

Knockdown of NOVA1 inhibits inflammation and migration of asthmatic airway smooth muscle cells to regulate PTEN/Akt pathway by targeting PTBP1

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ABSTRACT

NOVA1 (neuro-oncological ventral antigen 1) is a neuron specific RNA binding protein, belonging to the Nova family, which plays an important role in various diseases. However, the role of NOVA1 in childhood asthma remains unclear. This study was aimed to investigate the role of NOVA1 in TGF- β 1-induced ASMCs proliferation and migration as well as the potential mechanisms. In our study, the NOVA1 expression was significantly increased in asthmatic tissues and TGF- β 1-induced ASMCs. Inhibition of NOVA1 significantly inhibited TGF- β 1-induced ASMCs cell proliferation and migration, and alleviates TGF- β 1-induced inflammation. NOVA1 positively regulated the PTBP1 expression and si-NOVA1 inhibited the activation of PTEN/AKT signal pathway. Importantly, the overexpression of PTBP1 partially reversed the effect of NOVA1 on cell viability, migration, inflammation and the activation of PTEN/AKT signal pathway. Generally, our study demonstrated that si-NOVA1 inhibited TGF- β 1-induced inflammation and migration in ASMCs through PTBP1/PTEN/AKT pathway. Therefore, inhibition of NOVA1 may be useful for the prevention or treatment of asthma airway remodeling.

1. Introduction

Asthma is a common chronic respiratory disease, characterized by mucus hypersecretion, airway inflammation, airway hyper-responsiveness (AHR), and airway remodeling (Barnes, 2000). Airway smooth muscle cell (ASMC) was well-known to engage in the development and progression of asthma. It has been revealed that abnormal proliferation and migration of ASMCs is a hallmark of airway remodeling, contributing to the occurrence of asthma (Liu et al., 2018; Chen et al., 2019). Furthermore, under the action of inflammatory mediators such as transforming growth factor- β 1 (TGF- β 1), the migration of ASMC to airway epithelium leads to the pathological changes of airway remodeling. (Li et al., 2015; Huang et al., 2018). Therefore, the suppressing proliferation and migration of ASMC may reveal potential way to inhibit the pathophysiology of childhood asthma.

NOVA1 (neuro-oncological ventral antigen 1) is a neuron specific RNA binding protein, which is one of the members of NOVA family.

NOVA superfamily has two members, such as NOVA1 and NOVA2 (Yu et al., 2018; Li et al., 2017a). NOVA1 is expressed in the central nervous system and has been proved to play an important role in the survival of motor neurons and the development of motor system (Li et al., 2017b). Recent evidence shows that NOVA1 plays an important role in the development of many kinds of tumors (Ludlow et al., 2018; Luo et al., 2019). In addition, more and more evidence showed that NOVA1 also plays a key role in the development of inflammatory diseases. For example, NOVA1 is induced by tumor microenvironment inflammatory immune signals and inhibited by epigenetic disorders. It can be used as a prognostic biomarker and a potential therapeutic target in head and neck squamous cell carcinoma (Kim et al., 2019). However, the role of NOVA1 in the pathogenesis of asthma remains unclear, and needs further explored.

Polypyrimidine tract-binding protein 1 (PTBP1), a heterogeneous nuclear ribonucleoprotein (hnRNP), is a multi-functional RNA-binding protein widely known for its role in tissue-specific regulation of exon

Abbreviations: ASMCs, airway smooth muscle cells; AHR, airway hyper-responsiveness; PTBP1, polypyrimidine tract-binding protein 1; PTEN, phosphatase and tension homolog; TGF- β 1, transforming growth factor- β 1; DMEM, Dulbecco's modified Eagle's medium; HRP, horseradish peroxidase; CCK-8, cell counting kit-8; ELISA, enzyme-linked immunosorbent assay.

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splicing (Spellman et al., 2005; Sawicka et al., 2008; Wollerton et al., 2004). PTBP1 was reported as an oncogene in several different types of cancer including lung carcinoma (Llorian et al., 2010; Cho et al., 2019). In addition, PTBP1 has higher expression in lung diseases such as pulmonary hypertension and pulmonary fibrosis, which takes part in regulating the overall metabolic and proliferative (Caruso et al., 2017; Xu et al., 2019). Previous reported that PTBP1 is significantly increased both in injured arteries and in physiological saline and platelet derived growth factor-BB-treated vascular smooth muscle cells, inhibition of PTBP1 significantly attenuated vascular smooth muscle cells proliferation and neointima hyperplasia (Wang et al., 2019). However, the exact molecular mechanism of PTBP1 regulating airway remodeling in asthma is still unclear.

It is worth noting that PTEN has become a key regulatory factor in the pathogenesis of asthma (Kwak et al., 2003). The expression of PTEN in lung tissue of asthmatic mice was decreased. Overexpression of PTEN in trachea could significantly inhibit bronchitis and airway hyper-responsiveness in asthmatic mice (Liu et al., 2015). PTEN as a target of IgE, downregulates PTEN through MicroRNA-21-5p and Stimulates Airway Smooth Muscle Cell Remodeling (Fang et al., 2019). In addition, PTEN/Akt pathway is also involved in regulating the cell viability and migration of ASMCs, thus participating in airway remodeling in asthma (Luo et al., 2013a). The decrease of PTEN expression was associated with the enhancement of PI3K signal, which upregulated ASMC remodeling. The results suggest that PTEN may be a therapeutic target to reduce airway wall remodeling (Fang et al., 2019). Walker et.al have found a significant number of genes that are altered after TGF- β induced trans-differentiation of WI-38 fibroblasts into myofibroblasts, many of which were expected or predicted. We also identified novel genes and pathways that were affected after TGF- β treatment (Walker et al., 2019).

In this study, we demonstrated that NOVA1 expression was significantly increased both in asthmatic tissues and TGF- β 1-induced ASMCs. Inhibition of NOVA1 suppressed ASMCs inflammation and migrative abilities through inhibiting PTBP1 expression activated PTEN/Akt pathway in TGF- β 1-induced ASMCs. Our findings offer some theoretical basis for the development of new agents for asthma therapy.

2. Methods

2.1. Blood sample

In the present study, we recruited 50 cases of asthma patients, including 25 males and 25 females, ranging in age from 5 to 12, with an average age of 8.2 ± 3.5 years. Children who underwent physical examination in the outpatient department of children's health care in our hospital during the same period were selected as the control group, including 12 males and 13 females, aged 6–13, with an average age of (8.2 ± 3.1) years, who had no family history of asthma, no respiratory tract infection, no history of allergic diseases or other diseases within 1 month. Besides, this study was approved by the medical ethics committee of the hospital and the same with the Code of Ethics of Declaration of Helsinki. And all the patients and subjects were provided informed consent. According to the protocol and internationally mentioned research (Yick et al., 2014), blood sample were gained from all subjects and then cryopreserved for further study.

2.2. Cell culture and building airway remodeling cell model

Human ASMCs were purchased from Lonza (Walkersville, MD, USA), and grown in Dulbecco's modified Eagle's medium (DMEM; Sigma-Aldrich, St Louis, USA), containing 10 % fetal bovine serum and 100 U/mL penicillin, was placed in a 5% CO₂ incubator at 37 °C. The building cell model of airway remodeling refers to Cao et al. (Gao et al., 2018). Briefly, ASMCs were treated with 10 ng/mL TGF- β 1 (Sigma, St. Louis, MO, USA) for 24 h.

2.3. Cell transfection

PTBP1 cDNA was amplified and then inserted into pcDNA3.1 plasmid, which was used to upregulate PTBP1. si-PTEN, si-NOVA1 and si-NC were synthesized by GenePharma Co. (Shanghai, China). The ASMCs growing in 96-well plates (2×10^4 cells/well) for 48 h and were transfected with si-NOVA1 and si-NC, pcDNA3.1-PTBP1 and si-PTEN using the Lipofectamine 3000 (Invitrogen) following the manufacturer's protocols.

2.4. Extraction RNA and RT-qPCR

Total RNA from ASMCs was isolated using a Trizol reagent (Invitrogen). and then quantified by spectrophotometry. The RNA was then reverse transcribed into cDNA by using Primescript RT Reagents (TaKaRa, Dalian, China) according to the manufacturer's protocol. RT-PCR was performed on triplicate samples by using a GoTaq® qPCR Master Mix kit (Promega, Madison, WI, USA) on an ABI 7500 Real-time PCR System (Applied Biosystems, Foster City, CA, USA). The reaction protocol was as follows: initial denaturation at 94 °C for 2 min, followed by 40 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 20 s, and elongation at 68 °C for 20 s. GAPDH were used for reference genes for mRNAs. All the PCR sequences were presented in Table 1.

2.5. Western blotting

Total protein was extracted from the ASMCs by using RIPA buffer (SigmaAldrich, St. Louis, MO, USA). The concentrations of protein were measured through a BCA protein assay kit. And then protein was separated by 10–12 % SDS-polyacrylamide gels and resolved to polyvinylidene difluoride (PVDF) membranes (Millipore, Massachusetts, USA). After blocking in 5% skim milk for 1 h, primary antibodies against PTBP1 (1:1,000; Abcam, ab133734), collagen I (1:1000, Abcam, ab34710), collagen III (1:1000, Abcam, ab), PTEN (1:1,000; Abcam, ab267787), p-AKT (1:1,000; Abcam, ab18785), AKT (1:1,000; Abcam, ab8805) and GAPDH (1:5,000, Abcam, ab9485) were applied individually and incubated overnight at 4 °C. After that, the membranes were probed with horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz biotechnology, Inc.) at room temperature for 1 h, and visualized with ECL reagent (Millipore). GAPDH was used as the internal control.

2.6. Cell counting kit (CCK)-8 assay

The untreated and treated ASMCs were growing in 96-well plates (2×10^4 cells/well). After overnight culture, the cells were treated with si-NOVA1 for 48 h, and cell viability was measured at 12 h or 24 h later. CCK-8 reagent (10 μ L; Beyotime, Haimen, China) was added to each well, and after one hour of incubation, the optical density of each well at 450 nm was determined with a spectrophotometer (Bio-Rad, Hercules, CA, USA). The experiment was repeated three times.

Table 1
Primers for RT-PCR of genes.

Gene	Sequences (5'-3')
<i>NOVA1</i>	
Forward primer	TAAGGCACCTTCT-GAGTAGA
Reverse primer	GCGAGCACAGAATTAATACGAC
<i>PTBP1</i>	
Forward primer	CTGAGGATCCATGTCTGGTTATTCTAGTG
Reverse primer	TTACTCTCGAGTTACTGGGAATATCCGGTT
<i>GAPDH</i>	
Forward primer	ACCACAGTCCATGCCATCAC
Reverse primer	TCCACCACCCTGTTGCTGTA

2.7. Wound healing assay

Untreated and treated cells were cultured in 24-well plates (5×10^4 cells/well). A scratch was created by using a sterilized disposable straw in the center of the cell monolayer. After incubation for 48 h, images were taken under an inverted microscope from each well. The calculation formula of cell migration is as follows, The healing rate (%) = (scratch length of 0 h - scratch length of 48 h) / scratch length of 0 h $\times$ 100 %.

2.8. Transwell assay

After transfection, ASMCs in each group were cultured in a 24-well plate (5×10^4 cells/well). DMEM solution containing 10 % fetal bovine serum was added to the lower chamber of transwell chamber and cultured in a 37°C incubator. After 48 h, removed the upper compartment medium. The cells migrated to the lower chambers were fixed with formaldehyde and dyed with crystal violet. Randomly selected five fields, the number of migrated cells was counted under a light microscope (Olympus, Tokyo, Japan).

2.9. Statistical analysis

Results were presented as mean $\pm$ SD. Student's *t*-test and one-way ANOVA were used to analyze the significance of differences groups. The relationships between the relative expression levels of miR-506 and PTBP1 in asthmatic tissues was evaluated by Spearman's rank test. All analyses were performed with SPSS 22.0. All experiments have at least three biological repetitions. $P < 0.05$ was considered with statistically significant.

3. Results

3.1. The expression of NOVA1 induced by TGF- β 1 in ASMCs

In our study, we found that the NOVA1 expression was significantly increased in asthmatic tissues compared with normal tissues (Fig. 1A). We next treated ASMCs with TGF- β 1 to construct the asthma model.

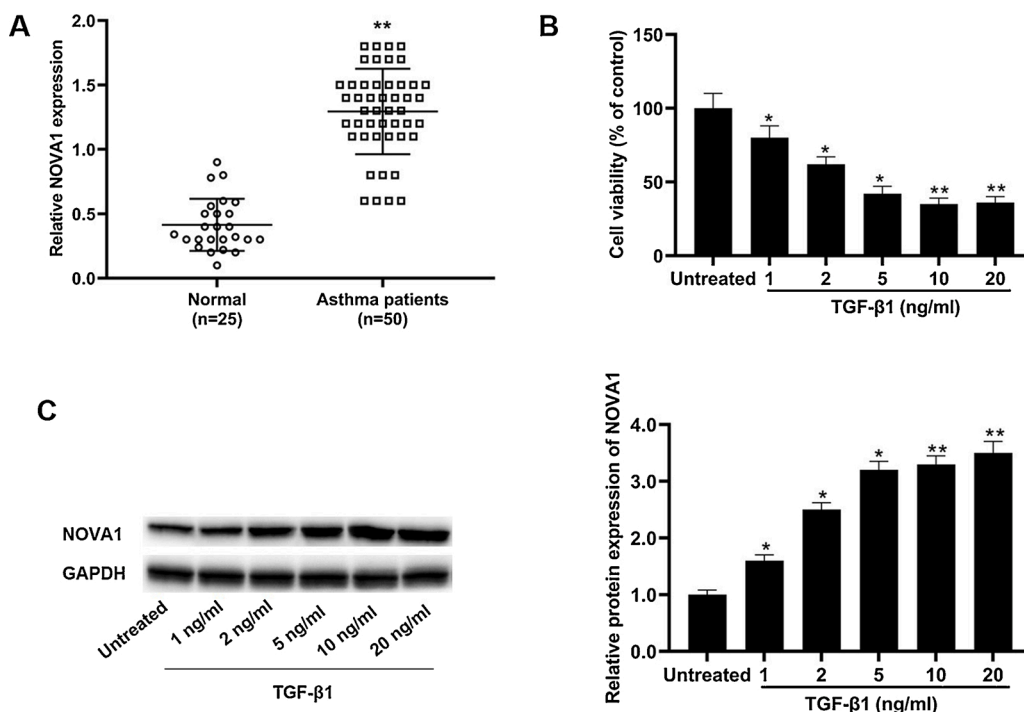


Fig. 1. The expression of NOVA1 induced by TGF- β 1 in ASMCs. ASMCs were treated with different concentrations of TGF- β 1 (0, 1, 2, 5, 10, 20 ng/mL) for 12 h. (A) The expression of NOVA1 in normal and asthma patients. (B) The cell viability of ASMCs in different concentrations of TGF- β 1. (C) The protein expression of NOVA1 in different concentrations of TGF- β 1. “*” means compared with Normal or untreated group $P < 0.05$, “**” means compared with normal or untreated group $P < 0.01$. GAPDH was used as an invariant internal control for calculating protein-fold changes.

According to the results, TGF- β 1 significantly inhibited ASMCs viability in a dose-dependent manner (Fig. 1B). Compared with the untreated group, TGF- β 1 treatment significantly increased the protein expression of NOVA1, and the quantitative images showed the same trend (Fig. 1C).

3.2. si-NOVA1 alleviated the TGF- β 1-induced inflammation in ASMCs

As indicated in Fig. 2A, the results showed that si-NOVA1 significantly decreased the expression of NOVA1 compared with the untreated group and si-RNA group. We choose the most efficient group (si-NOVA1-1) for the next experiment (Fig. 2A). Next, we explored the function of NOVA1 in inflammation of ASMCs. As shown in Fig. 2B, si-NOVA1 significantly decreased the expression of NOVA1 compared with si-NC group (Fig. 2B). TGF- β 1 significantly decreased the cell viability of chondrocytes, si-NOVA1 markedly increased the cell viability of ASMCs (Fig. 2C). Meanwhile, inhibition of NOVA1 decreased the content of IL-6 and TNF- α induced by TGF- β 1 (Fig. 2D and E). Also, the protein expression of iNOS and COX-2 was inhibited by si-NOVA1 treatment (Fig. 2F).

3.3. si-NOVA1 suppressed migration in TGF- β 1-induced ASMCs

It has been revealed that abnormal migration of ASMCs is contributing to the occurrence of asthma (Liu et al., 2018; Chen et al., 2019). As indicated in Fig. 3A and B, wound healing and transwell assays demonstrated that the migrative ability was alleviated by si-NOVA1 (Fig. 3A and B). Moreover, TGF- β 1 significantly increased the protein expressions of collagen I and collagen III. Whereas, the effect of TGF- β 1 was reversed by si-NOVA1 (Fig. 3C).

3.4. There was a positive correlation between NOVA1 and PTBP1, si-NOVA1 inhibited PTBP1/PTEN/Akt axis

Next, we explore the underlying mechanism by which NOVA1 inhibited the progression of asthma. Previous reported that PTBP1 regulates the activation of PTEN/Akt signaling pathway (Wang et al., 2018). To further address the underlying mechanism of NOVA1 exerts its effects in TGF- β 1-induced ASMCs, we next examined the relationship

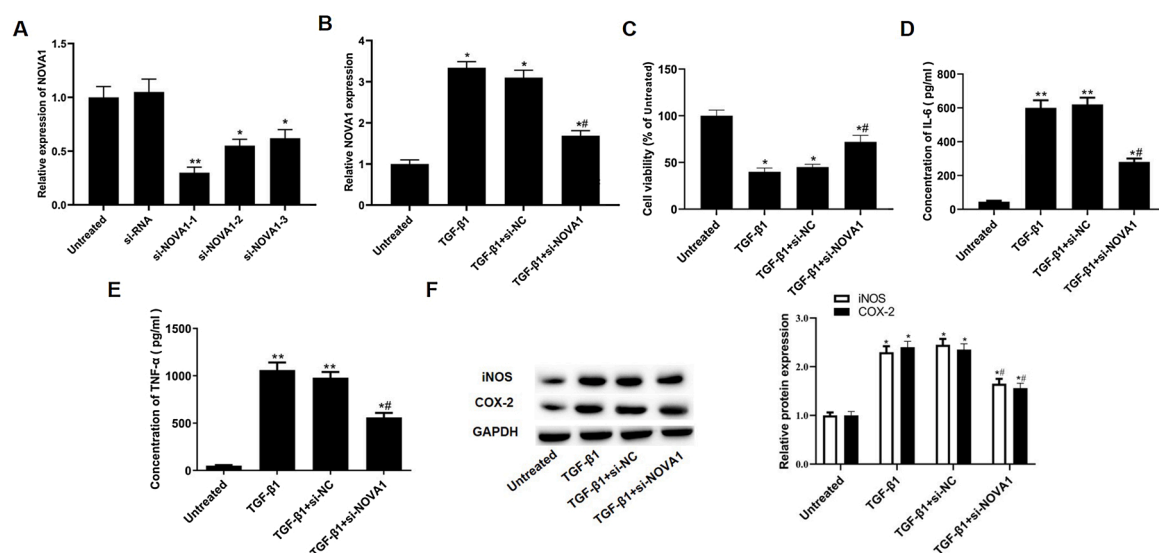


Fig. 2. si-NOVA1 alleviated the TGF-β1-induced inflammation in ASMCs. ASMCs were transfected with control si-RNA (si-NC) or si-NOVA1 for 48 h, and then treated with 10 ng/mL TGF-β1 for 12 h. (A) The expression of NOVA1 in untreated, si-RNA group and si-NOVA1 group. (B) The expression of NOVA1 in untreated, TGF-β1, TGF-β1+si-NC and TGF-β1+si-NOVA1 group. (C) The cell viability of ASMCs in untreated, TGF-β1, TGF-β1+si-NC and TGF-β1+si-NOVA1 group. (D) The levels of IL-6 untreated, TGF-β1, TGF-β1+si-NC and TGF-β1+si-NOVA1 group. (E) The levels of TNF-α in untreated, TGF-β1, TGF-β1+si-NC and TGF-β1+si-NOVA1 group. (F) The protein expression of COX-2 and iNOS in untreated, TGF-β1, TGF-β1+si-NC and TGF-β1+si-NOVA1 group. “*” means compared with untreated group $P < 0.05$, and “#” means compared with TGF-β1+si-NC group $P < 0.05$. GAPDH was used as an invariant internal control for calculating protein-fold changes.

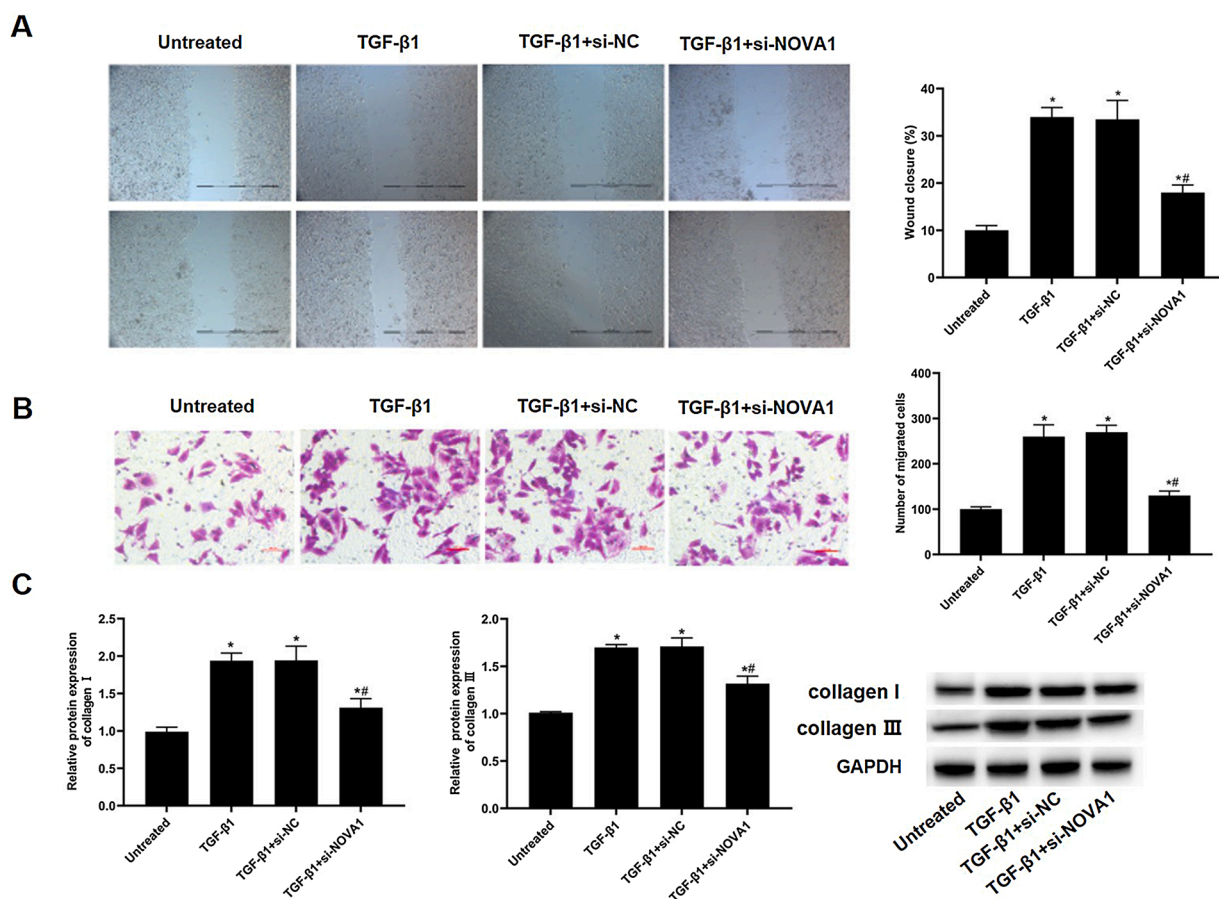


Fig. 3. si-NOVA1 suppressed migration in TGF-β1-induced ASMCs. ASMCs were transfected with control si-RNA (si-NC) or si-NOVA1 for 48 h, and then treated with 10 ng/mL TGF-β1 for 12 h. (A) The migrative ability was checked by wound healing assay. (B) and transwell migration assay. (C) The expressions of collagen I and collagen III protein were detected by western blot. “*” means compared with untreated group $P < 0.05$, and “#” means compared with TGF-β1+si-NC group $P < 0.05$. GAPDH was used as an invariant internal control for calculating protein-fold changes.

among NOVA1, PTBP1 and PTEN/Akt signal pathway in this process. As indicated that Fig. 4A, we found that si-NOVA1 significantly decreased the expression of PTEN and PTBP1, as well as increased the level of AKT phosphorylation in TGF- β 1-induced ASMCs (Fig. 4A). Additionally, the PTBP1 mRNA expression was significantly increased and positively correlated with NOVA1 expression in asthmatic tissues (Fig. 4D and E). These data demonstrated that PTBP1 might be a functional target of NOVA1. As indicated in Fig. 4F, the results showed that si-PTEN significantly decreased the expression of PTEN compared with the untreated group and si-RNA group. We choose the most efficient group (si-PTEN-2) for the next experiment (Fig. 4F).

3.5. PTBP1/PTEN/Akt signaling pathway involved in the regulation of NOVA1 on ASMCs

The results of western blotting indicated that si-NOVA1 elevated the protein levels of p-Akt, while decreased the protein of PTEN, however, PTBP1 overexpression and si-PTEN significantly reversed the effects of NOVA1 (Fig. 5A). Besides, inhibition of NOVA1 promoted the cell viability, while PTBP1 overexpression and si-PTEN inhibited the cell viability (Fig. 5B). Interestingly, si-NOVA1 significantly hindered the levels of IL-6, overexpression of PTBP1 and si-PTEN increased levels of IL-6 (Fig. 5C). Interestingly, the results revealed that si-NOVA1 inhibited cell viability and migration of ASMCs, while the role was reversed by OVE-PTBP1 or si-PTEN (Fig. 5D). Those results revealed that inhibition of NOVA1 suppressed cell viability and migration of ASMCs via modulating PTBP1/PI3K/Akt axis.

4. Discussion

Airway remodeling is one of the important characteristics of asthma, and its mechanism has not been fully clarified. Human ASMCs migration and proliferation participate in airway remodeling of asthma, leading to aggravation and deterioration of asthma. In the present study, we found that NOVA1 could modulate PTBP1 to inhibit ASMCs inflammation, cell

viability and migration by regulating PTBP1/PTEN/Akt pathway, so as to provide a brand-new perspective for asthma treatment.

Previous studies have reported that NOVA1 plays a vital role in pulmonary diseases such as lung cancer and pulmonary fibrosis (Ludlow et al., 2018; Sayed et al., 2019). For example, Ludlow demonstrated that NOVA1 promoted proliferation, invasion and migration via regulating hTERT splicing in non-small cell lung cancer (Ludlow et al., 2018). Zhu et al. suggested that NOVA1 reduced pulmonary fibrosis by NF- κ B-modulated apoptosis and inflammation. The present study demonstrated that NOVA1 expression was down-regulated in asthmatic tissues. Inhibition of NOVA1 expression significantly alleviates proliferative and migrative abilities of ASMCs. Thus, these data suggested that NOVA1 engaged in airway remodeling of asthma.

Previous studies showed that PTBP1 was significantly increased in asthmatic patents and act as a target of many regulators (Kang et al., 2019; Cui and Placzek, 2018). In our study, we found that PTBP1 is a functional target of NOVA1. And the PTBP1 expression was significantly increased and positively correlated with NOVA1 expression in asthmatic tissues. PTBP1 overexpression significantly promoted ASMCs proliferation and migration, those results were similar with Wang et al. and Zhang et al. who reported that inhibition of PTBP1 decreased vascular smooth muscle cells or lung cancer cells proliferation and migration (Cho et al., 2019; Wang et al., 2019). Furthermore, con-transfection of NOVA1 and PTBP1 reversed the effect of NOVA1 on the migration abilities of ASMCs. These data demonstrated that NOVA1 promoted ASMCs cell viability and migration through targeting PTBP1.

Studies showed that the phosphatase and tension homolog (PTEN) have the phosphatase activity to dephosphorylate PIP3 to generate PIP2 and negatively regulate the PI3K/Akt signaling pathway (Luo et al., 2013b; Vanhaesebroeck and Waterfield, 1999). Luo et al. has reported that PTEN overexpression inhibited proliferation and promoted apoptosis of human ASMCs cultured *in vitro* and was associated with downregulation of p-Akt level (Luo et al., 2013b). In addition, previous study reported that PTBP1 overexpression promoted cell proliferation in breast cancer and bladder cancer through activating PTEN/Akt signal

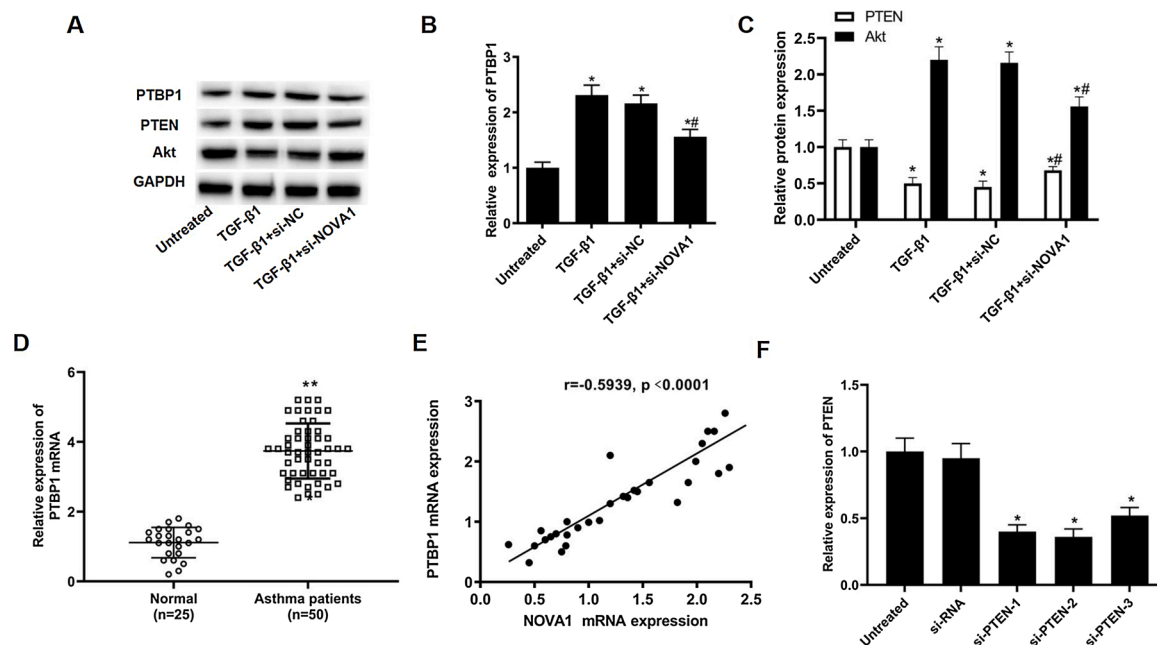


Fig. 4. There was a positive correlation between NOVA1 and PTBP1, si-NOVA1 inhibited PTBP1/PTEN/Akt axis. (A) Western blot was used to measure the expression of PTBP1, PTEN and p-Akt in untreated, TGF- β 1, TGF- β 1+si-NC and TGF- β 1+si-NOVA1 group. (B) The protein expression of PTBP1 in untreated, TGF- β 1, TGF- β 1+si-NC and TGF- β 1+si-NOVA1 group. (C) The protein expression of PTEN and p-Akt in untreated, TGF- β 1, TGF- β 1+si-NC and TGF- β 1+si-NOVA1 group. (D) The expression level of PTBP1 in asthmatic tissues was measured through RT-qPCR. (E) The relationship between NOVA1 and PTBP1 was analyzed. (F) The expression of PTEN in untreated, si-RNA group and si-PTEN group. “*” means compared with untreated group $P < 0.05$, and “#” means compared with TGF- β 1+si-NC group $P < 0.05$. GAPDH was used as an invariant internal control for calculating protein-fold changes.

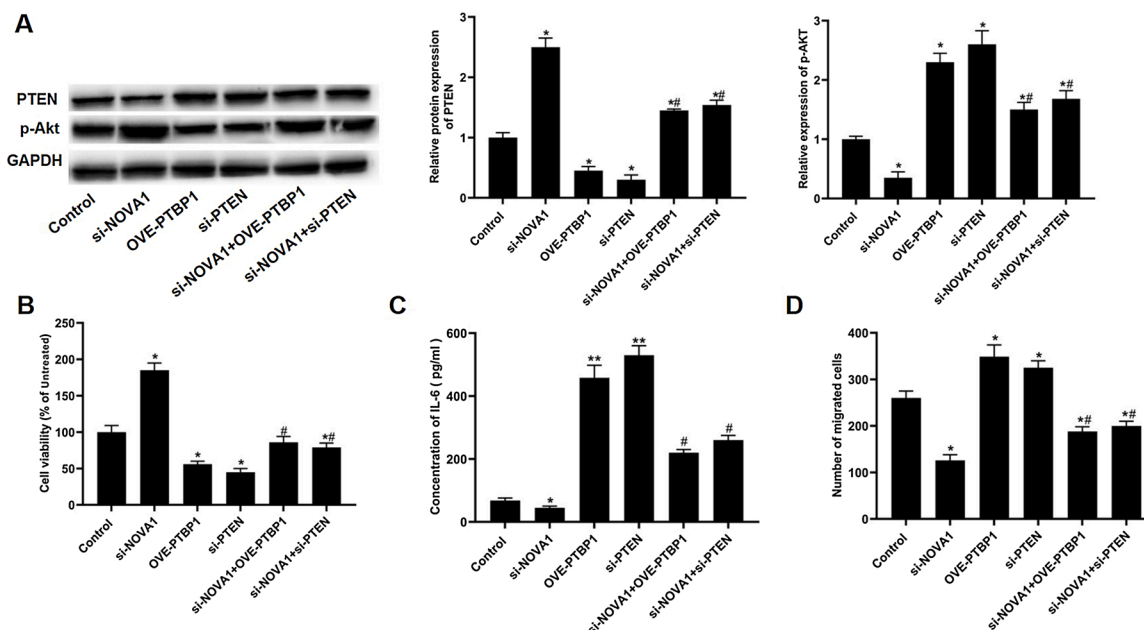


Fig. 5. PTBP1/PTEN/Akt signaling pathway involved in the regulation of NOVA1 on ASMCs. ASMCs were transfected with si-NOVA1 or pcDNA3.1-PTBP1 (OVE- PTBP1) or si-PTEN for 48 h, and then treated with 10 ng/mL TGF- β 1 for 12 h. (A). Western blot was used to measure the expression of PTEN and p-Akt in each group. (B) The cell viability of ASMCs in each group. (C) The levels of IL-6 in each group. (D) Cell migration was measured through transwell assay in each group. “*” means compared with control group $P < 0.05$, and “#” means compared with si-NOVA1 group $P < 0.05$. GAPDH was used as an invariant internal control for calculating protein-fold changes.

pathway (Wang et al., 2018; Takai et al., 2017). In our study, we found that PTBP1 overexpression significantly decreased the expression of PTEN and increased the level of Akt phosphorylation, yet those results were opposite with NOVA1 in human ASMCs. Those results revealed that inhibition of NOVA1 hindered cell viability and migration of ASMCs via modulating PTBP1/PTEN/Akt axis. Therefore, inhibition of PTBP1/PTEN/Akt signal may be one of the potential therapies for asthma. In this study, we found that TGF- β 1 decreased survival ability, which is contrary to most existing literatures. It has been reported that TGF- β 1 is usually described as a proliferative ASMC activating protein. The results showed that TGF- β 1 decreased cell viability. Therefore, its role in the development of asthma still needs to be further explored.

In summary, the present study demonstrates for the first time that NOVA1 is associated with airway remodeling. Inhibition of NOVA1 inhibited cell inflammation, cell viability and migration of ASMCs. Mechanically, NOVA1 reduced the activation of PTEN/Akt signal pathway through targeting PTBP1. These data offer some theoretical basis for the development of new agents for asthma therapy.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Statement of ethics

Prior to enrolment, all subjects signed written informed consents, and this study protocol has been approved by the Ethical Committee of the hospital.

Author's contributions

XLS conceived and designed the experiments, YC analyzed the results of the experiments and wrote the paper, YC, NW, LZ, CCL, JW and CLM performed the experiments, all authors have read and approved the manuscript.

Declaration of Competing Interest

The authors report no declarations of interest.

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References

- Barnes, P.J., 2000. Endogenous inhibitory mechanisms in asthma. *Am. J. Respir. Crit. Care Med.* 161 (supplement 2), S176–S181.
- Caruso, P., Dunmore, B.J., Schlosser, K., Schoors, S., Dos Santos, C., Perez-Iratxeta, C., et al., 2017. Identification of microRNA-124 as a major regulator of enhanced endothelial cell glycolysis in pulmonary arterial hypertension via PTBP1 (polypyrimidine tract binding protein) and pyruvate kinase M2. *Circulation* 136 (25), 2451–2467.
- Chen, Y.F., Huang, G., Wang, Y.M., Cheng, M., Zhu, F.F., Zhong, J.N., et al., 2019. Exchange protein directly activated by cAMP (Epac) protects against airway inflammation and airway remodeling in asthmatic mice. *Respir. Res.* 20 (1), 285.
- Cho, C.-Y., Chung, S.-Y., Lin, S., Huang, J.-S., Chen, Y.-L., Jiang, S.-S., et al., 2019. PTBP1-mediated regulation of AXI mRNA stability plays a role in lung tumorigenesis. *Sci. Rep.* 9 (1), 1–12.
- Cui, J., Placzek, W.J., 2018. PTBP1 enhances miR-101-guided AGO2 targeting to MCL1 and promotes miR-101-induced apoptosis. *Cell Death Dis.* 9 (5), 552.
- Fang, L., Wang, X., Sun, Q., Papakonstantinou, E., S'ng, C., Tamm, M., et al., 2019. IgE downregulates PTEN through microRNA-21-5p and stimulates airway smooth muscle cell remodeling. *Int. J. Mol. Sci.* 20 (4).
- Gao, Y., Wang, B., Luo, H., Zhang, Q., Xu, M., 2018. miR-217 represses TGF- β 1-induced airway smooth muscle cell proliferation and migration through targeting ZEB1. *Biomed. Pharmacother.* 108, 27–35.
- Huang, N., Liu, K., Liu, J., Gao, X., Zeng, Z., Zhang, Y., et al., 2018. Interleukin-37 alleviates airway inflammation and remodeling in asthma via inhibiting the activation of NF- κ B and STAT3 signalings. *Int. Immunopharmacol.* 55, 198–204.
- Kang, H., Heo, S., Shin, J.J., Ji, E., Tak, H., Ahn, S., et al., 2019. A miR-194/PTBP1/CCND3 axis regulates tumor growth in human hepatocellular carcinoma. *J. Pathol.* 249 (3), 395–408.
- Kim, E.K., Cho, Y.A., Seo, M.K., Ryu, H., Cho, B.C., Koh, Y.W., et al., 2019. NOVA1 induction by inflammation and NOVA1 suppression by epigenetic regulation in head and neck squamous cell carcinoma. *Sci. Rep.* 9 (1), 11231.
- Kwak, Y.G., Song, C.H., Yi, H.K., Hwang, P.H., Kim, J.S., Lee, K.S., et al., 2003. Involvement of PTEN in airway hyperresponsiveness and inflammation in bronchial asthma. *J. Clin. Invest.* 111 (7), 1083–1092.

- Li, L.H., Lu, B., Wu, H.K., Zhang, H., Yao, F.F., 2015. Apigenin inhibits TGF- β 1-induced proliferation and migration of airway smooth muscle cells. *Int. J. Clin. Exp. Pathol.* 8 (10), 12557–12563.
- Li, C., He, Y., Ma, H., Han, S., 2017a. NOVA1 acts as an oncogene in osteosarcoma. *Am. J. Transl. Res.* 9 (10), 4450–4457.
- Li, H., Lv, B., Kong, L., Xia, J., Zhu, M., Hu, L., et al., 2017b. Nova1 mediates resistance of rat pheochromocytoma cells to hypoxia-induced apoptosis via the Bax/Bcl-2/caspase-3 pathway. *Int. J. Mol. Med.* 40 (4), 1125–1133.
- Liu, Y., Yang, K., Shi, H., Xu, J., Zhang, D., Wu, Y., et al., 2015. MiR-21 modulates human airway smooth muscle cell proliferation and migration in asthma through regulation of PTEN expression. *Exp. Lung Res.* 41 (10), 535–545.
- Liu, Y., Miao, Y., Gao, X., Wang, Y.Y., Wang, H., Zheng, Y.W., et al., 2018. MicroRNA-200a affects the proliferation of airway smooth muscle cells and airway remodeling by targeting FOXC1 via the PI3K/AKT signaling pathway in ovalbumin-induced asthmatic mice. *Cell. Physiol. Biochem.* 50 (6), 2365–2389.
- Llorian, M., Schwartz, S., Clark, T.A., Hollander, D., Tan, L.-Y., Spellman, R., et al., 2010. Position-dependent alternative splicing activity revealed by global profiling of alternative splicing events regulated by PTB. *Nat. Struct. Mol. Biol.* 17 (9), 1114.
- Ludlow, A.T., Wong, M.S., Robin, J.D., Batten, K., Yuan, L., Lai, T.P., et al., 2018. NOVA1 regulates hTERT splicing and cell growth in non-small cell lung cancer. *Nat. Commun.* 9 (1), 3112.
- Luo, L., Gong, Y.Q., Qi, X., Lai, W., Lan, H., Luo, Y., 2013a. Effect of tumor suppressor PTEN gene on apoptosis and cell cycle of human airway smooth muscle cells. *Mol. Cell. Biochem.* 375 (1–2), 1–9.
- Luo, L., Gong, Y.Q., Qi, X., Lai, W., Lan, H., Luo, Y., 2013b. Effect of tumor suppressor PTEN gene on apoptosis and cell cycle of human airway smooth muscle cells. *Mol. Cell. Biochem.* 375 (1–2), 1–9.
- Luo, Y., Hao, T., Zhang, J., Zhang, M., Sun, P., Wu, L., 2019. MicroRNA-592 suppresses the malignant phenotypes of thyroid cancer by regulating lncRNA NEAT1 and downregulating NOVA1. *Int. J. Mol. Med.* 44 (3), 1172–1182.
- Sawicka, K., Bushell, M., Spriggs, K.A., Willis, A.E., 2008. Polypyrimidine-tract-binding Protein: A Multifunctional RNA-binding Protein. Portland Press Ltd.
- Sayed, M.E., Yuan, L., Robin, J.D., Tedone, E., Batten, K., Dahlsen, N., et al., 2019. NOVA1 directs PTBP1 to hTERT pre-mRNA and promotes telomerase activity in cancer cells. *Oncogene* 38 (16), 2937–2952.
- Spellman, R., Rideau, A., Matlin, A., Gooding, C., Robinson, F., McGlinchey, N., et al., 2005. Regulation of Alternative Splicing by PTB and Associated Factors. Portland Press Ltd.
- Takai, T., Yoshikawa, Y., Inamoto, T., Minami, K., Taniguchi, K., Sugito, N., et al., 2017. A novel combination RNAi toward Warburg effect by replacement with miR-145 and silencing of PTBP1 induces apoptotic cell death in bladder cancer cells. *Int. J. Mol. Sci.* 18 (1), 179.
- Vanhaesebroeck, B., Waterfield, M., 1999. Signaling by distinct classes of phosphoinositide 3-kinases. *Exp. Cell Res.* 253 (1), 239–254.
- Walker, E.J., Heydet, D., Veldre, T., Ghildyal, R., 2019. Transcriptomic changes during TGF- β -mediated differentiation of airway fibroblasts to myofibroblasts. *Sci. Rep.* 9 (1), 20377.
- Wang, X., Li, Y., Fan, Y., Yu, X., Mao, X., Jin, F., 2018. PTBP1 promotes the growth of breast cancer cells through the PTEN/Akt pathway and autophagy. *J. Cell. Physiol.* 233 (11), 8930–8939.
- Wang, Z., Gan, X., Qiu, C., Yang, D., Sun, X., Zeng, Z., 2019. Role of polypyrimidine tract-binding protein 1/yin yang 2 signaling in regulating vascular smooth muscle cell proliferation and neointima hyperplasia. *Toxicol. Appl. Pharmacol.* 383, 114747.
- Wollerton, M.C., Gooding, C., Wagner, E.J., Garcia-Blanco, M.A., Smith, C.W., 2004. Autoregulation of polypyrimidine tract binding protein by alternative splicing leading to nonsense-mediated decay. *Mol. Cell* 13 (1), 91–100.
- Xu, T., Yan, W., Wu, Q., Xu, Q., Yuan, J., Li, Y., et al., 2019. MiR-326 inhibits inflammation and promotes autophagy in silica-induced pulmonary fibrosis through targeting TNFSF14 and PTBP1. *Chem. Res. Toxicol.* 32 (11), 2192–2203.
- Yick, C.Y., Zwiderman, A.H., Kunst, P.W., Grünberg, K., Mauad, T., Chowdhury, S., et al., 2014. Gene expression profiling of laser microdissected airway smooth muscle tissue in asthma and atopy. *Allergy* 69 (9), 1233–1240.
- Yu, X., Zheng, H., Chan, M.T.V., Wu, W.K.K., 2018. NOVA1 acts as an oncogene in melanoma via regulating FOXO3a expression. *J. Cell. Mol. Med.* 22 (5), 2622–2630.