Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Downregulation of NOX4 improves airway remodeling and inflammation by the TGF-β1-Smad2/3 pathway in asthma

Aili Xuan[1], Mei Yang[1], Qun Xia[1], Qian Sun[2*]

[1] Department of Pediatrics, The first affiliated hospital of Bengbu Medical College, Bengbu 233004, Anhui, China
[2] Department of radiation oncology, The first affiliated hospital of Bengbu Medical College, Bengbu 233004, Anhui, China

ARTICLE INFO

Original paper

Article history:
Received: July 22, 2023
Accepted: August 12, 2023
Published: September 30, 2023

Keywords:

NOX4, asthma, airway remodeling, airway inflammation, TGF-β1-Smad2/3 pathway

ABSTRACT

Asthma is a respiratory inflammatory disease, and nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) is involved in the progression of respiratory diseases. However, the role of NOX4 in asthma remains unclear. In the present study, we aimed to explore the effects of NOX4 on airway remodeling and inflammation. NOX4 expression was measured using immunocytochemistry (IHC), western blot, and real-time PCR (qPCR). Lung tissues were stained using the H&E assay. ELISA was used to examine the levels of airway remodeling-related indicators, and qPCR was used to detect airway inflammatory factors. The results indicated that NOX4 is highly expressed in lung tissues, bronchoalveolar lavage fluid (BALF), and serum of OVA-treated mice. Inhibition of NOX4 alleviated OVA-induced airway remodeling and inflammation. Similarly, TGF-β1 was also upregulated in BALF and serum OVA-induced mice. Inhibition of TGF-β1 signaling also improved airway remodeling and inflammation induced by OVA. Moreover, the downregulation of NOX4 inactivated the TGF-β1-Smad2/3 pathway, and TGF-β1 decreased Smad2/3 expression. Moreover, inhibition of the TGF-β1 was enhanced, while TGF-β1 reversed the effects on airway remodeling and inflammation induced by NOX4 inhibition. Taken together, the downregulation of NOX4 improves airway remodeling and inflammation via inactivation of the TGF-β1-Smad2/3 pathway in asthma mice, suggesting that NOX4 may be a therapeutic target for asthma.

Doi: http://dx.doi.org/10.14715/cmb/2023.69.9.31

Introduction

Asthma is a prevalent lung disease characterized by inflammation of the lower respiratory tract. It poses a significant global health burden, affecting individuals of all ages and backgrounds. The hallmark features of asthma include reversible airway obstruction, airway inflammation, and increased airway sensitivity (hyperresponsiveness) (1). These pathological changes result in various clinical symptoms, such as dyspnea (shortness of breath), chest tightness, persistent coughing, and even cyanosis in severe cases. Extensive research has highlighted the interconnectedness of asthma with other diseases, emphasizing its complex nature. For instance, studies have established pathological associations between asthma and conditions such as rhinitis (inflammation of the nasal cavity), reflux esophagitis (inflammation of the esophagus due to backward flow of stomach acid), obstructive sleep apnea (repetitive episodes of breathing cessation during sleep), and psychiatric diseases (2). These comorbidities contribute to the heterogeneity of asthma, making it a challenging condition to diagnose and treat effectively. Despite its prevalence and impact on quality of life, asthma remains underdiagnosed and undertreated in many cases (3,4). This discrepancy arises from several factors, including the variability in asthma symptoms and the lack of standardized diagnostic criteria. Additionally, healthcare disparities and limited access to healthcare services further contribute to the underdiagnosis and undertreatment of asthma. The primary goals of asthma management revolve around preventing mortality, reducing symptoms, and maintaining normal daily activities (5). Treatment strategies are tailored based on the severity of the disease and individual differences to achieve optimal therapeutic outcomes (6). The current therapeutic approaches for asthma include the use of bronchodilators to relieve acute symptoms and anti-inflammatory medications, such as corticosteroids, to control airway inflammation. However, despite the available treatment options, asthma remains a challenging condition to cure due to the incomplete understanding of its underlying pathogenesis. The mechanisms that initiate and sustain the inflammatory processes in the airways are not yet fully elucidated, hindering the development of targeted therapies. Consequently, there is a pressing need for further research to unravel the intricacies of asthma pathophysiology and identify novel therapeutic targets. In summary, asthma is a common lung disease characterized by airway inflammation, reversible obstruction, and increased airway sensitivity. It is intricately associated with other medical conditions, making its diagnosis and treatment complex. The management of asthma focuses on symptom control and maintaining normal daily activities. However, due to the limited understanding of its pathogenesis, asthma currently lacks a definitive cure. Addressing this knowledge gap through ongoing research will pave the way for more precise diagnostic criteria and innova-

* Corresponding author. Email: QianSun2108042@163.com

tive therapeutic interventions, ultimately improving the outcomes and quality of life for individuals living with asthma.

Nicotinamide adenine dinucleotide phosphate oxidase 4 (NOX4) is a member of the NOX family, a key enzyme that leads to the production of reactive oxygen species (ROS) (7). NOX4 is the only subtype of the NOX family that can produce hydrogen peroxide (H_2O_2). It is initially detected to be highly expressed in the kidneys and then found to be expressed in other types of cells, such as endothelial cells, smooth muscle cells, and osteoclasts (8,9). Aberrant expression of NOX4 is associated with numerous diseases, including renal diseases, lung diseases, and cancers (10-12). The role of NOX4 in pulmonary diseases is contradictory. It has both destructive and protective effects on the respiratory tract (11). NOX4 is closely linked to ciliary function, smooth muscle function, and fibroblast differentiation in asthma (13-15). However, the effect of NOX4 on airway remodeling and inflammation of asthma and the underlying mechanism remains unknown.

In the study, we investigated the role of NOX4 in asthma and the underlying mechanisms. We found that NOX4 is highly expressed in OVA-induced mice, which suppression improved airway remodeling and inflammation. Mechanically, the effect of NOX4 is acted through the TGF-β1-Smad2/3 pathway. These findings provided a novel target for asthma therapy.

Materials and Methods

Animals

The animal study was approved by the Ethics Committee of The first affiliated hospital of Bengbu Medical College. BALB/c mice (6-8 weeks, female; Hunan SJA Laboratory Animal Co., Ltd., Changsha, China) were housed at 22 ± 1°C temperature, 45-55% humidity, and 12 h light/dark cycle SPF conditions. Then the mice were randomly divided into 4 groups: control, OVA, OVA+ DPI, OVA + SB431542, and OVA + DPI + SB431542 groups. The mice in the control and OVA groups only received nasal perfusion with the same amount of normal saline. To establish asthma mice, they were intraperitoneally injected with 0.2 ml of normal saline containing 50 μg ovalbumin (OVA, Aladdin, Shanghai, China) and aluminum hydroxide (1 mg; Sigma-Aldrich, St. Louis, MO, USA) at 0, 7, and 14 days. Starting on day 21, OVA mice were given aerosolization of 1 % OVA normal saline for 30 min every day, continuous for 3 weeks. The mice in the control were injected with 0.2 ml saline at 0, 7, and 14 days intraperitoneally and aerosolized using saline for the same time. To inhibit NOX4, 0.5mg/kg DPI (MedChemExpress, Shanghai, China) was intraperitoneally injected into mice (16). To inhibit TGF-β1 signaling, 0.5mL of SB431542 (MedChemExpress) at 2 μg/mL was used for nasal inhalation. To increase TGF-β1 expression, 0.1 μg TGF-β1 (PeproTech) was intraperitoneally injected into mice. The mice were sacrificed by intraperitoneal injection with 200 mg/kg pentobarbital sodium (Sigma-Aldrich, St. Louis, MO, USA). Serum, bronchoalveolar lavage fluid (BALF), and lung tissues were collected for further study.

Immunocytochemistry (IHC) assay

The lung tissues were fixed with formaldehyde for 24 h, embedded in paraffin, making pathological sections. Paraffin sections were dewaxed with xylene and gradient alcohol. Then the sections were incubated with 3% H_2O_2 for 10 min to remove endogenous catalase. After digesting with citric acid buffer, the sections were blocked using normal goat serum. The sections were incubated with primary antibodies (anti-NOX4 (ab109225, 1/500) and anti-TGF-β1 (ab215715, 1/500), Abcam, Cambridge, MA, USA) at 4°C overnight, followed by incubation with secondary antibody (ab150113, 1/500, Abcam, Cambridge, MA, USA) at 37°C for 0.5 h. After washing, the sections were stained with DAB and hematoxylin. The images were visualized under a microscope.

H&E staining assay

Isolated lung tissues were fixed with 4% paraformaldehyde for 24 h and cut into 5 μm sections after paraffin embedding. All sections were stained with Hematoxyline for 10 min and eosin for 1 min. The stained sections were visualized under a microscope. The airway wall area (Wat), the perimeter of the bronchial basement membrane (pbm), and smooth muscle thickness were measured using the Image Pro Plus software.

Western blot

Proteins were extracted with pre-cooled RIPA lysis buffer (Solarbio, Beijing, China). After protein concentration was detected using a BCA Protein Assay Kit (Solarbio, Beijing, China), equal proteins were separated via 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidene fluoride (PVDF) membrane (Millipore, Bedford, MA, USA). The membranes were blocked with 5 % non-fat milk for 1 h, incubated with primary antibodies (anti-NOX4: ab109225, 1/2000; anti-TGFBR1: ab235578, 1/1000; anti-TGF-β1: ab275715, 1/1000; p-Smad2/3: ab202445, 1/1000; anti-β-actin: ab8227, 1/3000; Abcam, Cambridge, MA, USA) overnight at 4°C, and then incubated with the secondary antibody (ab6721, 1/3000; Abcam, Cambridge, MA, USA) for 1 h at room temperature. The bands were visualized using an ECL substrate (Solarbio, Beijing, China). β-actin was used as an internal reference.

Real-time PCR (qPCR)

Total RNA was isolated from lung tissues homogenates using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). The isolated RNA was used for reverse-transcribed and qPCR using M-MLV reverse transcriptase (Tiangen, Beijing, China) and SYBR Green (Solarbio, Beijing, China). qPCR was performed on a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). β-actin was the internal control. The relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The sequences of the primers were listed as follows: NOX4 F: 5′-TCTGGCTCT-CCATGAATGTC-3′ and R: 5′CTGCTTGGAACCTTCTGTGA-3′, β-actin F5′-AAAGACCTGTACGCCAACACAGTGCTGTCTGG-3′ and R5′CGTCATACTCCTGCTTGCTGATCCACATCTGC-3′.

ELISA

The expression of TGF-β1, IL-4, IL-13, ET-1, and VEGF in BALF and serum was detected using their specific ELISA kits (Jiancheng, Nanjing, China) following the manufacturer's protocol. Cysteinyl leukotrienes 1 (CysLT1) and cysteinyl leukotriene receptor 1 (CysLTR1)

levels in lung tissues were also detected using the CysLT1 ELISA kits (Tongwei, Shanghai, China) and the CysLTR1 ELISA kit (Gaining, Shanghai, China) following the manufacturer's protocol, respectively (17). Lung tissues were homogenized in normal saline. The supernatant was obtained after centrifugation at 1000×g for 10 min. CysLT1 and CysLTR1 levels were detected using the supernatant.

Statistical analysis

Data from three independent experiments were analyzed using GraphPad Prism 7.0 software (La Jolla, CA, USA) and presented as the mean ± standard deviation. Comparisons were analyzed using Student's t-test and one-way analysis of variance (ANOVA) followed by Tukey's test. $P < 0.05$ was considered statistically significant.

Results

Effects of NOX4 on airway remodeling and inflammation

To evaluate the role of NOX4 in asthma mice, the expression of NOX4 in lung tissues, BALF, and serum was evaluated using IHC staining assay, western blot, and qPCR. As shown in Figure 1A, NOX4 was significantly highly expressed in lung tissues, BALF, and serum, while DPI reduced NOX4 levels. Furthermore, OVA observably induced the increase of inflammatory cells in the airway, Wat/bpm, as well as the smooth muscle thickness, while DPI markedly reversed the increase induced by OVA (Figure 1B). The levels of airway remodeling factors ET-1, IL-4, IL-13, and VEGF were significantly evaluated by OVA in both BALF and serum, while DPI notably reduced their levels (Figure 1C). The number of total cells, eosinophils, neutrophils, lymphocytes, and macrophages increased by OVA, which were markedly abrogated by DPI (Figure 1D). Moreover, OVA significantly elevated CysLT1 and CysLTR1 levels, and DPI markedly rescued the increase (Figure 1E).

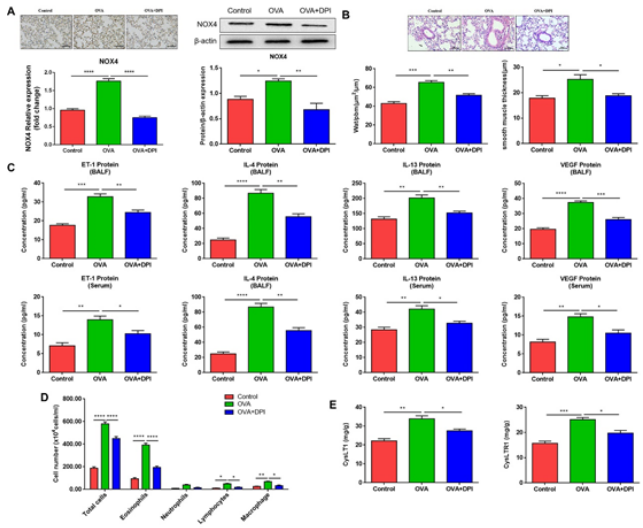

Figure 1. Effects of NOX4 on airway remodeling and inflammation. (A) The expression of NOX4 in lung tissues was measured by IHC assay and western blot, while the levels of NOX4 were tested by qPCR in BALF and serum. (B) H&E staining assay of lung tissues in each group. quantification of Wat/pbm ratio and smooth muscle thickness were performed. (C) The levels of ET-1, IL-4, IL-13, and VEGF in BALF and serum were examined by ELISA. (D) The number of inflammatory cells in BALF was counted. (E) The expression of CysLT1 and CysLTR1 was examined by ELISA. ****P<0.0001. ***P<0.001. **P<0.01. *P<0.05.

Effects of TGF-β1 signaling inhibition on airway remodeling and inflammation

As illustrated in Figure 2A, we found that the expression of TGFBR1 was markedly upregulated in BALF and serum of mice in the OVA group, while SB431542 abolished the upregulation, suggesting the TGF-β1 pathway was inactivated. Then, the results of the H&E staining assay showed that the inflammatory cells in the airway, Wat/pbm ratio, and smooth muscle thickness were all significantly increased by OVA, which were significantly reversed by SB431542 (Figure 2B). The levels of ET-1, IL-4, IL-13, and VEGF in BALF and serum were significantly evaluated by OVA, but inhibiting of TGF-β1 signaling markedly abolished the effects induced by OVA (Figure 2C). OVA markedly increased the number of total cells, eosinophils, neutrophils, lymphocytes, and macrophages, while SB431542 significantly reversed the increase (Figure 2D). Additionally, the levels of CysLT1 and CysLTR1 were significantly enhanced in OVA mice, which were notably abrogated in the mice in the OVA+SB431542 group (Figure 2E).

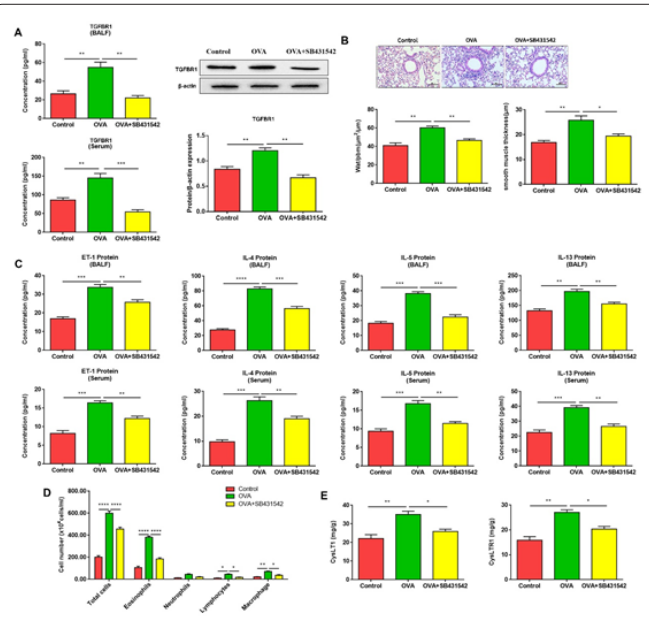

Figure 2. Effects of TGF-β1 pathway on airway remodeling and inflammation. (A) TGFBR1 expression in BALF and serum was tested by ELISA, and its expression in lung tissues was tested using a western blot. (B) Lung tissues in each group were stained using H&E, and Wat/pbm and smooth muscle thickness were quantified. (C) The levels of ET-1, IL-4, IL-13, and VEGF in BALF and serum were examined by ELISA. (D) The number of total inflammatory cells, eosinophils, neutrophils, lymphocytes, and macrophages in BALF was counted. (E) The expression of CysLT1 and CysLTR1 was examined by ELISA. ****P<0.0001. ***P<0.001. **P<0.01. *P<0.05.

NOX4 is positively related to the TGF-β1-Smad2/3 pathway

The data of the western blot indicated that TGF-β1 and p-Smad2/3 levels were upregulated in OVA mice, while DPI abolished the upregulation (Figure 3A). Furthermore, we found that TGF-β1 treatment increased p-Smad2/3 levels in OVA mice (Figure 3B). Additionally, DPI reversed the upregulation of p-Smad2/3 levels induced by TGF-β1

treatment (Figure 3C).

SB431542 enhanced the effects of NOX4 on airway remodeling and inflammation

We explored the role of both DPI and SB431542 in OVA mice. Compared with the OVA+DPI group, the expression of NOX4 and TGF-β1 was downregulated in the OVA+DPI+SB431542 group (Figure 4A). The levels of ET-1, IL-4, IL-13, and VEGF induced by OVA were decreased by DPI and further decreased by SB431542 (Figure 4B). Additionally, the number of inflammatory cells, CysLT1, and CysLTR1 levels were all reduced by DPI, which were further reduced by SB431542 (Figure 4C and D).

TGF-β1 treatment reversed the effects of NOX4 on airway remodeling and inflammation

Finally, we performed the rescue experiments. TGF-β1

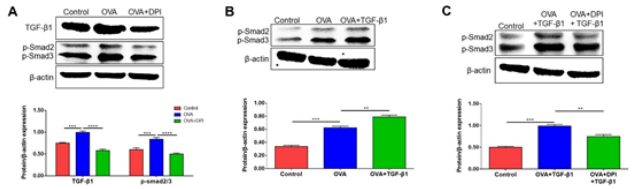

Figure 3. NOX4 is positively related to the TGF-β1-Smad2/3 pathway. (A) The p-Smad2 and p-Smad3 levels were evaluated using a western blot in OVA treated with DPI. (B) The p-Smad2 and p-Smad3 levels were evaluated using western blot in OVA treated with TGF-β1. (C) The p-Smad2 and p-Smad3 levels were evaluated using a western blot in OVA treated with DPI and TGF-β1. ****P<0.0001. ***P<0.001. **P<0.01.

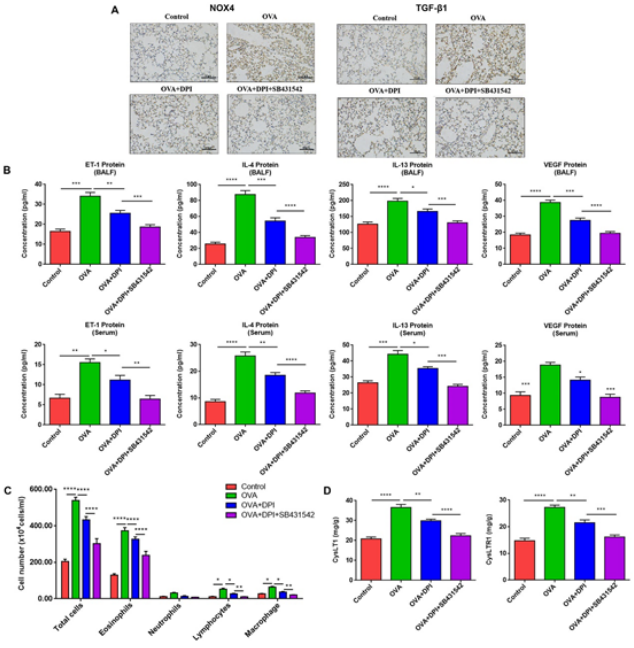

Figure 4. SB431542 enhanced the effects of NOX4 on airway remodeling and inflammation. (A) The levels of NOX4 and TGF-β1 were evaluated using an IHC staining assay. (B) ELISA was utilized to examine the levels of ET-1, IL-4, IL-13, and VEGF in BALF and serum. (C) The number of total inflammatory cells, eosinophils, neutrophils, lymphocytes, and macrophages in BALF was counted. (D) CysLT1 and CysLTR1 levels were tested using ELISA. ****P<0.0001. ***P<0.001. **P<0.01. *P<0.05.

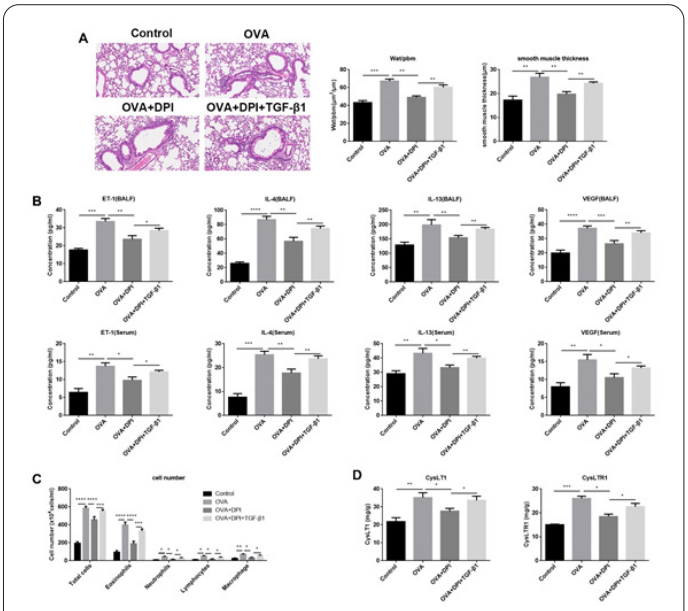

Figure 5. TGF-β1 treatment reversed the effects of NOX4 on airway remodeling and inflammation. (A) The lung tissues were stained using an H&E assay. Wat/pbm ratio and smooth muscle thickness were quantified. (B) ELISA was performed to measure the levels of ET-1, IL-4, IL-13, and VEGF in BALF and serum. (C) The number of inflammatory cells in BALF was counted. (D) The expression of CysLT1 and CysLTR1 was examined by ELISA. ****P<0.0001. ***P<0.001. **P<0.01. *P<0.05.

increased Wat/bpm ratio and the smooth muscle thickness in DPI-treated mice (Figure 5A). TGF-β1 abrogated the levels of airway remodeling factors ET-1, IL-4, IL-13, and VEGF reduced by DPI (Figure 5B). The number of total cells, eosinophils, neutrophils, lymphocytes, and macrophages was reduced by DPI, which was markedly abrogated by TGF-β1 (Figure 5C). Moreover, DPI significantly reduced CysLT1 and CysLTR1 levels in OVX mice, and TGF-β1 markedly rescued the effects induced by DPI (Figure 5D).

Discussion

Herein, we investigated the role of NOX4 in asthma and the underlying mechanism. We found that downregulated NOX4 contributed to alleviating airway remodeling and inflammation. Inactivating the TGF-β1-Smad2/3 pathway enhanced the effects of NOX4 inhibition on airway remodeling and inflammation.

In recent years, the incidence of asthma is increasing, but its occurrence mechanism is not identified. Airway remodeling and airway inflammation are known as two main factors of asthma pathogenesis (18). Airway remodeling is a change in the structure, cellular, and molecular composition of the airway wall and is generally believed to result from long-term airway inflammation (19). The interaction of inflammatory cells such as eosinophils, neutrophils, lymphocytes, and macrophages causes the onset of asthma (20,21). The release of IL-4, IL-5, and IL-13 by immune system cells is an important feature of high Type 2 T helper lymphocytes (Th2) asthma (22). CysLT1 mediates most of the pathological processes of asthma through its receptor CysLTR1, especially inflammation (23). ET-1 is associated with airway remodeling, especially in the absence of obvious Th2 asthma (24). Moreover, VEGF regulates vascular growth in airway remodeling, thus contributing

to the inflammatory response in Th2-type asthma (25). In this study, we used OVA to induce asthma, we found OVA increased inflammatory cell number, Wat/bpm, smooth muscle cells, IL-4, IL-13, ET-1, VEGF, CysLT1, and CysLTR1 levels. The findings suggested that we successfully established an asthma model, and asthma was associated with airway remodeling and inflammation.

Dysregulation of NOX4 is related to asthma. Through genome-wide microarray analysis and qPCR experiment, NOX4 is identified to be upregulated in airway smooth muscle in asthma, and its expression in COPD is higher than in asthma (14,26). NOX4 also regulates the function of pulmonary artery smooth muscle cells to influence asthma (27). Additionally, NOX4 is expressed in bronchial epithelial cells, and inhibition of NOX4 eliminates ciliary dysfunction in mice with asthma (13). Herein, we clarified that NOX4 was highly expressed in serum, BALF, and lung tissues of asthma mice. Furthermore, we used DPI to inhibit NOX4 expression. The results showed that DPI reversed the effects on airway remodeling and inflammation induced by OVA, suggesting that downregulated NOX4 improved airway remodeling and inflammation, and further attenuated the progression of asthma.

TGF-β is known to play a crucial role in asthma by regulating inflammation, cell growth, and fibrosis. TGF-β1 is an isoform of TGF-β that participates in airway remodeling (28). The levels of TGF-β1 produced by inflammatory cells are increased in airway lavage fluid (29). The results of bronchial biopsies TGF-β1 is expressed in most eosinophils (30). Additionally, TGF-β1 signaling transduction commonly depends on the Smad pathway. TGF-β1 activates its receptors in a paracrine and autocrine manner, leading to the phosphorylation of the Smad proteins (31). In the context of asthma, the TGF-β1/Smad pathway modulates airway remodeling, inflammation, choroidal neovascularization, and airway smooth muscle cell proliferation (32-34). In addition, NOX4 has been reported to regulate the TGF-β1/Smad pathway in multiple diseases, such as acute kidney injury, lung fibrosis, and atrial fibrosis (35-37). NOX4 promotes TGF-β1-induced ROS production, airway smooth muscle cell growth, and hypertrophy, therefore affecting the pathological process of asthma (38). Herein, we found that SB431542 decreased TGFR1 expression, suggesting inhibiting the TGF-β1 pathway in mice with asthma. Moreover, DPI decreased OVA-induced p-Smad2 and p-Smad3 levels, whereas TGF-β1 increased OVA-induced p-Smad2 and p-Smad3 levels. Functionally, SB431542 enhanced the effect on airway remodeling and inflammation induced by DPI, and TGF-β1 reversed the effects induced by DPI. The findings suggested that inhibition of NOX4 expression improved airway remodeling in asthmatic mice by the TGF-β1-Smad2/3 pathway.

The results of this study shed light on the significant role of NOX4 in the pathogenesis of asthma. The findings demonstrate that the levels of NOX4 were elevated in various compartments, including bronchoalveolar lavage fluid (BALF), serum, and lung tissues, in mice with asthma. This upregulation of NOX4 suggests its involvement in the development and progression of asthma. Furthermore, the study reveals that the downregulation of NOX4 has a beneficial impact on airway remodeling and inflammation in the context of asthma. The inhibition of NOX4 activity resulted in improved airway structure and reduced inflammation, indicating its potential as a therapeutic target. Notably, these beneficial effects were mediated through the inactivation of the TGF-β1-Smad2/3 pathway, which is known to play a pivotal role in asthma pathogenesis. The identification of NOX4 as a key player in asthma pathophysiology opens new avenues for the development of targeted therapies. By specifically targeting NOX4, it may be possible to intervene in the disease process and alleviate the symptoms associated with asthma. This novel idea holds promise for the future management of asthma, as current treatment options often focus on symptomatic relief rather than addressing the underlying mechanisms driving the disease.

Conclusion

In summary, the findings presented in this manuscript underscore the significance of NOX4 in asthma and provide compelling evidence for its potential as a therapeutic target. Further investigations are warranted to explore the precise molecular mechanisms underlying NOX4-mediated airway remodeling and inflammation, as well as to assess the safety and efficacy of targeting NOX4 in clinical settings. Ultimately, these efforts may lead to the development of innovative and more effective treatment strategies for individuals suffering from asthma.

Ethical Compliance

The animal study was approved by the Ethics Committee of the first affiliated hospital of Bengbu Medical College.

Conflict of Interests

The authors declared no conflict of interest.

Acknowledgment

This work was supported by the Natural Science Research Key Project of Bengbu Medical College under Grant (number BYKY2019052ZD); Natural Science Research Project of Anhui Educational Committee under Grant (No. KJ2021A0715).

Data Availability Statement

The datasets in this study are available from the corresponding author upon reasonable request.

References

1. Mims JW. Asthma: definitions and pathophysiology. Int Forum Allergy Rh 2015; 5 Suppl 1: S2-S6.
2. Boulet LP, Boulay ME. Asthma-related comorbidities. Expert Rev Resp Med 2011; 5(3): 377-393.
3. Hamid I, Janbaz KH, Iqbal R, et al. Therapeutic potential of Ceratonia siliqua extract for the management of asthma and hypertension. Cell Mol Biol 2021; 67(5): 6-15.
4. Li G, Su C, Zhou L, Tong Z, Xia Q, Chen Q. Exploring the Function of Fritillariae thunbergii Bulbus on the Inflammation Response in Cough Variant Asthma Mice Model Based on Metabolomics. J Biomed Nanotechnol 2022; 18(11): 2573-2584.
5. Kwah JH, Peters AT. Asthma in adults: Principles of treatment. Allergy Asthma Proc 2019; 40(6): 396-402.
6. Castillo JR, Peters SP, Busse WW. Asthma Exacerbations: Pathogenesis, Prevention, and Treatment. J Aller Cl Imm-Pract 2017; 5(4): 918-927.
7. Brandes RP, Weissmann N, Schroder K. Nox family NADPH oxidases: Molecular mechanisms of activation. Free Radical Bio Med 2014; 76: 208-226.

8. Buvelot H, Jaquet V, Krause KH. Mammalian NADPH Oxidases. Methods Mol Biol 2019; 1982: 17-36.
9. Holterman CE, Read NC, Kennedy CR. Nox and renal disease. Clin Sci 2015; 128(8): 465-481.
10. Yang Q, Wu FR, Wang JN, et al. Nox4 in renal diseases: An update. Free Radical Bio Med 2018; 124: 466-472.
11. Li ZM, Xu SY, Feng YZ, et al. The role of NOX4 in pulmonary diseases. J Cell Physiol 2021; 236(3): 1628-1637.
12. Tang CT, Gao YJ, Ge ZZ. NOX4, a new genetic target for anticancer therapy in digestive system cancer. J Digest Dis 2018; 19(10): 578-585.
13. Wan WY, Hollins F, Haste L, et al. NADPH Oxidase-4 Overexpression Is Associated With Epithelial Ciliary Dysfunction in Neutrophilic Asthma. Chest 2016; 149(6): 1445-1459.
14. Sutcliffe A, Hollins F, Gomez E, et al. Increased nicotinamide adenine dinucleotide phosphate oxidase 4 expression mediates intrinsic airway smooth muscle hypercontractility in asthma. Am J Resp Crit Care 2012; 185(3): 267-274.
15. Al-Azzam N, Teegala LR, Pokhrel S, et al. Transient Receptor Potential Vanilloid channel regulates fibroblast differentiation and airway remodeling by modulating redox signals through NADPH Oxidase 4. Sci Rep-Uk 2020; 10(1): 9827.
16. Duret C, Merlos R, Wauthoz N, Sebti T, Vanderbist F, Amighi K. Pharmacokinetic evaluation in mice of amorphous itraconazole-based dry powder formulations for inhalation with high bioavailability and extended lung retention. Eur J Pharm Biopharm 2014; 86(1): 46-54.
17. Lin Y, Yao J, Wu M, et al. Tetrandrine Ameliorates Airway Remodeling of Chronic Asthma by Interfering TGF-beta1/Nrf-2/HO-1 Signaling Pathway-Mediated Oxidative Stress. Can Respir J 2019; 2019: 7930396.
18. Xie X, Yu Z, Huang A, Lai G, Liu D, Zou S. Role of Smooth Muscle Cells Regulated by Vitamin D in Bronchial Asthma Airway Remodeling and Efficacy of Nanomedicine on Bronchial Asthma. J Biomed Nanotechnol 2022; 18(7): 1837-1843.
19. Jin L, Gong H, Zhang Q. The clinical differences between cough variant asthma cells and humoral immunology indicators. Cell Mol Biol 2022; 68(4): 188-193.
20. Guida G, Riccio AM. Immune induction of airway remodeling. Semin Immunol 2019; 46(101346.
21. Saradna A, Do DC, Kumar S, Fu QL, Gao P. Macrophage polarization and allergic asthma. Transl Res 2018; 191(1-14.
22. Agache I, Sugita K, Morita H, Akdis M, Akdis CA. The Complex Type 2 Endotype in Allergy and Asthma: From Laboratory to Bedside. Curr Allergy Asthm R 2015; 15(6): 29.
23. Singh RK, Tandon R, Dastidar SG, Ray A. A review on leukotrienes and their receptors with reference to asthma. J Asthma 2013; 50(9): 922-931.
24. Gregory LG, Jones CP, Mathie SA, Pegorier S, Lloyd CM. Endothelin-1 directs airway remodeling and hyper-reactivity in a murine asthma model. Allergy 2013; 68(12): 1579-1588.
25. Bakakos P, Patentalakis G, Papi A. Vascular Biomarkers in Asthma and COPD. Curr Top Med Chem 2016; 16(14): 1599-1609.
26. Hollins F, Sutcliffe A, Gomez E, et al. Airway smooth muscle NOX4 is upregulated and modulates ROS generation in COPD. Resp Res 2016; 17(1): 84.
27. Hoidal JR, Brar SS, Sturrock AB, et al. The role of endogenous NADPH oxidases in airway and pulmonary vascular smooth muscle function. Antioxid Redox Sign 2003; 5(6): 751-758.
28. Howell JE, McAnulty RJ. TGF-beta: its role in asthma and therapeutic potential. Curr Drug Targets 2006; 7(5): 547-565.
29. Brown SD, Baxter KM, Stephenson ST, Esper AM, Brown LA, Fitzpatrick AM. Airway TGF-beta1 and oxidant stress in children with severe asthma: association with airflow limitation. J Allergy Clin Immun 2012; 129(2): 388-396, 391-396.
30. Minshall EM, Leung DY, Martin RJ, et al. Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma. Am J Resp Cell Mol 1997; 17(3): 326-333.
31. Malhotra N, Kang J. SMAD regulatory networks construct a balanced immune system. Immunology 2013; 139(1): 1-10.
32. Yao Z, Fu Y. Glycyrrhizic acid restrains airway inflammation and remodeling in asthma via the TGF-beta1/Smad signaling pathway. Exp Ther Med 2021; 21(5): 461.
33. Yang F, Sun Y, Bai Y, Li S, Huang L, Li X. Asthma Promotes Choroidal Neovascularization via the Transforming Growth Factor Beta1/Smad Signalling Pathway in a Mouse Model. Ophthalmic Res 2022; 65(1): 14-29.
34. Chen M, Lv Z, Huang L, et al. Triptolide inhibits TGF-beta1-induced cell proliferation in rat airway smooth muscle cells by suppressing Smad signaling. Exp Cell Res 2015; 331(2): 362-368.
35. Cho S, Yu SL, Kang J, et al. NADPH oxidase 4 mediates TGF-beta1/Smad signaling pathway induced acute kidney injury in hypoxia. Plos One 2019; 14(7): e219483.
36. Li C, Sun X, Li A, Mo M, Zhao Z. S-Allylmercaptocysteine attenuates Bleomycin-induced pulmonary fibrosis in mice via suppressing TGF-beta1/Smad and oxidative stress pathways. Int Immunopharmacol 2020; 79: 106110.
37. Yeh YH, Kuo CT, Chang GJ, Qi XY, Nattel S, Chen WJ. Nicotinamide adenine dinucleotide phosphate oxidase 4 mediates the differential responsiveness of atrial versus ventricular fibroblasts to transforming growth factor-beta. Circ-Arrhythmia Elec 2013; 6(4): 790-798.
38. Sturrock A, Huecksteadt TP, Norman K, et al. Nox4 mediates TGF-beta1-induced retinoblastoma protein phosphorylation, proliferation, and hypertrophy in human airway smooth muscle cells. Am J Physiol-Lung C 2007; 292(6): L1543-L1555.