FSTL1 aggravates OVA-induced allergic airway inflammation by activating NLRP3 inflammasome

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Research

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Abstract

Background Asthma is a common respiratory disease characterized by chronic airway inflammation. As a novel inflammatory mediator, follistatin-like protein 1 (FSTL1) can activate immune reaction, suggesting that it may contribute to inflammatory disorders such as asthma. Besides, there are growing evidences that nucleotide-binding domain and leucine-rich repeat protein 3 (NLRP3) / Interleukin (IL)-1β axis participates in asthma. In this study, we investigated the role of FSTL1 in allergic airway inflammation and its underlying mechanism of activating NLRP3 inflammasome. Methods Circulating FSTL1 and IL-1β levels were quantified in serum of asthmatic patients and controls. Whole-body ablation Fstl1 heterozygous mice (Fstl1 +/-) and control group were assessed after the experimental treatment. The effects of FSTL1 on NLRP3 inflammasome were also tested in primary macrophages of mice in vitro. Results The concentration of FSTL1 and IL-1β in serum of asthmatic patients were elevated compared with controls and were positively correlated. FSTL1 deficiency ameliorated infiltration of inflammatory cells,corresponding pathological changes,cytokine responses (IL-1β, IL-5,IL-13), mucous hypersecretion and hyper-responsiveness of airway after Ovalbumin (OVA) exposure in the mouse model. Additionally, inhibition of NLRP3 with MCC950 attenuated FSTL1-induced activation of NLRP3 inflammasome and airway inflammation in vivo and vitro. Conclusions Our data showed that FSTL1 played an important role in allergic airway inflammation by activating NLRP3 inflammasome, providing the possibility that FSTL1 could be applied as a therapeutic strategy on asthma.

Introduction

Asthma is a common respiratory disease affecting 1–18% of the population in different countries, and is also recognized as the most common chronic disease in children[1].It is a chronic inflammatory disease characterized by airway inflammatory infiltration mainly consisting of eosinophils and $CD4^+$ T cells of the Th2 subset, mucus overproduction, epithelial destruction, smooth muscle hypertrophy and airway hyper-responsiveness (AHR). Although current therapeutic strategies such as beta agonists and corticosteroids are commonly used, there are still high morbidity and mortality rates in many patients. Therefore, the search for novel therapeutic targets and strategies are ongoing.

Fstl1, also referred as FRP or TSC-36, is a TGF-β1-inducible secreted glycoprotein belonging to the SPARC family, and is widely generated in nonhematopoietic cells especially in the mesenchymal lineage[2]. The role of FSTL1 has not been fully elucidated. Growing literatures have shown that it is a cardioprotective factor[3, 4], pro-fibrotic agent[5, 6], and is also a bi-directional regulator of tumor based upon the origin of the tumorigenic cell line[7–10]. More importantly, FSTL1 has been demonstrated as a novel proinflammatory molecule, which can activate immune cells and promote gene expression and release of some proinflammatory cytokines/chemokines[2]. Our previous studies revealed that asthmatic patients expressed high levels of FSTL1 in plasma and Bronchoalveolar lavage fluid (BALF) compared to healthy controls[11, 12], so we speculate that FSTL1 may participate in the pathologic process of asthmatic airway inflammation.

The NLRP3 inflammasome complex, composed by NLRP3, adaptor molecule apoptosis-associated speck-like protein containing a caspase recruitment domain (ASC) and pro-caspase 1, is a multiprotein oligomer that is identified to play a key role in innate immunity. After the pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) are recognized, inflammasome components assemble, self-oligomerize, then active caspase-1 and cleave precursor pro-IL-1β into its mature bioactive form IL-1β. NLRP3 inflammasome takes effect in host defense against infection, whereas it may lead to serious inflammatory conditions associated with a wide spectrum of inflammatory diseases when aberrantly being activated. The function of NLRP3 inflammasome-mediated IL-1β on allergic airway inflammation is controversial, but more evidences support its role in exacerbating disease. Many studies have proved the contribution of IL-1β to airway inflammatory infiltration (both neutrophilia and eosinophilia), differentiating and activating Th2 cells that lead to Th2 cytokines release[13–16], recruitment of dendritic cell into lymph nodes[17], promoting Th17 inflammatory responses corelating with the severity of asthma[18], making hypersecretion from goblet cells and initiating the airway smooth muscle (ASM) hyper-reactivity[19, 20]. MCC950 is a small-molecule and selective cytokine release inhibitor, which can specifically block NLRP3 activation and reduce IL-1β production[21]. It is thus a potential therapeutic method for NLRP3-associated syndromes and may alleviate IL-1β-induced airway inflammation.

In this study, we confirm the proinflammatory role of FSTL1 by using an OVA-induced asthmatic model in murine. We also find that FSTL1 can activate NLRP3 and increase NLRP3 inflammasome-mediated IL-1β secretion in vivo and in vitro. We further provide an evidence that targeting FSTL1 may offer a potential therapeutic approach for asthmatics.

Materials And Methods

Subjects

30 asthma patients were enrolled into the study. They were all inpatients of Qilu Hospital of Shandong University, and were diagnosed with asthma according to the Global Initiative for Asthma (2019 edition). We also recruited 30 subjects who visited our hospital for a routine medical checkup but did not have any respiratory condition as no asthmatic controls. All subjects (asthmatics and controls) had no history of heart failure, renal failure, autoimmune diseases, lung disease, tumor and infection within 2 weeks. The study was approved by the Ethics Review Committee for Human Studies at Qilu Hospital of Shandong University(Grant NO. KYLL-2017(ks)-112).

Cell isolation and culture

Primary macrophages (PMs) were prepared referred to previous methods[22]. 12-week-old C57BL/6 mouse was intraperitoneally (i.p.) injected with 2ml of 3.0% sterile thioglycolate medium. 96 hours later, mouse was euthanized, lavished intraperitoneally with 5-10 ml of Dulbecco's Modified Eagle Medium (DMEM) for 3 consecutive times to collect peritoneal exudate cells. Then, the eluent was centrifuged at 1000rpm for 5 min at 4°C, and the cell pellet was resuspended in 5-10 ml of DMEM supplemented with

10% fetal bovine serum (FBS), 100U/ml penicillin and 0.1 mg/ml streptomycin. Then cells were seeded in six-well culture plates at a density of 1 ×10^5 cells per well. After incubation for 2 h at 37°C in a 5% CO_2 humidified atmosphere, cells were washed with PBS for 3 times to remove the unattached cells, and the remaining cells were further cultured in complete medium as peritoneal macrophages for subsequent experiments. Purity and viability of PMs (both over 95%) was evaluated microscopically by Wright–Giemsa staining and Trypan blue exclusion, respectively.

Mice

C57BL/6 mice, 6-8 weeks of age, were maintained under standard laboratory conditions in the Animal Experimental Center of Shandong University for 1 week before experiments. All protocols were approved by the Institutional Animal Care and Use Committee of Shandong University. $Fstl1^{flox/+}$ mice were generous gifts from Xiang Gao (Nanjing University, Nanjing, China). $Fstl1^{+/-}$ mice were generated as previously described[23].

Model and Grouping

WT mice were randomly divided into 5 groups (n = 6/group) as follows: control group, OVA group, FSTL1 intranasal group, PBS intranasal group and FSTL1+MCC950 group. $FSTL1^{+/-}$ mice were randomly divided into 2 groups (n = 6/group): control group and OVA group. OVA-induced allergic airway inflammatory model was established as previously reported[24], with some modifications (Fig. 2a). Briefly, mice were sensitized by i.p. injection of 50 ug OVA (grade V, Sigma, St. Louis, MO, USA) without adjuvant in 100ul PBS on days 0, 7, 14 and followed by intranasal administration of 10 ug OVA in 40 ul PBS on days 21–28. Control mice were treated in corresponding manner with isometric PBS alone. Mice of FSTL1 group were intranasally administered with 10ug FSTL1 (Sino Biological, Beijing, China) in 40 ul PBS daily for 15 d[25], and PBS intranasal mice were treated with PBS instead. FSTL1+MCC950 group were i.p. injected with 200ug MCC950 (Target Molecule, MA, USA) 2h before each FSTL1 administration.

Assessment of AHR

24 hours after the final treatment, mice were anesthetized (i.p. by 1% pentobarbital, 50 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), intubated, exposed to aerosolized methacholine (Sigma-Aldrich, St. Louis, MO, USA) at increasing concentration of 0, 4, 8, 12 or 16mg/ml, and were respectively measured using the FlexiVent system (SCIREQ, Canada). The results were expressed as changes from baseline data.

BALF collection and analysis

BALF was collected to analyze cellular components and cytokines in supernatants. As previous mentioned[26, 27] after ligating one side of the bronchus, we flushed the other side of lungs with 0.5mL ice-cold PBS for 3 times via tracheal catheter. Approximately, over 80% of the instilled volume was recovered. Then, the BALF samples were centrifuged, supernatants were collected and stored at -80℃ for the following measurement of cytokines. The cell pellets were resuspended in 1ml PBS, and 0.1 ml

suspension was taken out to count the total number of nucleated cells under a cytometer. The differential cell counts were smeared on slides and processed by Wright–Giemsa staining (Sigma-Aldrich, St. Louis, MO, USA). At least 200 cells were classified and counted for each slide by brightfield microscope according to cells morphology, and the counting researchers were blinded to the grouping situations. The other side of lungs without lavage were stored in the Ultra-low temperature freezer or fixed in 10 % formalin for use.

Histological analysis

The lung tissues of mice were fixed in 10% formalin for more than 24h, then dehydrated, paraffin-embedded, and cut into 5μm sections. The sections were dewaxed, rehydrated and stained with Hematoxylin-eosin (H&E) staining and Periodic acid-schiff (PAS) staining. According to previously described methods[28], the severity of peribronchial and perivascular inflammation was graded by lung inflammatory scores of 0-4 , and the goblet cell hyperplasia was evaluated by numerical scores of 0-4 determined by PAS-positive cell in each airway.

Immunohistochemistry(IHC)

Sections were deparaffinized, rehydrated, pretreated with 10mM sodium citrate (pH 6.1) for

antigen recovery, prevented from the endogenous peroxidase by 3% H_2O_2 and blocked with 5% bovine serum. Next, the slides were incubated with primary antibodies of anti-FSTL1 antibody (l:200; Abcam, USA), anti-NLRP3 antibody (l:200; Abcam, USA), anti-pro-caspase 1 antibody (l:200; Proteintech Group Inc., Wuhan, China), anti-Muc5AC antibody (l:250; Boster, China) respectively or PBS overnight at 4˚C. After extensive wash, the sections were incubated with the corresponding HRP-conjugated secondary antigens for 1 hour at room temperature and then developed with DAB solution (Boster, China). At last, the slides were counterstained with hematoxylin for about 2 min. The positive area of the target protein was measured by Image-Pro Plus 6.0 software (Media Cybernetics, USA) at ×400 magnification.

ELISA

The levels of IL-4, IL-5, IL-13、IL-1β in BALF supernatant of mice were detected using ELISA kits(CUSABIO, China) according to the manufacturer's instructions. FSTL1 and IL-1β levels in human serum were also measured by ELISA (Abcam, USA). All the calibrations and analyses were performed in duplicate.

Western blot analysis

Prepared lung tissues and PBS washed adherent cells were homogenized by grinding and lysing using ice-cold RIPA buffer in the presence of protease inhibitors. Then, the homogenate was centrifuged at 12000 rpm for 10 min at 4 °C, and soluble supernatants were taken for protein concentration determination by BCA protein assay kit (Boster, China). After that, equal amounts of protein samples (25μg of total protein each) were boiled at 95 °C for 5 min and separated onto SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride (PVDF) membranes. The

membranes were blocked with 5% non-fat milk for 1 h at room temperature, and then incubated with diluted primary antibodies against NLRP3 (1:1000; CST, MA, USA), pro-caspase1 (1:1,000; Abcam, USA), IL-1β(1:1000; Abcam, USA), GAPDH (1:5000; Abcam, USA) overnight at 4˚C, followed by incubation with HRP-conjugated secondary antibody for 2h at room temperature. The binding of all the antibodies was detected using an enhanced chemiluminescence (ECL) kit (Pierce Biotechnology, USA). All experiments were repeated in triplicate.

Statistical analysis

All data are presented as mean ± standard deviation (mean ± SD). The quantitative analysis of figures of IHC, PAS staining was performed using GraphPad Prism 6 (GraphPad Software Inc, San Diego, CA, USA). The quantitative analysis of IHC was performed using ImageJ. Student's t-test and one-way analysis of variance (ANOVA) were applied to assess differences among groups. Post hoc analysis of SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) was used to evaluate statistical significance. Results were considered statistically significant at p value less than 0.05 ($P < 0.05$).

Results

1. **Up-regulation of FSTL1 and IL-1β expression in serum of asthmatics.**

Characteristics of the asthmatic patients and the controls were described in Table 1, and there are no differences in age and gender between two groups. The levels of circulating FSTL1 and IL-1β in serum were quantified, and they were elevated dramatically in serum from asthma patients compared to the controls ($P<0.01$) (Fig. 1a, b). In addition, there is a positive correlation between the concentration of FSTL1 and IL-1β in serum ($P<0.001$)(Fig. 1c).

2. **FSTL1$^{+/-}$ mice exhibited attenuated infiltration of inflammatory cells and pathological changes after OVA exposure.**

To verify the effect of FSTL1 on airway inflammation in asthma, lung tissues of mice were sectioned , stained with H&E staining, and analyzed for the degree of inflammation. The OVA-sensitized and challenged WT mice exhibited extensive infiltration of inflammatory cells into the peribronchial and perivascular connective tissues areas as compared with the PBS-treated control mice. However, the infiltration was dramatically reduced in FSTL1$^{+/-}$ mice after OVA exposure (Fig. 3a). Besides, the OVA-exposed WT group exhibited significant increases in total cell numbers mainly caused by an influx of monocytes and eosinophils when compared to the control group, and these increases were dramatically alleviated in FSTL1$^{+/-}$ mice after OVA exposure(Fig. 4a-e).

3. **FSTL1 deficiency reduced goblet cell hyperplasia and mucous hypersecretion in the OVA-induced mice.**

PAS and IHC detection with Muc5AC were used to assess the effect of FSTL1 on the mucus production caused by goblet cell hyperplasia in the bronchi. Although many mucus-containing epithelial cells were apparent in the OVA-WT mice when compared to the controls, Strikingly decreasing accumulation of these cells was detected in the OVA-FSTL1$^{+/-}$ mice (Fig. 3b, c).

4. **FSTL1 deficiency inhibited Th cytokine responses in BALF.**

After OVA treatment, the secretion levels of IL-1β, IL-4, IL-5, IL-13 were significantly elevated in BALF from WT mice as compared with the control group. And data shown in Figure 4f-i indicated that the levels of IL-1β, IL-5 and IL-13 in BALF from the OVA-FSTL1+/- mice were significantly decreased compared with the OVA-WT mice. The level of IL-4 in BALF from FSTL1$^{+/-}$ mice was also reduced, but comparing with the control group there was no statistical difference.

5. **FSTL1$^{+/-}$ mice showed less airway resistance in response to methacholine compared with WT mice.**

The airway responsiveness was assessed within 24 hours after the final challenge. Measurement of lung resistance (RL) showed a significantly increasing in response to methacholine inhalation in the OVA-induced asthmatic mice model, but dramatical reduction of RL induced by OVA was observed in FSTL1$^{+/-}$ mice (Fig. 2c), demonstrating that FSTL1 may ameliorate OVA-induced AHR.

6. **OVA-induced NLRP3 inflammasome activation was reduced in FSTL1-deficient mice.**

To determine the effect of FSTL1 on regulating NLRP3 inflammasome in vivo, we examined the expression of the NLRP3 inflammasome components in the lung of mice. As shown in Fig. 5a and 5d , we found dramatically increased NLRP3 immunohistochemical staining in the lung of OVA-WT mice compared with the control group, especially in the alveolar macrophages (AMs), but it was prominently declined in FSTL1$^{+/-}$ mice after OVA exposure. There were no significant differences of pro-caspase 1 detected between FSTL1$^{+/-}$ mice and WT mice after OVA exposure (Fig. 5b, e). But the over-expression of IL-1β induced by OVA exposure was dramatically down-regulated in FSTL1$^{+/-}$ mice compared with WT mice(Fig. 5c, f). Taken together, we suspected that FSTL1 increased the expression of NLRP3, not pro-caspase 1, but it may promote self-cleavage of pro-caspase 1 and then lead to IL-1β production.

7. **FSTL1 promoted activation of NLRP3 inflammasome in PMs.**

Further experiments were performed in vitro to demonstrate the effect of FSTL1 on NLRP3 inflammasome. PMs from peritoneal lavage of C57BL/6 mice were prepared, then stimulated with FSTL1 at different concentrations or at different time points. At last, the expression of NLRP3 were detected. As shown in Fig. 7a , the expression of NLRP3 increased in a dose-dependent
manner when PMs were treated by FSTL1. Besides, we detected the components at different time points, showing that the level of NLRP3 significantly elevated following FSTL1 stimulation for 8h (Fig. 7b). We further blocked the effect of FSTL1 with small interfering RNA (siRNA), and the expression of NLRP3, P10

and IL-1β were decreased accordingly as FSTL1 was silenced. These results demonstrated that FSTL1 could activate NLRP3 inflammasome in vitro (Fig. 7c).

8. **MCC950 treatment attenuated FSTL1-induced activation of NLRP3 inflammasome and airway inflammation.**

We used a small molecule NLRP3 inhibitor, MCC950, to test whether inflammasome blockade alters FSTL1-induced inflammatory recruitment. Firstly, we tested its blockade action in PMs, the expression of NLRP3,P10 and IL-1β was decreased after MCC950 treatment. We designed animal experiment to take further step to reveal the inflammatory inhibition role of MCC950. After FSTL1 intranasal, the inflammation degree of lung tissues from mice increased compared with the PBS control group. However, this airway inflammation was significantly inhibited by pre-injection of MCC950.

Discussion

Asthma is a heterogeneous disease, usually characterized by chronic airway inflammation. Limiting the development of inflammatory processes is essential for asthma control, improving the symptoms and ameliorating lung function. Previous studies from our and other teams have found that FSTL1 played an important role in airway remodeling in asthma[11, 25]. Here, we further investigated the role of FSTL1 in asthmatic airway inflammation for the first time. Our results support that targeting FSTL1 may be a potential treatment strategy for asthmatic patients.

FSTL1, a secreted glycoprotein, participates in many biological processes and involves in a variety of inflammatory diseases and inflammatory conditions as shown in recent evidences [2, 29]. Literatures referred to both anti- and pro-inflammatory effects for FSTL1 have been covered; most support its pro-inflammatory effect, such as in rheumatoid arthritis[30, 31], osteoarthritis[32, 33],juvenile rheumatoid arthritis[34]、Acute Coronary Syndrome[35]、intervertebral disc disease and obeosity[36],[37]. In our study, we demonstrated the pro-inflammatory role of FSTL1 in asthma. Circulation levels of FSTL1 in blood serum of the asthmatic patients were higher compared to the healthy controls. The histological analysis revealed that FSTL1 was induced in mice lung after OVA sensitization. Deletion FSTL1 using the Lys–Cre mouse line significantly attenuated airway inflammation, manifested by down-regulation of airway inflammatory cell infiltration into the lung、inflammatory cell count and inflammatory factors production in BALF. Besides, reduced mucus secretion and inhibited airway reactivity were observed in FSTL1 deficiency mice of asthma. Instead, inflammatory cell infiltration was increased in mice accepted the nasal drip with FSTL1.

The mechanism by which FSTL1 exerts its pro-inflammatory effects is not yet illuminated, which may cover a wide range of signaling pathways and immune regulation. Yury Chaly et al pointed that FSTL1 could be taken up by endotoxin-stimulated monocytes and macrophages, thereby enhancing NLRP3 inflammasome mediated IL-1β secretion from above cells[38]. In this article, we identified that FSTL1 also played its part in promoting asthmatic airway inflammation through activating NLRP3 inflammasome. The NLRP3 inflammasome plays a pivotal role in innate immunity,as well as in some adaptive immune

processes in response to a wide variety of stimuli. Recent research has demonstrated that asthma should be regarded as a crosstalk between the adaptive and innate immune system, both of which play key roles in the initiation and progression of allergic immune responses, rather than simply an adaptive immune disease[39]. We observed that the expression of NLRP3 in the lungs of mice was increased after OVA treatment or intranasal stimulation of FSTL1, while decreased in FSTL1$^{+/-}$ mice compared with WT mice after OVA treatment.

The effect of NLRP3 inflammasome on asthma is controversial,but current evidence would favor a promoting role. Chronic activation of inflammasome induced by many endogenous molecules in tissue-resident immune cells effects pathology such as chronic inflammation or fibrotic responses[40]. Lung-resident macrophages were revealed to have immunomodulatory function because of its ability to link innate and adaptive immune responses during allergic airway inflammation. AMs are important candidates for inducing airway inflammation. Lee YG et al pointed that depletion of AMs attenuated Th2-type allergic inflammation and remodeling in airway in a mouse allergic asthma model[41]. AMs express high levels of NLRP3 mRNA, and are the major source of locally produced IL-1β and IL-18[42]. There are considerable evidences that IL-1β plays essential roles in the progression of asthma. IL-1β is not only associated with neutrophil inflammation, steroid resistance, asthma severity[43], frequent exacerbations[44, 45], but also involved in Th2-type inflammatory responses. KenArae et al reported that DC-derived IL-1β promoted OVA-specific Th2 cell activation, resulting in aggravation of allergic airway eosinophilia depending on an IL-4/IL-13-STAT6 pathway[46]. Antibodies to IL-1β could down-regulate the expression of IL-4, IL-5 and adhesion molecules that recruit inflammatory cells to the lung[13]. Administration of the anti-IL-1β-neutralizing antibody dramatically reduced the increases of IL-4, IL-5, IL-13, tumor necrosis factor-α (TNF-α), Interferon-γ (IFN-γ), IL-17 and KC protein in lung tissues[47]. IL-1R1$^{-/-}$ mice showed reduced pulmonary antibody responses, eosinophilia, and goblet cell mucus production, which are mediated by the Th2 cytokines IL-4, IL-5, and IL-13[16].

Our study showed that there was an obviously positive correlation between FSTL1 and IL-1β in the serum of asthmatic patients. By up-regulating FSTL1 in vitro macrophages, the expressions of NLRP3 and IL-1β were correspondingly increased; after inhibition of NLRP3 with MCC950, FSTL1-induced IL-1β was blocked. FSTL1 stimulation increased NLRP3 and IL-1β expression in biopsies of mice, while the expression was decreased after targeting deletion of FSTL1 in asthmatic model. After intraperitoneal injection of MCC950 in mice, Fstl1-induced airway inflammation was significantly reduced.

However, further work will be needed to illuminate the mechanisms by which FSTL1 is involved in the pathogenesis of asthma. We found FSTL1 promoted the release of inflammatory mediators in asthma by activating NLRP3/IL-1β axis, but it remains unclear whether FSTL1 directly acts on other cells such as T cells, airway epithelial cells and ASM cells, which in turn cause production of a range of inflammatory medium. And the underlying mechanisms need to be identified. Besides, there was no study to look at if differences of FSTL1 levels exist in patients with acute and non-acute exacerbations or in different asthma phenotypes.

Conclusions

Taken together, our results have underscored the key role of FSTL1 in promoting allergic airway inflammation, and that it worked through activating NLRP3 inflammasome. In conclusion, this study and our continuing efforts may provide a novel treatment strategy and/or a diagnostic biomarker for asthma.

Abbreviations

FSTL1: follistatin-like protein 1; NLRP3: nucleotide-binding domain and leucine-rich repeat protein 3; IL: Interleukin；OVA: Ovalbumin；AHR: airway hyper-responsiveness; BALF: Bronchoalveolar lavage fluid； ASC: adaptor molecule apoptosis-associated speck-like protein containing a caspase recruitment domain；PAMPs: pathogen-associated molecular patterns; DAMPs: damage-associated molecular patterns; ASM: airway smooth muscle; i.p.: intraperitoneally; DMEM: Dulbecco's Modified Eagle Medium; FBS: fetal bovine serum; PMs: peritoneal macrophages; H&E:Hematoxylin-eosin; PAS: Periodic acid-schiff; IHC: Immunohistochemistry; PAGE: polyacrylamide gel electrophoresis; PVDF: polyvinylidene difluoride; ECL: enhanced chemiluminescence; ANOVA: analysis of variance; RL: lung resistance; Ams: alveolar macrophages; siRNA: small interfering RNA; TNF-α: tumor necrosis factor-α; IFN-γ: Interferon-γ； FEV1: forced expiratory volume in one second; FVC: forced vital capacity; SD: standard diviation.

Declarations

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Authors' contributions

DL and WY designed the whole experiment and drafted the manuscript. WY, XJW, LS, ZJP, ZRH, ZJT performed the major research. LT, WJF, WJX, CLL collected and analyzed the data. All authors have read and approved the final submitted paper.

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Availability of data and materials

The data generated during the study are available from the corresponding author on reasonable request.

Consent for publication

Not applicable.

Ethics approval and consent to participate

This study was approved by Ethics Review Committee for Human Studies at Qilu Hospital of Shandong University and Institutional Animal Care and Use Committee of Shandong University (Grant NO. KYLL-2017(ks)-112).

Competing interests

The authors declare that they have no competing interests.

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Tables

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

	Healthy subjects	Asthma patients
Sex (M/F)	16/14	17/13
Age (y)	58.90±8.98	60.5±10.91
FEV1 (%predicted)	-	48.50±18.95
FEV1/FVC (%)	-	55.54±11.93

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

Figures

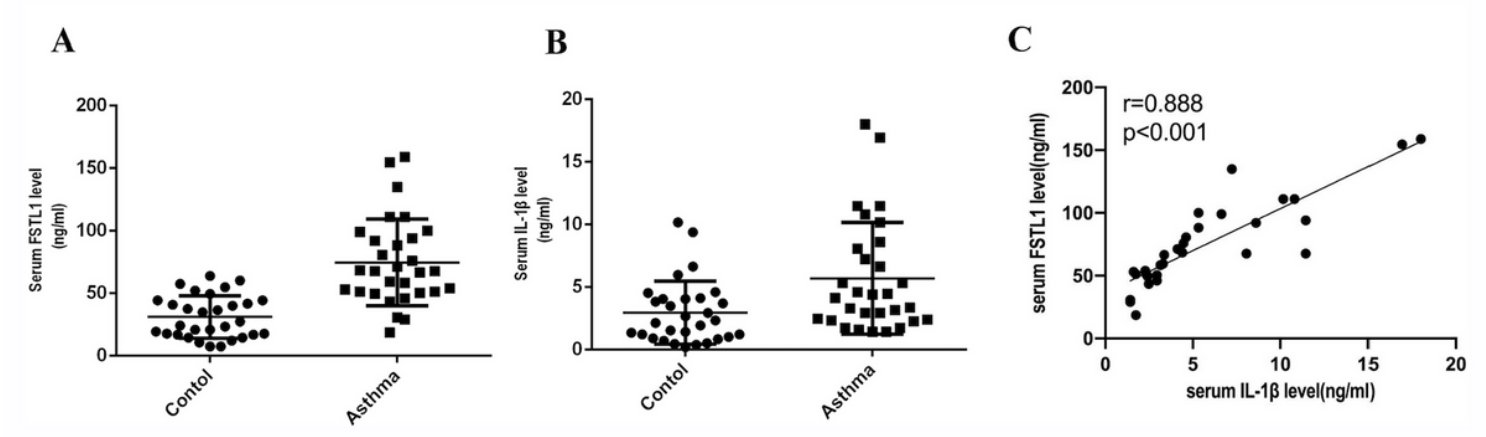

Figure 1

FSTL1 levels in serum of controls and asthmatics (a). IL-1β levels in serum of controls and asthmatics (b). Correlation between FSTL1 and IL-1β levels in serum of asthmatics(c). Correlation was determined by Spearman rank correlation analysis. Data is represented as mean±SD.

Figure 2

The OVA and drug treatment procedures a). Mice were sensitized with OVA by i.p. injection at days 0, 7 and 14, and an OVA challenge was performed by intranasal administration every day from day 21 to day 28. FSTL1 chanllenge and MCC950 inhibition were carried out as shown. The control mice were treated only with PBS. FSTL1 were highly expressed in bronchial epithelium in asthmatic mice compared to controls(b). AHR developed in the different groups (c). Airway resistance was shown as P values. The data represent means ± S.D. (n=6). (*p<0.05 and **p<0.01 vs. the control group. #p<0.05 and ##p<0.01 vs. the model group).

Figure 3

Histologic examination of lung tissues. The lung sections were stained with H&E to evaluate the airway inflammation (3a). PAS staining and IHC detection with Muc5AC were used to asess mucin production (3b, c). Tissue was examined by light microscopy (original magnification ×400). Substantial inflammation and mucin production caused by OVA were reduced by FSTL1 deficiency. Semiquantitative analysis of the severity of airway inflammation and the abundance of PAS-positive cells were defined as described in Materials and Methods. (**p<0.01 vs. the control group. #p<0.05 and ##p<0.01 vs. the model group).

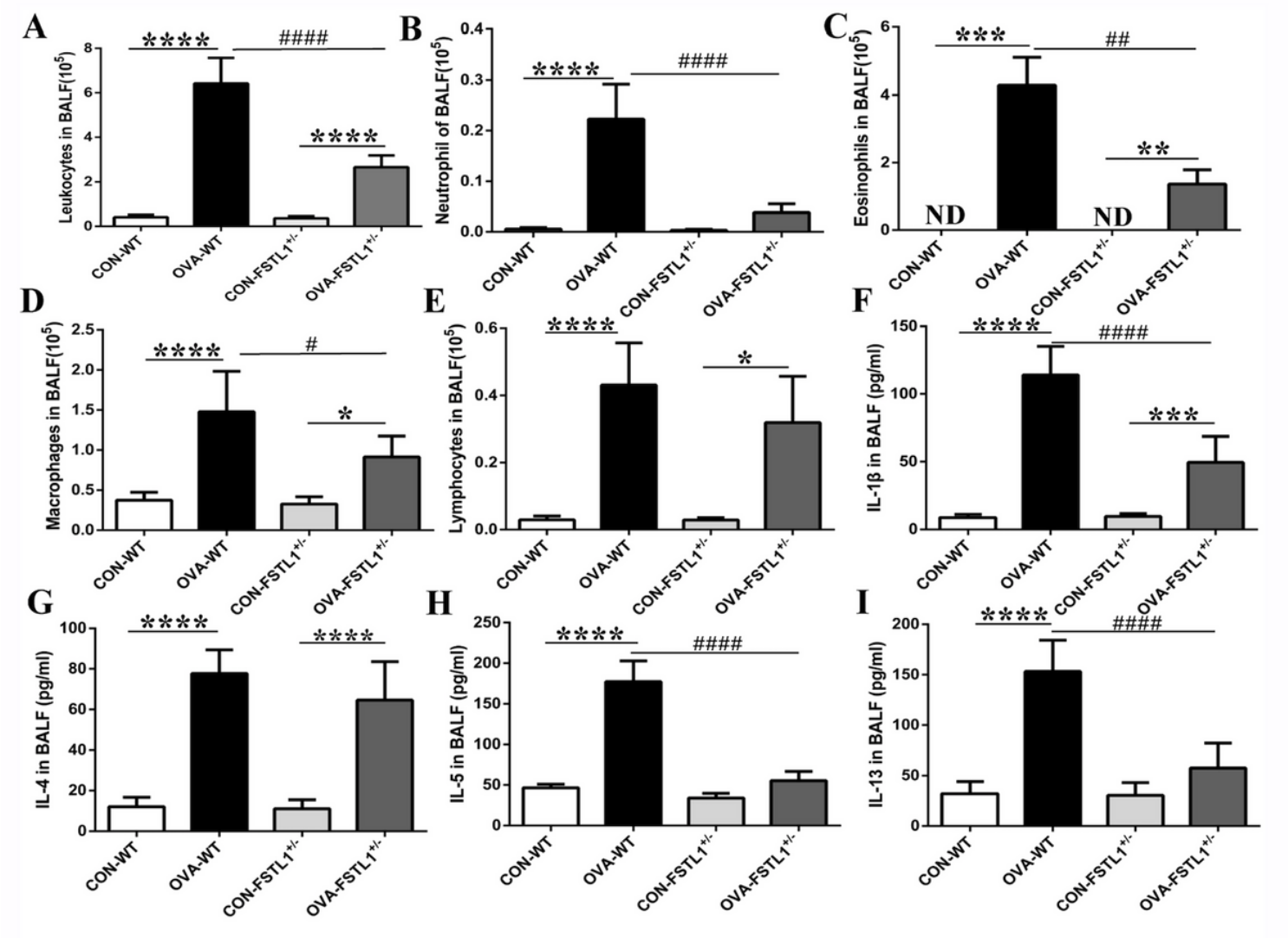

Figure 4

Total leucocytes and the percentage of different cells (eosinophils, lymphocytes, neutrophils and macrophages) were observed within the BALF (a-e). Cytokine levels in BALF supernatant from each group were detected by ELISA. FSTL1 deficiency reduces the level of IL-5, IL-13 and IL-1β, but has no statistical effect on the IL-4 (f-i). Values were shown as the means ± S.D. of six mice. (*p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001 vs. the control group. #p<0.05 and ##p<0.01, ####p<0.0001 vs. the model group).

Figure 5

The lung sections of mice were prepared and stained with IHC to evaluate the protein expression of NLRP3, pro-caspase 1, and IL-1β in lung tissues. A semiquantitative analysis was performed. The data represent means ± S.D. (n=6). (****p<0.0001 vs. the control group. ###p<0.001, ####p<0.0001 vs. the model group).

Figure 6

Fstl1 induces airway inflammation, and it can be blocked by NLRP3 inhibitor MCC950. WT mice were administered with Fstl1 intranasally daily for 15 d before sacrifiifice. A control WT group only receive PBS. FSTL1+MCC950 group were i.p. injected with 200ug MCC950 2h before each FSTL1 administration. H&E staining was applyed to evaluate the airway inflammation (a). IHC of NLRP3 and pro-caspase 1 were used to evaluate the protein expression (b, c). Values were shown as the means ± S.D. of six mice. (***P<0.001 compared with the normal group, ##p<0.01 compared with the FSTL1 group).

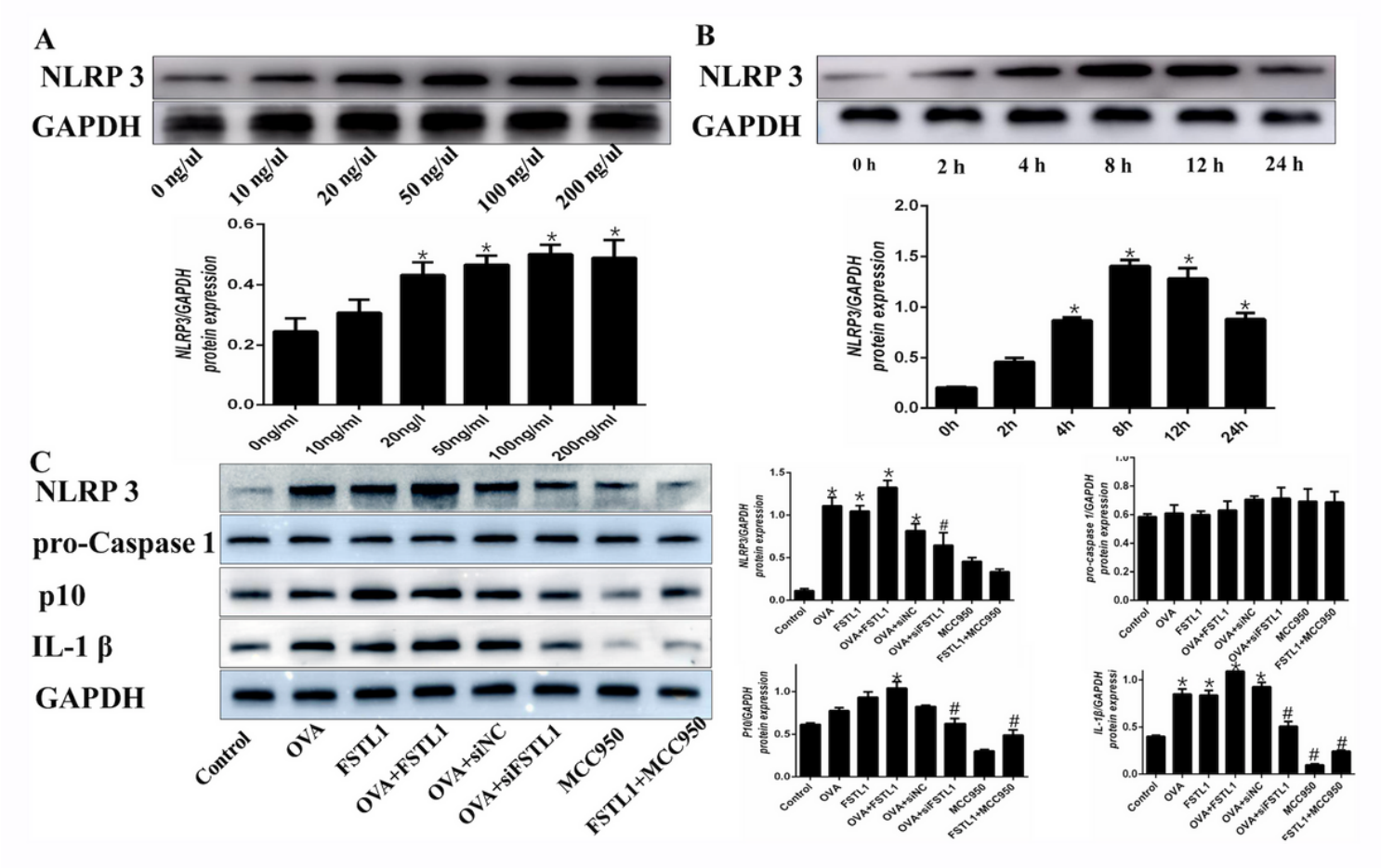

Figure 7

PMs were stimulated with different doses of FSTL1 for 12h (7a). Protein expression of NLRP3 were detected by western blotting. PMs were stimulated by 50ng/ul FSTL1 for indicated times and protein expressions of NLRP3 were detected by western blotting (7b). Then we treat cells with OVA, FSTL1, siFSTL1 and MCC950 individually or in combination to determine the expression of NLRP3, pro-caspase 1, P10, and IL-β (7c). The experiments were independently repeated at least three times. (*p<0.05, compared to control, #p<0.05 vs. the model group.)