCGTTGTTTCATACA-3'); IL-13 (forward: 5'-CCCTGGATTCC CTGACCAAC-3', reverse: 5'-TTTTGGTATCGGGGAGGCTG-3'); IFN- γ (forward: 5'-TCAAGTGGCATAGATGTGGAAGAA-3', reverse: 5'-TGGCT CTGCAGGATTTTCATG-3'); E-cadherin (forward: 5'-GTTTACCCAGGA GGTGTTTGTAG-3', reverse: 5'-GTGTTGACGTCTCATCTCTGC-3'); N-cadherin (forward: 5'-CCTCCAACGGGCATCTTCAT-3', reverse: 5'-TGTC

ACTGCATGTGCTCTC-3'); Vimentin (forward: 5'-AGCAGTATGAAAGC GTGGCT-3', reverse: 5'-CCGGTACTCGTTTGACTCCT-3'); GAPDH (forward: 5'-CATGGCCTTCGTGTTTCTA-3', reverse: 5'-TGCTTCACCACC TTCTTGATG-3'). The relative expression of the target genes was calculated by the $2^{-\Delta\Delta CT}$ method with normalization to GAPDH.

2.9. Western blot and ELISA assay

Mouse lungs and cultured cells were lysed in RIPA lysis buffer with 10 mM PMSF for 30 min on ice. The total protein concentration was determined by the BCA Protein Assay Kit (Generay, Shanghai, China). 30 μ g of proteins per lane were separated by SDS-PAGE and immunoblotting using standard protocols. Primary antibodies against I κ B, NF- κ B p65 (p65), phospho-NF- κ B p65 (p-p65), STAT3, p-STAT3, E-cadherin, N-cadherin, and Vimentin were all from Abcam (Cambridge, MA, USA).

Levels of IL-4, IL-6, IL-13, and IFN- γ in mouse BALF and in the supernatant of ASMCs were detected by using commercially available ELSA kits (ExCell Bio, Shanghai, China) according to the manufacturer's protocols.

2.10. Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Differences between groups were analyzed by GraphPad Prism 5 software (GraphPad, San Diego, CA) with one-way ANOVA followed by Bonferroni's multiple comparison tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. IL-37 attenuates asthmatic symptoms in mice

We firstly confirmed the role of rhIL-37 in OVA-induced mouse models of allergic asthma. The results showed that compared with the control group, OVA significantly increased AHR, which was significantly reduced by IL-37 (Fig. 1A). Also, the numbers of total inflammatory cells, macrophages, lymphocytes, neutrophils, and eosinophils in mouse BALF in the OVA group were higher than the control group; compared with the OVA group, IL-37 notably reduced these cell numbers (Fig. 1B). In addition, HE staining revealed that compared with the control group, the inflammation was more obvious in the OVA

group; compared with the OVA group, IL-37 treatment markedly decreased the inflammation (Fig. 1C). These results indicate that IL-37 can attenuate OVA-induced asthmatic symptoms.

3.2. IL-37 modulates Th1/Th2 balance and epithelial-mesenchymal-transition (EMT) process in OVA-induced allergic mouse models

Since the imbalance of Th1/Th2 contributes to the initiation and mediation of asthma [23], we further evaluated the effect of IL-37 on the levels of Th1 and Th2 cytokines. As shown in Fig. 2A–D, the mRNA expression of IL-4, IL-6 and IL-13 induced by OVA was significantly reduced by IL-37, whereas IFN- γ mRNA expression was upregulated by IL-37 in mouse lungs. Moreover, the protein levels of these cytokines in BALF were similar to the mRNA expression in lung tissues (Fig. 2E–H). These results suggest that IL-37 regulates Th1/Th2 balance in asthma mouse models.

EMT is a process by which epithelial cells lose polarity and intercellular contacts, and exhibit a mesenchymal phenotype. Increased EMT has a disruptive effect on the capacity to regenerate an effective epithelial barrier, which in turn facilitates a chronic cycle of inflammation and airway remodeling [24]. We further analyzed the levels of epithelial (E-cadherin) and mesenchymal markers (N-cadherin and Vimentin) in mouse lungs by Real-time PCR. The results showed that E-cadherin expression was notably downregulated, and the levels of N-cadherin and Vimentin were increased in the OVA group compared with the control group. Compared with the OVA group, IL-37 significantly elevated the expression of E-cadherin, and decreased the levels of N-cadherin and Vimentin (Fig. 2I–K). Taken together, these data demonstrated that IL-37 can inhibit the EMT process in mouse asthma models.

3.3. IL-37 inhibits NF- κ B and STAT3 signalings in allergic mice

To further explore the molecular mechanism of IL-37 in OVA-induced allergic asthma, we determined the effects of IL-37 on NF- κ B and STAT3 signalings. The results showed that OVA reduced the expression of I κ B, elevated the levels of p65, p-p65, STAT3 and p-STAT3. Compared with the OVA group, IL-37 notably increased I κ B expression, and reduced the levels of p65, p-p65, STAT3 and p-STAT3 (Fig. 2L). These results suggest that IL-37 suppresses the activation of NF- κ B and STAT3 signalings in allergic mice.

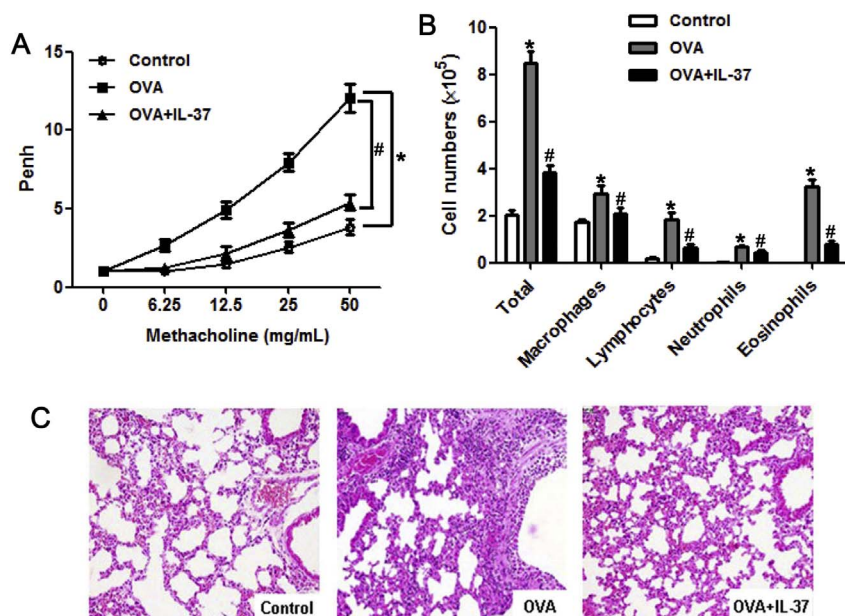


Fig. 1. IL-37 attenuates OVA-induced asthmatic symptoms in mice. (A) AHR of mice in response to inhaled methacholine was determined. (B) IL-37 inhibited OVA-induced inflammatory cell recruitment in BALF. (C) Representative images of H&E staining of lung tissues. $N = 8$, * $P < 0.05$ vs control group, # $P < 0.05$ vs OVA group.

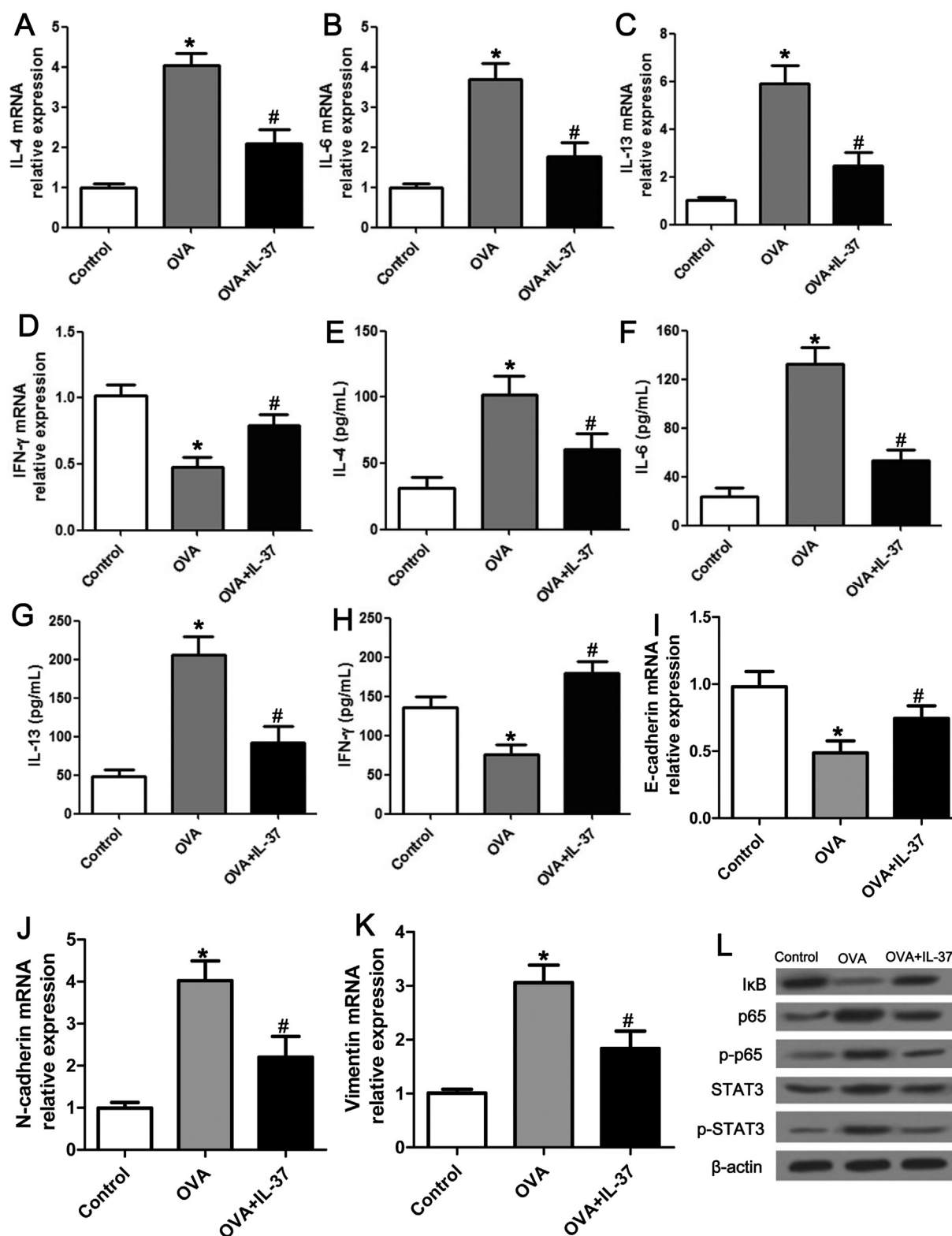


Fig. 2. IL-37 regulates OVA-induced the imbalance of Th1/Th2 balance and EMT, and suppresses the activation of NF- κ B and STAT3. Real-time PCR analysis for the mRNA expression of IL-4 (A), IL-6 (B), IL-13 (C), and IFN- γ (D) in lung tissues. ELISA assay for the protein levels of IL-4 (E), IL-6 (F), IL-13 (G), and IFN- γ (H) in mouse BALF. The mRNA expression of E-cadherin (I), N-cadherin (J), and Vimentin (K) in lung tissues was assayed by Real-time PCR. (L) The protein levels of I κ B, p65, p-p65, STAT3, and p-STAT3 were determined by Western blot. N = 8, *P < 0.05 vs control group, #P < 0.05 vs OVA group.

3.4. IL-37 suppresses TGF- β 1-induced proliferation, migration, EMT, and inflammatory response in ASMCs

We further investigated the role of IL-37 in TGF- β 1-induced ASMCs.

As shown in Fig. 3A and B, the proliferation and migration induced by TGF- β 1 were dramatically inhibited by IL-37. EMT plays a vital role to facilitate the migration of ASMCs [24]. Hence, we assessed changes in expression of E-cadherin, N-cadherin and Vimentin and the results

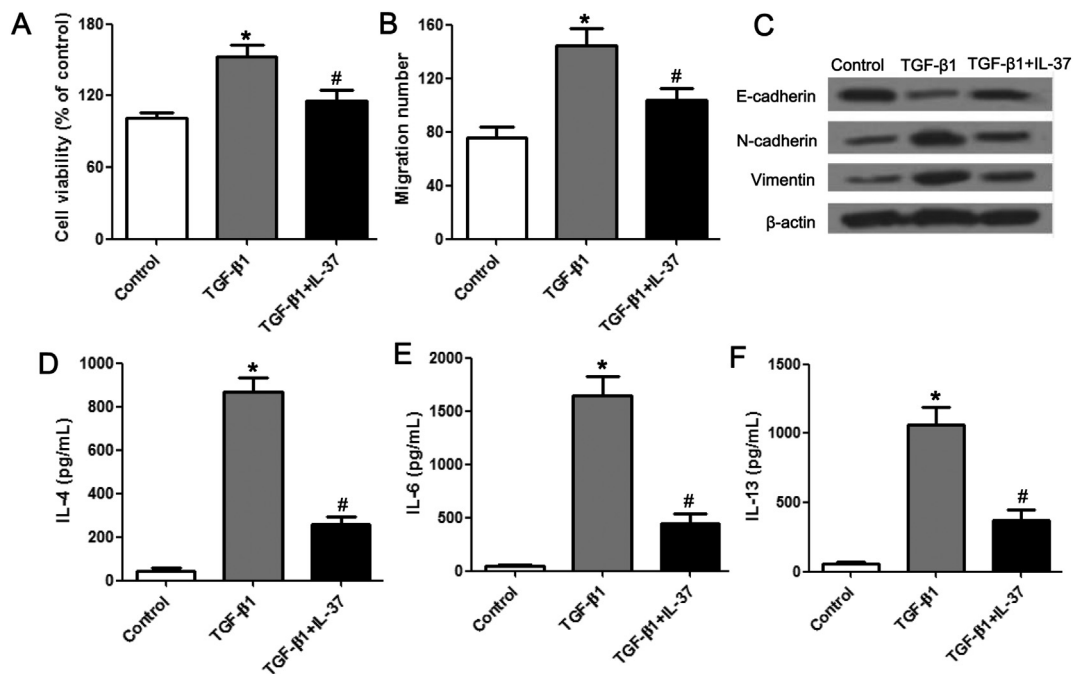


Fig. 3. IL-37 suppresses TGF-β1-induced proliferation, migration, EMT, and inflammatory response in ASMCs. (A) Cell viability was determined by MTT. (B) Tranwell analysis for the migration of ASMCs in different groups. (C) The protein levels of E-cadherin, N-cadherin, and Vimentin were detected by Western blot in ASMCs. Levels of IL-4 (D), IL-6 (E), and IL-13 (F) were examined in the supernatant of ASMCs. N = 3, **P* < 0.05 vs control group, #*P* < 0.05 vs TGF-β1 group.

demonstrated that compared with the TGF-β1 group, IL-37 notably elevated E-cadherin expression, while decreased the expression of N-cadherin and Vimentin (Fig. 3C). Additionally, compared with the control group, TGF-β1 remarkably upregulated the levels of IL-4, IL-6, and IL-13. However, IL-37 treatment notably decreased these pro-inflammatory cytokine levels (Fig. 3D–F). Collectively, these data indicate that IL-37 suppresses TGF-β1-induced proliferation, migration, EMT, and inflammatory response in ASMCs.

3.5. IL-37 functions in TGF-β1-induced ASMCs via inhibiting the activation of NF-κB and STAT3

To confirm whether IL-37 functions in TGF-β1-stimulated ASMCs via suppressing NF-κB and STAT3 signalings, we firstly measured the levels of IκB, p65, p-p65, STAT3 and p-STAT3. The results showed that compared with the TGF-β1 group, IL-37 markedly elevated IκB expression and decreased the levels of p65, p-p65, STAT3 and p-STAT3 (Fig. 4A). Furthermore, we determined the effects of NF-κB inhibitor PDTC on TGF-β1-induced cell proliferation, migration, EMT, and inflammation. The proliferation and migration induced by TGF-β1 were significantly suppressed by PDTC (Fig. 4B and C). Compared with the TGF-β1 group, PDTC markedly upregulated E-cadherin expression, and decreased the levels of N-cadherin, Vimentin and IL-6 (Fig. 4E and F). In addition, we also overexpressed STAT3 expression by pcDNA3.1-STAT3 plasmid in ASMCs. The results showed that pcDNA3.1-STAT3 but not the empty plasmid significantly upregulated STAT3 expression (Fig. 4D). Compared with IL-37 treated only, STAT3 overexpression dramatically enhanced cell proliferation, migration, EMT, and IL-6 level (Fig. 4B, C, E, and F). Taken together, these results suggest that IL-37 attenuates TGF-β1-induced ASMC proliferation, migration, EMT, and inflammation via suppressing the activation of NF-κB and STAT3.

4. Discussion

Asthma is one of the most common chronic inflammatory diseases of childhood with an increasing prevalence worldwide [25]. Inhaled corticosteroids is a well-known therapy to treat asthma, however, this

strategy can reduce bone mineral density and growth of children [25]. Hence, it is urgent to explore more effective treatment of pediatric asthma. Previous study has demonstrated that sex hormones can affect the response to inhaled antigen [26]. In our study, the mice were randomly divided into three groups, which could offset the bias of sex hormones. Studies have shown that IL-37 was downregulated in asthmatic children compared with healthy control, and it could alleviate hallmarks of experimental asthma in mice [14,20]. Consistent with these results, we also found IL-37 weakened OVA-induced AHR and inflammation in mice (Fig. 1).

The imbalance of Th1/Th2 plays important roles in the development of asthma [27]. Th2 cells are the key T helper cell subset that contributes to the pathogenesis of asthma [28]. IL-4 triggers the differentiation of Th2 cells, leading to the enhancement of their effector cytokines including IL-4, IL-6, and IL-13 [28,29]. Th2 cytokines is responsible for the induction of allergen-specific immunoglobulin E production and inflammatory mediator release [30]. Th1 cells can counter regulate Th2 responses [28]. IFN-γ is a vital cytokine secreted by Th1 cells. Han et al. [31] demonstrated that rhinovirus infection, which is closely related to asthma development in children, fails to induce lung IFN-γ expression in immature mice. Intranasal IFN-γ administration attenuated rhinovirus-induced IL-13 expression and mucous metaplasia [31]. In this study, we found compared with the OVA group, IL-37 significantly reduced the levels of IL-4, IL-6 and IL-13 both in BALF and lung tissues of mice. IFN-γ level was notably elevated by IL-37 compared with the OVA group (Fig. 2). Regulatory T (Treg) cells and Th17 cells are also associated with asthmatic inflammation. Regulatory T (Treg) cells play vital roles in suppressing airway inflammation. The numbers of Treg cells were decreased in patients with allergic asthma [32]. Th17 cells participate in airway neutrophil infiltration, which contributes to the pathogenesis of severe asthma [33].

The excessive proliferation of ASMCs causes increased airway smooth muscle mass, leading to persistent airway inflammation and remodeling responses [4]. Benayoun et al. [34] revealed that ASMCs were found closer to the epithelium in asthma, which implies that ASMC also undergo a migration process in asthma. EMT plays vital roles in airway repair and remodeling in many respiratory diseases

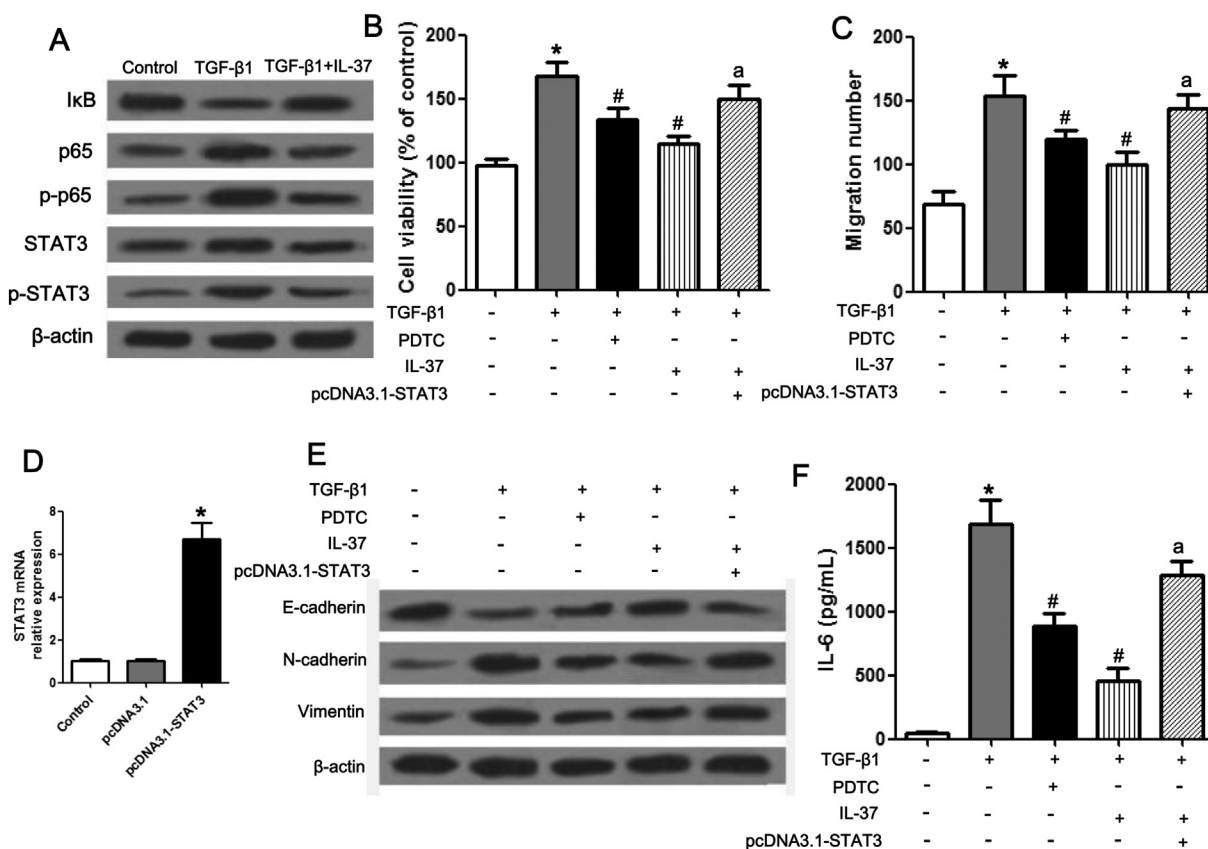


Fig. 4. IL-37 alleviates TGF-β1-induced cell proliferation, migration, EMT, and inflammation via inhibiting the activation of NF-κB and STAT3 signaling pathway. (A) Western blot was used to detect the protein levels of IκB, p65, p-p65, STAT3, and p-STAT3 in ASMCs. (B) MTT assay for the viability of ASMCs. (C) The migration of ASMCs with designed treatment was analyzed by Transwell. (D) STAT3 mRNA expression was analyzed by Real-time PCR. (E) Levels of E-cadherin, N-cadherin, and Vimentin were determined by Western blot in ASMCs. (F) IL-6 levels in the supernatant of ASMCs were assayed by ELISA. N = 3, *P < 0.05 vs control group, #P < 0.05 vs TGF-β1 group, ^aP < 0.05 vs TGF-β1 + IL-37 group.

including asthma. EMT is involved in the alteration of extracellular matrix, cell migration, and differentiation [35]. Our results demonstrated that IL-37 notably suppressed the EMT process in asthma mouse (Fig. 2I–K). Additionally, we further evaluated the role of IL-37 in TGF-β1-induced ASMCs. We found the proliferation, migration, EMT, and pro-inflammatory cytokines (IL-4, IL-6, and IL-13) induced by TGF-β1 were notably suppressed by IL-37 treatment (Fig. 3).

Suppression of NF-κB signaling is related to the modulation of Th1/Th2 imbalance in allergic airway mouse models [29]. Also, NF-κB pathway inactivation is responsible for the inhibition of TGF-β1-induced ASMC proliferation and migration [36]. IL-37 relieves inflammatory responses via inhibiting NF-κB activation in human aortic valve interstitial cells [19]. However, the relationship between IL-37 and NF-κB signaling remains unknown in asthma. Therefore, we determined the effect of IL-37 on the activation of NF-κB in OVA-induced mouse models as well as in TGF-β1-stimulated ASMCs. The results showed that IL-37 remarkably upregulated IκB expression and down-regulated levels of p65 and p-p65 both in mouse models and in ASMCs, which indicates that IL-37 suppresses NF-κB activation in asthma (Figs. 2L and 4). Moreover, to confirm whether IL-37 alleviates asthma via NF-κB inactivation, PDTC, a NF-κB inhibitor, was used in TGF-β1-induced ASMCs. We found PDTC showed the similar effects as IL-37 treatment, although these effects were weaker than IL-37 (Fig. 4). These results indirectly demonstrated that IL-37 functions partly via suppressing the activation of NF-κB signaling. We speculated IL-37 may also function via other pathways. Studies showed that STAT3 was required for allergic inflammation in a murine model of asthma [17]. The suppression of inflammatory responses by IL-37 is associated with STAT3 inactivation [20]. We further explored the effect of IL-37 on STAT3 signaling. The results demonstrated that IL-37 decreased the

levels of STAT3 and p-STAT3 both in vivo and in vitro. Additionally, STAT3 overexpression notably abrogated the effects of IL-37 on TGF-β1-induced proliferation, migration, EMT, and inflammatory response in ASMCs (Fig. 4). All these data suggest IL-37 functions via suppressing the activation of NF-κB and STAT3. In conclusion, our study shows that IL-37 alleviates airway inflammation in asthmatic mice and inhibits TGF-β1-induced proliferation, migration, EMT, and inflammatory response in ASMCs via suppressing the activation of NF-κB and STAT3. This study provides further insight into the molecular mechanism of IL-37 in allergic asthma.

Conflict of interest

None.

References

- [1] C.H. Fanta, Asthma, *N. Engl. J. Med.* 360 (10) (2009) 1002–1014.
- [2] S. Shimura, Y. Andoh, M. Haraguchi, K. Shirato, Continuity of airway goblet cells and intraluminal mucus in the airways of patients with bronchial asthma, *Eur. Respir. J.* 9 (7) (1996) 1395–1401.
- [3] A. Bush, L. Fleming, S. Saglani, Severe asthma in children, *Respirology* 22 (5) (2017) 886–897.
- [4] M.M. Perry, J.E. Baker, D.S. Gibeon, I.M. Adcock, K.F. Chung, Airway smooth muscle hyperproliferation is regulated by microRNA-221 in severe asthma, *Am. J. Respir. Cell Mol. Biol.* 50 (1) (2014) 7–17.
- [5] S. Dragon, S.J. Hirst, T.H. Lee, A.S. Gounni, IL-17A mediates a selective gene expression profile in asthmatic human airway smooth muscle cells, *Am. J. Respir. Cell Mol. Biol.* 50 (6) (2014) 1053–1063.
- [6] C.A. Ojiaku, E.J. Yoo, R.A. Panettieri Jr., Transforming growth factor beta1 function in airway remodeling and hyperresponsiveness. The missing link? *Am. J. Respir. Cell Mol. Biol.* 56 (4) (2017) 432–442.
- [7] D.B. Wright, T. Triana, S. Siddiqui, C.D. Pascoe, J.R. Johnson, B.G. Dekkers, S. Dakshinamurti, R. Bagchi, J.K. Burgess, V. Kanabar, O.O. Ojo, Phenotype

- modulation of airway smooth muscle in asthma, *Pulm. Pharmacol. Ther.* 26 (1) (2013) 42–49.
- [8] C.A. Dinarello, C. Nold-Petry, M. Nold, M. Fujita, S. Li, S. Kim, P. Bufler, Suppression of innate inflammation and immunity by interleukin-37, *Eur. J. Immunol.* 46 (5) (2016) 1067–1081.
 - [9] Q. Ji, K. Meng, K. Yu, S. Huang, Y. Huang, X. Min, Y. Zhong, B. Wu, Y. Liu, S. Nie, J. Zhang, Y. Zhou, Q. Zeng, Exogenous interleukin 37 ameliorates atherosclerosis via inducing the Treg response in ApoE-deficient mice, *Sci. Rep.* 7 (1) (2017) 3310.
 - [10] B. Wu, K. Meng, Q. Ji, M. Cheng, K. Yu, X. Zhao, H. Tony, Y. Liu, Y. Zhou, C. Chang, Y. Zhong, Z. Zhu, W. Zhang, X. Mao, Q. Zeng, Interleukin-37 ameliorates myocardial ischaemia/reperfusion injury in mice, *Clin. Exp. Immunol.* 176 (3) (2014) 438–451.
 - [11] Q. Zeng, R. Song, D.A. Fullerton, L. Ao, Y. Zhai, S. Li, D.B. Ballak, J.C. Cleveland Jr., T.B. Reece, T.A. McKinsey, D. Xu, C.A. Dinarello, X. Meng, Interleukin-37 suppresses the osteogenic responses of human aortic valve interstitial cells in vitro and alleviates valve lesions in mice, *Proc. Natl. Acad. Sci. U. S. A.* 114 (7) (2017) 1631–1636.
 - [12] L. Ye, B. Jiang, J. Deng, J. Du, W. Xiong, Y. Guan, Z. Wen, K. Huang, Z. Huang, IL-37 alleviates rheumatoid arthritis by suppressing IL-17 and IL-17-triggering cytokine production and limiting Th17 cell proliferation, *J. Immunol.* 194 (11) (2015) 5110–5119.
 - [13] L. Ye, L. Ji, Z. Wen, Y. Zhou, D. Hu, Y. Li, T. Yu, B. Chen, J. Zhang, L. Ding, J. Du, Z. Huang, IL-37 inhibits the production of inflammatory cytokines in peripheral blood mononuclear cells of patients with systemic lupus erythematosus: its correlation with disease activity, *J. Transl. Med.* 12 (2014) 69.
 - [14] R. Charrad, A. Berraies, B. Hamdi, J. Ammar, K. Hamzaoui, A. Hamzaoui, Anti-inflammatory activity of IL-37 in asthmatic children: correlation with inflammatory cytokines TNF- α , IL- β , IL-6 and IL-17A, *Immunobiology* 221 (2) (2016) 182–187.
 - [15] L. Lunding, S. Webering, C. Vock, A. Schroder, D. Raedler, B. Schaub, H. Fehrenbach, M. Wegmann, IL-37 requires IL-18R α and SIGIRR/IL-1R8 to diminish allergic airway inflammation in mice, *Allergy* 70 (4) (2015) 366–373.
 - [16] Y.P. Hwang, S.W. Jin, J.H. Choi, C.Y. Choi, H.G. Kim, S.J. Kim, Y. Kim, K.J. Lee, Y.C. Chung, H.G. Jeong, Inhibitory effects of l-theanine on airway inflammation in ovalbumin-induced allergic asthma, *Food Chem. Toxicol.* 99 (2017) 162–169.
 - [17] M.C. Simeone-Penney, M. Severgnini, P. Tu, R.J. Homer, T.J. Mariani, L. Cohn, A.R. Simon, Airway epithelial STAT3 is required for allergic inflammation in a murine model of asthma, *J. Immunol.* 178 (10) (2007) 6191–6199.
 - [18] A.C. Gavino, K. Nahmod, U. Bharadwaj, G. Makedonas, D.J. Twardy, STAT3 inhibition prevents lung inflammation, remodeling, and accumulation of Th2 and Th17 cells in a murine asthma model, *Allergy* 71 (12) (2016) 1684–1692.
 - [19] Q. Zhan, Q. Zeng, R. Song, Y. Zhai, D. Xu, D.A. Fullerton, C.A. Dinarello, X. Meng, IL-37 suppresses MyD88-mediated inflammatory responses in human aortic valve interstitial cells, *Mol. Med.* 23 (2017).
 - [20] C.A. Nold-Petry, C.Y. Lo, I. Rudloff, K.D. Elgass, S. Li, M.P. Gantier, A.S. Lotz-Havla, S.W. Gersting, S.X. Cho, J.C. Lao, A.M. Ellisdon, B. Rotter, T. Azam, N.E. Mangan, F.J. Rossello, J.C. Whisstock, P. Bufler, C. Garlanda, A. Mantovani, C.A. Dinarello, M.F. Nold, IL-37 requires the receptors IL-18R α and IL-1R8 (SIGIRR) to carry out its multifaceted anti-inflammatory program upon innate signal transduction, *Nat. Immunol.* 16 (4) (2015) 354–365.
 - [21] D. Sun, J. Wang, N. Yang, H. Ma, Matrine suppresses airway inflammation by downregulating SOCS3 expression via inhibition of NF- κ B signaling in airway epithelial cells and asthmatic mice, *Biochem. Biophys. Res. Commun.* 477 (1) (2016) 83–90.
 - [22] Q. Wang, H. Li, Y. Yao, G. Lu, Y. Wang, D. Xia, J. Zhou, HB-EGF-promoted airway smooth muscle cells and their progenitor migration contribute to airway smooth muscle remodeling in asthmatic mouse, *J. Immunol.* 196 (5) (2016) 2361–2367.
 - [23] V. Mishra, V. Baranwal, R.K. Mishra, S. Sharma, B. Paul, A.C. Pandey, Titanium dioxide nanoparticles augment allergic airway inflammation and Socs3 expression via NF- κ B pathway in murine model of asthma, *Biomaterials* 92 (2016) 90–102.
 - [24] F. Moheimani, H.M. Roth, J. Cross, A.T. Reid, F. Shaheen, S.M. Warner, J.A. Hirota, A. Kicic, T.S. Hallstrand, M. Kahn, S.M. Stick, P.M. Hansbro, T.L. Hackett, D.A. Knight, Disruption of beta-catenin/CBP signaling inhibits human airway epithelial-mesenchymal transition and repair, *Int. J. Biochem. Cell Biol.* 68 (2015) 59–69.
 - [25] M.S. Jassal, Special considerations—asthma in children, *Int. Forum Allergy Rhinol.* 5 (Suppl. 1) (2015) S61–7.
 - [26] Y. Rizzo-Vasquez, A.P. Ligeiro de Oliveira, C.P. Page, D. Spina, W. Tavares-de-Lima, Role of sex hormones in allergic inflammation in mice, *Clin. Exp. Allergy* 37 (3) (2007) 459–470.
 - [27] Y.H. Hwang, M.J. Paik, S.T. Yee, Diisononyl phthalate induces asthma via modulation of Th1/Th2 equilibrium, *Toxicol. Lett.* 272 (2017) 49–59.
 - [28] M. Berker, L.J. Frank, A.L. Gessner, N. Grassl, A.V. Holtermann, S. Hoppner, C. Kraef, M.D. Leclaire, P. Maier, D.A. Messerer, L. Mohrmann, J.P. Niekke, D. Schoch, D. Soll, C.M. Woopen, Allergies - A T cells perspective in the era beyond the Th1/Th2 paradigm, *Clin. Immunol.* 174 (2017) 73–83.
 - [29] T.T. Bui, C.H. Piao, S.M. Kim, C.H. Song, H.S. Shin, C.H. Lee, O.H. Chai, Citrus tachibana leaves ethanol extract alleviates airway inflammation by the modulation of Th1/Th2 imbalance via inhibiting NF- κ B signaling and histamine secretion in a mouse model of allergic asthma, *J. Med. Food* 20 (7) (2017) 676–684.
 - [30] M. Kubo, Innate and adaptive type 2 immunity in lung allergic inflammation, *Immunol. Rev.* 278 (1) (2017) 162–172.
 - [31] M. Han, J.Y. Hong, S. Jaipalli, C. Rajput, J. Lei, J.L. Hinde, Q. Chen, N.M. Hershenon, J.K. Bentley, M.B. Hershenon, IFN- γ blocks development of an asthma phenotype in rhinovirus-infected baby mice by inhibiting type 2 innate lymphoid cells, *Am. J. Respir. Cell Mol. Biol.* 56 (2) (2017) 242–251.
 - [32] J. Skuljec, M. Chmielewski, C. Happle, A. Habener, M. Busse, H. Abken, G. Hansen, Chimeric antigen receptor-redirected regulatory T cells suppress experimental allergic airway inflammation, a model of asthma, *Front. Immunol.* 8 (2017) 1125.
 - [33] A. Jia, Y. Wang, W. Sun, B. Xiao, Y. Wei, L. Qiu, L. Mu, L. Xu, J. Li, X. Zhang, D. Liu, C. Peng, D. Zhang, X. Xiang, MBD2 regulates Th17 cell differentiation and experimental severe asthma by affecting IRF4 expression, *Mediat. Inflamm.* 2017 (2017) 6249685.
 - [34] L. Benayoun, A. Druilhe, M.C. Dombret, M. Aubier, M. Pretolani, Airway structural alterations selectively associated with severe asthma, *Am. J. Respir. Crit. Care Med.* 167 (10) (2003) 1360–1368.
 - [35] A. Ge, Y. Ma, Y.N. Liu, Y.S. Li, H. Gu, J.X. Zhang, Q.X. Wang, X.N. Zeng, M. Huang, Diosmetin prevents TGF- β 1-induced epithelial-mesenchymal transition via ROS/MAPK signaling pathways, *Life Sci.* 153 (2016) 1–8.
 - [36] M. Chen, J.T. Shi, Z.Q. Lv, L.J. Huang, X.L. Lin, W. Zhang, R.Y. Liang, Y.Q. Li, S.P. Jiang, Triptolide inhibits TGF- β 1 induced proliferation and migration of rat airway smooth muscle cells by suppressing NF- κ B but not ERK1/2, *Immunology* 144 (3) (2015) 486–494.