FOXF1 attenuates TGF-β1-induced bronchial epithelial cell injury by inhibiting CDH11-mediated Wnt/β-catenin signaling

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Abstract. Forkhead box F1 (FOXF1) has been reported to be associated with lung development. However, the role of FOXF1 in asthma is still not fully understood. In the present study, the biological role and the potential mechanism of FOXF1 was explored in transforming growth factor β1 (TGF-β1)-induced bronchial epithelial cell injury. Reverse transcription-quantitative PCR and western blotting were performed to detect the expression levels of FOXF1 and cadherin (CDH) 11 in TGF-β1-induced bronchial epithelial cells. Proliferation, apoptosis and inflammation were assessed using Cell Counting Kit-8 assay, flow cytometry, western blotting and ELISA. Fibrosis and epithelial-mesenchymal transition (EMT) were evaluated using immunofluorescence and western blotting. The expression levels of the proteins involved in the Wnt/β-catenin pathway were detected by western blotting. The results indicated that FOXF1 expression was downregulated, while CDH11 expression was upregulated in TGF-β1-treated BEAS-2B cells. FOXF1 overexpression promoted proliferation, inhibited induction of apoptosis and suppressed the inflammatory response of BEAS-2B cells exposed to TGF-β1. In addition, FOXF1 overexpression restrained TGF-β1-induced bronchial epithelial fibrosis and EMT and inhibited the activation of the Wnt/β-catenin pathway. CDH11 overexpression reversed the effects of FOXF1 overexpression on proliferation, apoptosis, fibrosis, EMT and inflammation by regulating the Wnt/β-catenin pathway. Collectively, the results of the present study suggested that FOXF1 regulated TGF-β1-induced BEAS-2B cell injury by inhibiting CDH11-mediated Wnt/β-catenin signaling. This may provide a novel therapeutic strategy for the treatment of asthma.

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Introduction

Asthma, also known as bronchial asthma, is a common chronic airway inflammatory disease. It is characterized by reversible airflow obstruction, airway inflammation, persistent airway hyperresponsiveness and airway remodeling and the release of inflammatory mediators that can cause tissue damage and airway dysfunction (1-3). Asthma is a globally significant non-communicable disease with major public health consequences for both children and adults and high morbidity and mortality (4). Pediatric asthma, also known as pediatric bronchial asthma, is the most common chronic disease noted in children worldwide and its mortality has reached a high level with a mortality rate ranging from 0-0.7 per 100,000 individuals in children (5). An increasing trend of this disease has been noted in recent years (6,7). Therefore, it is important to explore the molecular mechanisms involved in pediatric asthma and develop more effective and feasible strategies for this disease.

The forkhead box (FOX) transcription factor family is composed of a group of evolutionarily conserved transcriptional regulators that play an important role in embryonic growth and development and maintaining cellular homeostasis (8-10). FOXF1, a member of the FOX family of transcription factors, has been previously shown to be important for lung development, homeostasis, and injury responses (11,12). It has been reported that haploinsufficiency of *FOXF1* leads to severe lung malformations and suppresses the development of pulmonary capillaries during both embryonic and early postnatal periods (13). In addition, heterozygous deletions and point mutations in the *FOXF1* gene locus were observed in >40% of patients with alveolar-capillary dysplasia and misalignment of pulmonary veins (14). An additional study reported that endothelial-specific deletion of both *FOXF1* alleles causes notable inflammatory cell infiltration, pulmonary edema and pulmonary hemorrhage in adult mice (15). Although the key role of FOXF1 in the development of lung injury has been established, the molecular mechanisms regulated by FOXF1 during asthma remain unclear. Therefore, the present study aimed to explore the expression levels of

FOXF1 in asthma and the mechanisms by which FOXF1 influences the development of this disease.

Materials and methods

Cell culture and treatment. The human bronchial epithelial cell line BEAS-2B was purchased from the American Type Culture Collection. The cells were cultured in DMEM containing 10% FBS, 1% penicillin/streptomycin in an incubator at 37˚C in the presence of 5% CO_2 for 24 h. Subsequently, BEAS-2B cells were stimulated with 10 ng/ml TGF-β1 for 24 h at 37˚C. LiCl (β-catenin activator; 20 mM; Sigma-Aldrich; Merck KGaA) was used to treat the cells for 3 h at 37˚C. Untreated cells were regarded as the control group.

Cell transfection. FOXF1-specific pcDNA overexpression vector (pcDNA-FOXF1; 20 μg), cadherin (CDH) 11-specific pcDNA overexpression vector (pcDNA-CDH11; 20 μg), and the corresponding negative control (pcDNA3.1; 20 μg) were synthesized by Suzhou GenePharma Co., Ltd. These recombinant plasmids were transfected into BEAS-2B cells using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) for 48 h at 37˚C according to the manufacturer's protocols. Cells were collected after 48 h transfection for the following experiments.

Cell Counting Kit-8 (CCK-8) assay. BEAS-2B cell viability was evaluated using the CCK-8 assay. The transfected cells were seeded into 96-well plates at a density of $5x10^4$ cells/ml and cultured in DMEM with 10% FBS at 37˚C. A total of 10 μl CCK-8 solution (Sangon Biotech Co., Ltd.) was added to each well following 24, 48 and 72 h of culture and incubated for 2 h. The absorbance was measured at 450 nm with a microplate reader (Bio-Rad Laboratories, Inc.).

Flow cytometry. Apoptosis was detected by the FITC Annexin V/propidium iodide (PI) Apoptosis Detection Kit I (Guangzhou RiboBio Co., Ltd.). Briefly, the cells were collected by centrifugation (1,000 x g for 5 min) at room temperature, washed with precooled PBS at 4˚C and re-suspended in binding buffer (Guangzhou RiboBio Co., Ltd.). The cells were incubated with 5 μl Annexin V-FITC (20 μg/ml) at room temperature for 15 min and with 10 μl PI (10 mg/ml) in a dark room for 5 min at room temperature. Apoptotic cells were subsequently analyzed using a BD FACS Calibur flow cytometer (BD Biosciences) and FlowJo software (v10.4; FlowJo LLC) was used for apoptosis analysis.

ELISA analysis. The cell supernatants of BEAS-2B cells were collected. The concentration levels of tumor necrosis factor-α (TNF-α), interleukin (IL)-6 and IL-1β in each group were measured using Human TNF-alpha Quantikine ELISA kit (cat. no. DTA00D), Human IL-6 Quantikine ELISA kit (cat. no. D6050) and Human IL-1 beta/IL-1F2 Quantikine ELISA kit (cat. no. DLB50) (R&D Systems, Inc.), respectively. The optical density of each well was assayed at 450 nm using a microplate spectrophotometer.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from BEAS-2B cells using the TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) in accordance with the manufacturer's protocols. The quality and concentration of RNA were detected using NanoDrop 2000 (Thermo Fisher Scientific) at 260 and 280 nm. Reverse transcription of first-strand cDNAs was performed using PrimeScript RT Master Mix (Perfect Real Time; Takara Bio, Inc.) according to the manufacturer's instructions. cDNA amplification was performed by RT-qPCR using the SYBR Premix Ex Taq™ II kit (Takara Bio, Inc.). The following thermocycling conditions were used for qPCR: Pre-denaturation at 95˚C for 1 min, followed by 40 cycles of denaturation at 95˚C for 15 sec, annealing at 60˚C for 40 sec and extension at 72˚C for 15 sec. The primer sequences for PCR are: FOXF1, forward 5'-GCCATCCAGAGTTCACCCAC-3', and reverse 5'-GAAGCCGAGCCCGTTCAT-3'; CDH11, forward 5'-GGG TTGCCCAAGCTTAATGG-3', and reverse 5'-TTTGATGTC TTTGCGGGGGA-3'; GAPDH, forward 5'-GGGAAACTG TGGCGTGAT-3', and reverse 5'-GAGTGGGTGTCGCTG TTGA-3'. The results were normalized to the expression levels of GAPDH and were measured using the $2^{-\Delta\Delta Cq}$ method (16).

Immunofluorescence staining. The collected BEAS-2B cells were fixed in 4% polyoxymethylene for 1 h at room temperature and permeabilized with 0.5% Triton X-100 for 10 min at room temperature. Following blocking with 2% BSA for 1 h at room temperature, cells were incubated with an anti-alpha smooth muscle actin primary antibody (α-SMA; 1:500; cat. no. ab32575; Abcam) at 4˚C overnight. Subsequently, the secondary antibody (1:400; cat. no. ab150077; Abcam) was added and incubated for 1 h at room temperature. The cells were subsequently counterstained with DAPI (Beyotime Institute of Biotechnology) for 10 min at 37˚C and examined using a confocal microscope.

Western blotting. The total protein was extracted from the cells using RIPA buffer (Hunan Auragene Biotechnology Co., Ltd.). The BCA Protein Assay kit (Beijing Dingguo Changsheng Biotechnology Co., Ltd.) was used to detect the protein concentration according to the manufacturer's protocols. An equal amount of protein (60 μg/lane) was loaded on 8% SDS-polyacrylamide gels and subsequently transferred to a pure nitrocellulose blotting membrane (Pall Life Sciences). Following blocking with 5% non-fat milk in 0.1% Tris-buffered saline with 0.1% Tween-20 for 1 h at room temperature, the membranes were incubated with primary antibodies at 4˚C overnight for the following proteins: FOXF1 (cat. no. ab168383), Bcl-2 (cat. no. ab32124), Bax (cat. no. ab32503), poly(ADP ribose) polymerase (PARP; cat. no. ab191217), cleaved PARP (cat. no. ab32064), α-SMA (cat. no. ab108531), fibronectin (cat. no. ab2413), collagen IV (cat. no. ab6586), E-cadherin (cat. no. ab40772), N-cadherin (cat. no. ab76011), vimentin (cat. no. ab92547), CDH11 (cat. no. ab151302) (all 1:1,000), β-catenin (1:5,000; cat. no. ab32572), c-Myc (1:1,000; cat. no. ab32072), c-jun (1:1,000; cat. no. ab40766), and β-actin (1:1,000; cat. no. ab8227) (all Abcam). The membranes were washed and incubated with horseradish peroxidase-labeled secondary antibody (Cell Signaling Technology, Inc.) for 1 h at room temperature. Finally, bands were visualized using an enhanced chemiluminescence detection system (Merck KGaA) and immunoreactivity was detected using Image J software (version 1.49; National Institutes of Health).

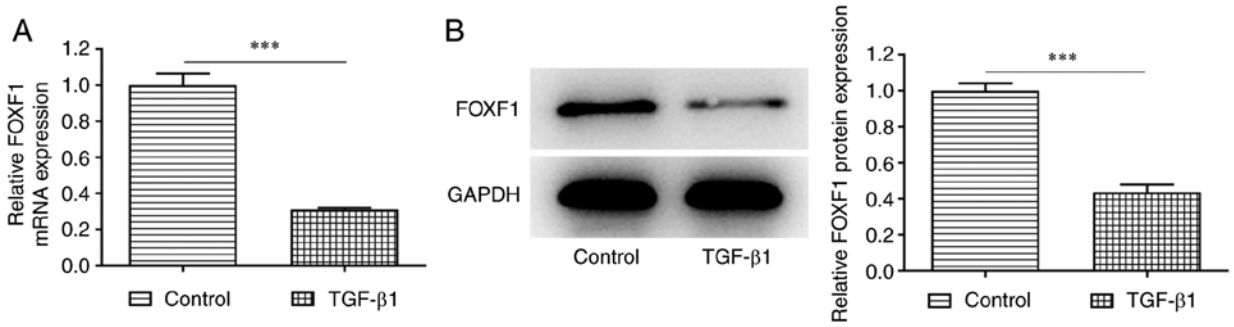

Figure 1. TGF-β1 reduces the FOXF1 expression in BEAS-2B cells. (A) mRNA and (B) protein expression levels of FOXF1 in BEAS-2B cells were detected using reverse transcription-quantitative PCR and western blotting, respectively. ***P<0.001. FOXF1, forkhead box F1; TGF-β1, transforming growth factor β1.

Statistical analysis. Statistical analysis was conducted using SPSS 22.0 (IBM Corp.) and GraphPad Prism 6 (GraphPad Software, Inc.). The unpaired Student's t-test was used for the comparison between two groups. The differences among multiple groups were analyzed using one-way ANOVA with a post hoc Bonferroni multiple comparison test. The data are presented as mean ± standard deviation of three independent experiments. P<0.05 was considered to indicate a statistically significant difference.

Results

TGF-β1 induces downregulation of FOXF1 expression in BEAS-2B cells. To explore the role of FOXF1 in bronchial epithelial cells, the expression levels of FOXF1 were initially investigated in untreated and TGF-β1-treated BEAS-2B cells. RT-qPCR and western blotting indicated a significant decrease in both mRNA and protein expression levels of FOXF1 in TGF-β1-treated BEAS-2B cells compared with the corresponding levels observed in the control cells (Fig. 1). To conclude, FOXF1 expression was low in TGF-β1-treated BEAS-2B cells.

Upregulation of FOXF1 expression reduces TGF-β1-induced damage and release of inflammatory factors in BEAS-2B cells. To investigate the roles of FOXF1 in proliferation, apoptosis and inflammation in BEAS-2B cells, FOXF1 was overexpressed in untreated and TGF-β1-treated BEAS-2B cells. The transfection efficiency was evaluated using RT-qPCR and western blotting, which revealed that FOXF1 expression was significant increased following transfection of pcDNA-FOXF1 compared with transfection control (Fig. 2A and B). Subsequently, the CCK-8 assay was used to assess proliferation. The results indicated that TGF-β1 treatment significantly inhibited proliferation in BEAS-2B cells compared with the control group; whereas overexpression of FOXF1 significantly promoted proliferation in TGF-β1-treated BEAS-2B cells compared with the TGF-β1 + pcDNA3.1 negative control cells (Fig. 2C). Furthermore, TGF-β1 treatment significantly increased the apoptotic rate of BEAS-2B cells compared with the control, while the apoptotic rate of TGF-β1-treated BEAS-2B cells transfected with pcDNA-FOXF1 was significantly reduced compared with the transfection control (Fig. 2D and E). In addition, TGF-β1 treatment significantly reduced Bcl-2 expression and significantly enhanced the expression levels of Bax and PARP compared with the control group. Whereas a significant increase in Bcl-2 and significant decreases of Bax and cleaved-PARP expression were observed after transfection with pcDNA-FOXF1 compared with the TGF-β1 + pcDNA3.1 group (Fig. 2F). In addition, FOXF1 overexpression significantly suppressed the levels of IL-6, TNF-α and IL-1β in TGF-β1-treated BEAS-2B cells compared with TGF-β1 + pcDNA3.1- treated cells (Fig. 2G). Overall, FOXF1 alleviated TGF-β1-stimulated viability injury and apoptosis in BEAS-2B cells.

Upregulation of FOXF1 inhibits fibrosis and epithelial-mesenchymal transition (EMT) in TGF-β1-treated BEAS-2B cells. The biological role of FOXF1 was investigated with regard to the induction of BEAS-2B cell fibrosis and EMT. Treatment of the cells with TGF-β1 markedly enhanced the relative fluorescence intensity of α-SMA in BEAS-2B cells, which was subsequently reduced following FOXF1 overexpression (Fig. 3A). Moreover, TGF-β1 treatment contributed to significantly increased levels of α-SMA, fibronectin and collagen IV compared with the control group; while FOXF1 overexpression exhibited significant reversal of the expression of these same markers compared with the TGF-β1 + pcDNA3.1 group (Fig. 3B). Furthermore, compared with the control group, significantly decreased levels of E-cadherin and significantly increased levels of N-cadherin and vimentin were observed in TGF-β1-treated BEAS-2B cells. FOXF1 overexpression was again able to significantly reverse the effects of TGF-β1 treatment on BEAS-2B cells compared with the TGF-β1 + pcDNA3.1 group (Fig. 3C). In summary, FOXF1 suppressed TGF-β1-elicited fibrosis and EMT in BEAS-2B cells.

FOXF1 regulates the Wnt/β-catenin signaling pathway by inhibiting CDH11 expression. The mechanism underlying the regulatory role of FOXF1 in TGF-β1-treated BEAS-2B cells was investigated. As presented in Fig. 4A and B, TGF-β1 treatment of the cells significantly enhanced the levels of CDH11 compared with the control group; whereas, FOXF1 overexpression led to a significant reduction of CDH11 levels in TGF-β1-treated BEAS-2B cells compared with the TGF-β1 + pcDNA3.1 group. Subsequently, CDH11 was overexpressed in untreated and TGF-β1-treated BEAS-2B cells. The transfection efficiency was evaluated using RT-qPCR and western blotting and CDH11 expression was significantly increased in transfected cells compared with the controls (Fig. 4C and D). Western blotting analysis indicated that TGF-β1 treatment significantly increased the

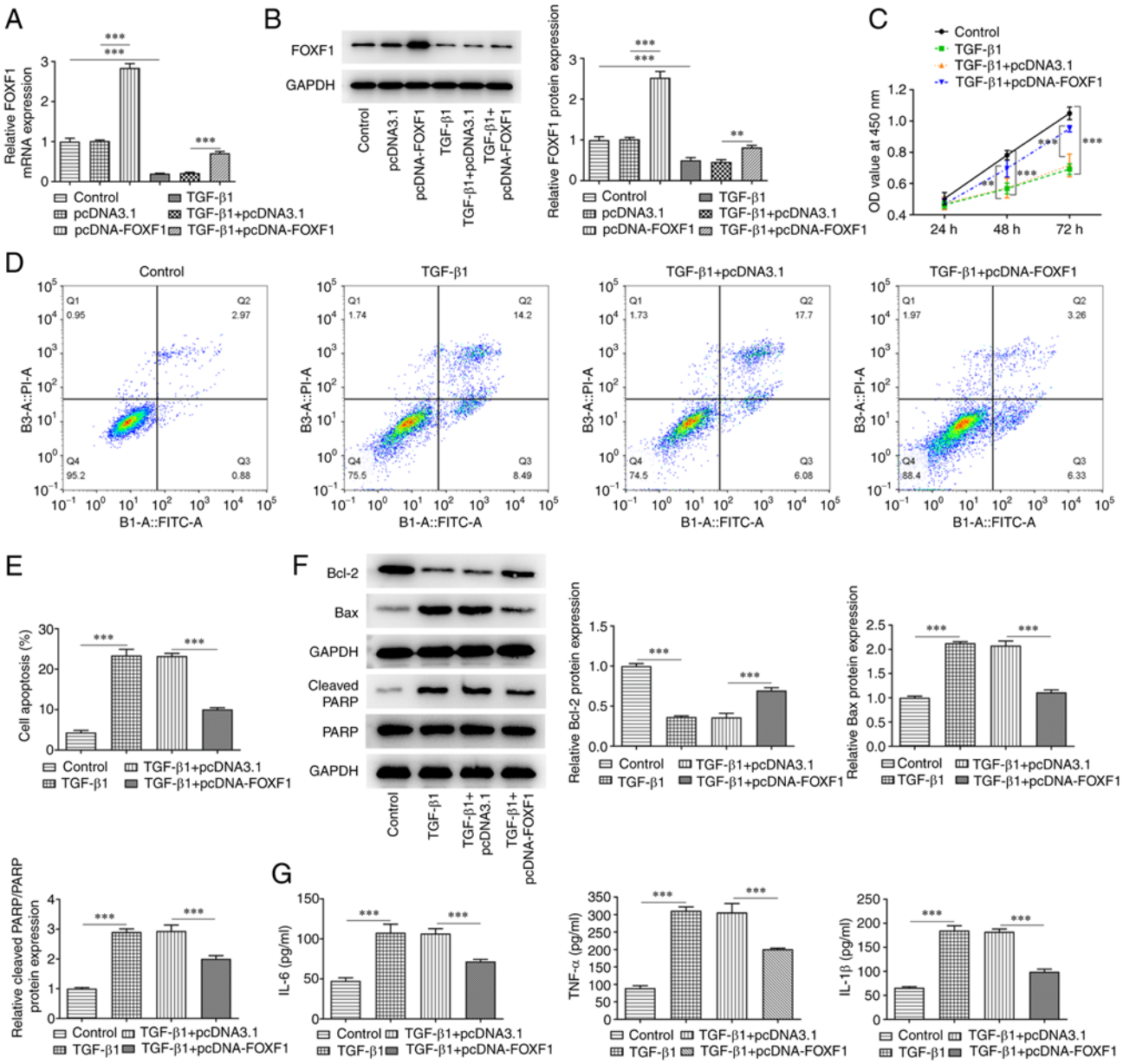

Figure 2. Upregulation of FOXF1 reduces TGF-β1-induced damage and release of inflammatory factors in BEAS-2B cells. (A) mRNA and (B) protein expression level of FOXF1 in TGF-β1-treated BEAS-2B cells were detected using reverse transcription-quantitative PCR and western blotting, respectively. (C) Cell Counting Kit-8 assay was used to assess proliferation. Flow cytometry was carried out to (D) identify and (E) quantify apoptosis in TGF-β1-treated BEAS-2B cells transfected with pcDNA-FOXF1. (F) Western blotting was performed to detect the protein expression level of Bcl-2, Bax, PARP and cleaved PARP. (G) Levels of IL-6, TNF-α and IL-1β were detected using ELISA. **P<0.01 and ***P<0.001. FOXF1, forkhead box F1; TGF-β1, transforming growth factor β1; PI, propidium iodide; FITC-A, FITC-Annexin-V; PARP, poly(ADP ribose) polymerase; IL, interleukin; TNF-α, tumor necrosis factor-α.

expression levels of β-catenin, c-Myc, and c-Jun in BEAS-2B cells compared with the control group. FOXF1 overexpression reversed the effects of TGF-β1 on β-catenin levels in TGF-β1 + pcDNA-FOXF1 group compared with TGF-β1 + pcDNA3.1 group, though minimal changes were noted to the expression levels of c-Myc and c-Jun. However, CDH11 overexpression significantly reversed the effects of FOXF1 overexpression on the expression levels of β-catenin, c-Myc and c-jun (Fig. 4E). Collectively, FOXF1 down-regulated CDH11 to inactivate Wnt/β-catenin signaling.

FOXF1 inhibits CDH11-mediated Wnt/β-catenin signaling in TGF-β1-treated BEAS-2B cells. The CCK-8 assay revealed that CDH11 overexpression and LiCl treatment significantly reduced the optical density values of TGF-β1-treated BEAS-2B cells transfected with the FOXF1 overexpression plasmid compared with the TGF-β1 + pcDNA-FOXF1 group (Fig. 5A). Moreover, a significant increase was noted in the apoptotic rate of TGF-β1-treated BEAS-2B cells co-transfected with pcDNA-FOXF1 and pcDNA-CDH11 or treated with LiCl compared with that in cells transfected with pcDNA-FOXF1 alone (Fig. 5B and C). In addition, western blotting indicated that pcDNA-CDH11 and LiCl treatment significantly reversed the effects of FOXF1 overexpression on the protein expression levels of Bcl-2, Bax, and cleaved PARP/PARP in TGF-β1-treated BEAS-2B cells compared with cells

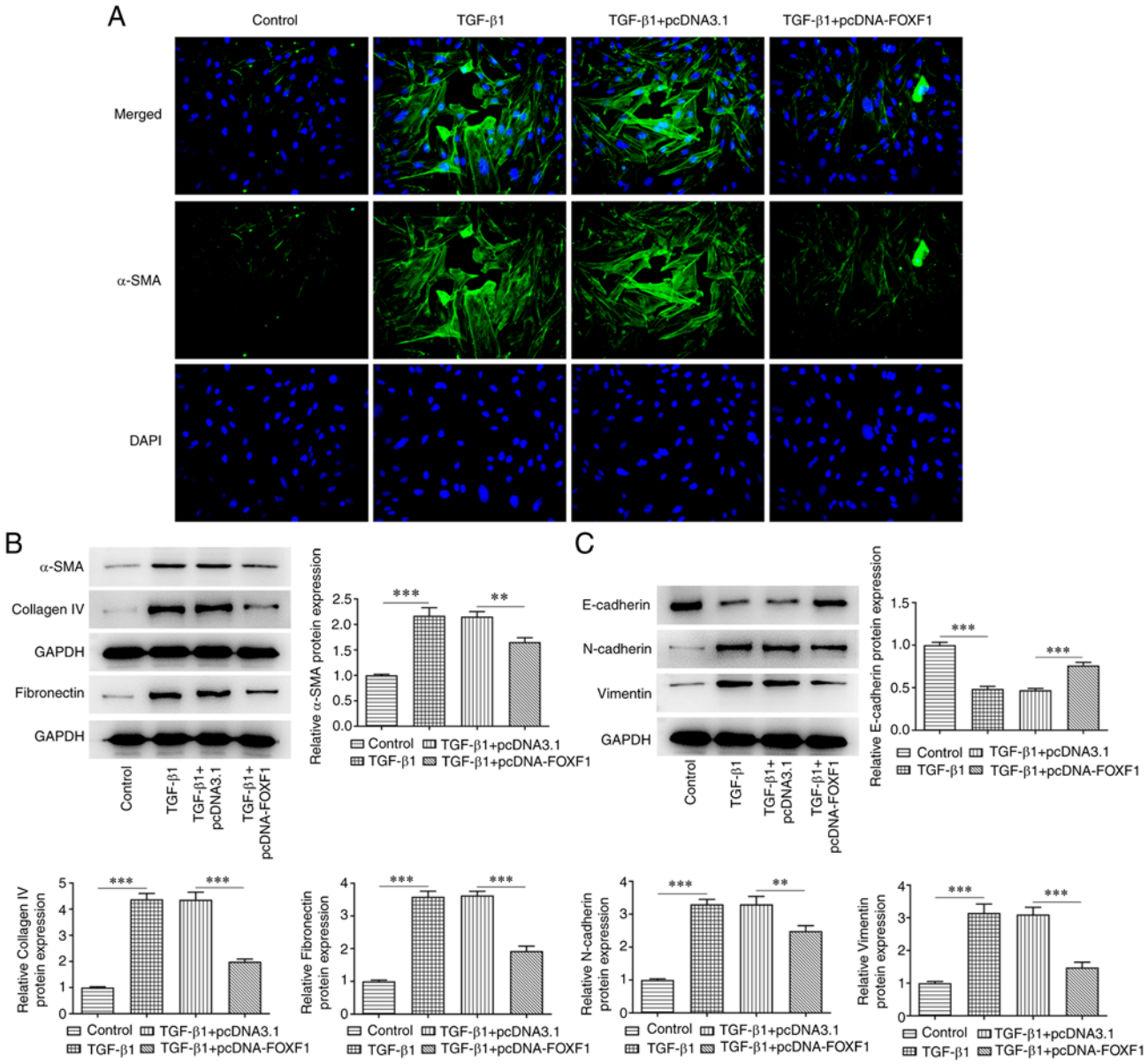

Figure 3. Upregulation of FOXF1 suppresses TGF-β1-induced BEAS-2B cell fibrosis and epithelial-mesenchymal transition. (A) Immunofluorescence was used to detect the expression of α-SMA (magnification, 200x). (B) Western blotting was performed to detect the protein expression level of α-SMA, fibronectin and collagen IV. (C) The levels of E-cadherin, N-cadherin and vimentin were assessed using western blotting. **P<0.01, ***P<0.001. FOXF1, forkhead box F1; TGF-β1, transforming growth factor β1; α-SMA, smooth muscle α-actin.

transfected with pcDNA-FOXF1 alone (Fig. 5D). Furthermore, pcDNA-CDH11 and treatment with LiCl markedly enhanced the relative fluorescence intensity of α-SMA in BEAS-2B cells following decreased fluorescence after transfection with the FOXF1 overexpression vector (Fig. 5E). In agreement with these findings, it was observed that the previously significantly downregulated expression levels of α-SMA, fibronectin and collagen IV were significantly elevated in TGF-β1-treated BEAS-2B cells overexpressing FOXF1 following transfection with pcDNA-CDH11 and treatment with LiCl compared with cells transfected with pcDNA-FOXF1 alone (Fig. 6A). Furthermore, the significantly increased levels of E-cadherin and the significantly reduced levels of N-cadherin and vimentin noted in TGF-β1-treated BEAS-2B cells overexpressing FOXF1 were significantly reversed following transfection with pcDNA-CDH11 and treatment with LiCl compared with cells transfected with pcDNA-FOXF1 alone (Fig. 6B). The latter treatments significantly increased the levels of IL-6, TNF-α and IL-1β in TGF-β1-treated BEAS-2B cells transfected with the FOXF1 overexpression plasmid (Fig. 6C). Overall, CDH11 elevation of activation of Wnt/β-catenin signaling reversed the impacts of FOXF1 on TGF-β1-treated BEAS-2B cells.

Discussion

Asthma is a chronic airway inflammatory disease, with children being mainly susceptible to the disease (17). The release of inflammatory mediators can cause tissue damage and airway dysfunction (18). Various types of medical treatments are available for childhood asthma; however, their efficacy remains unsatisfactory (19-21). In the present study, a mechanism of action of asthma was examined that demonstrated FOXF1 may

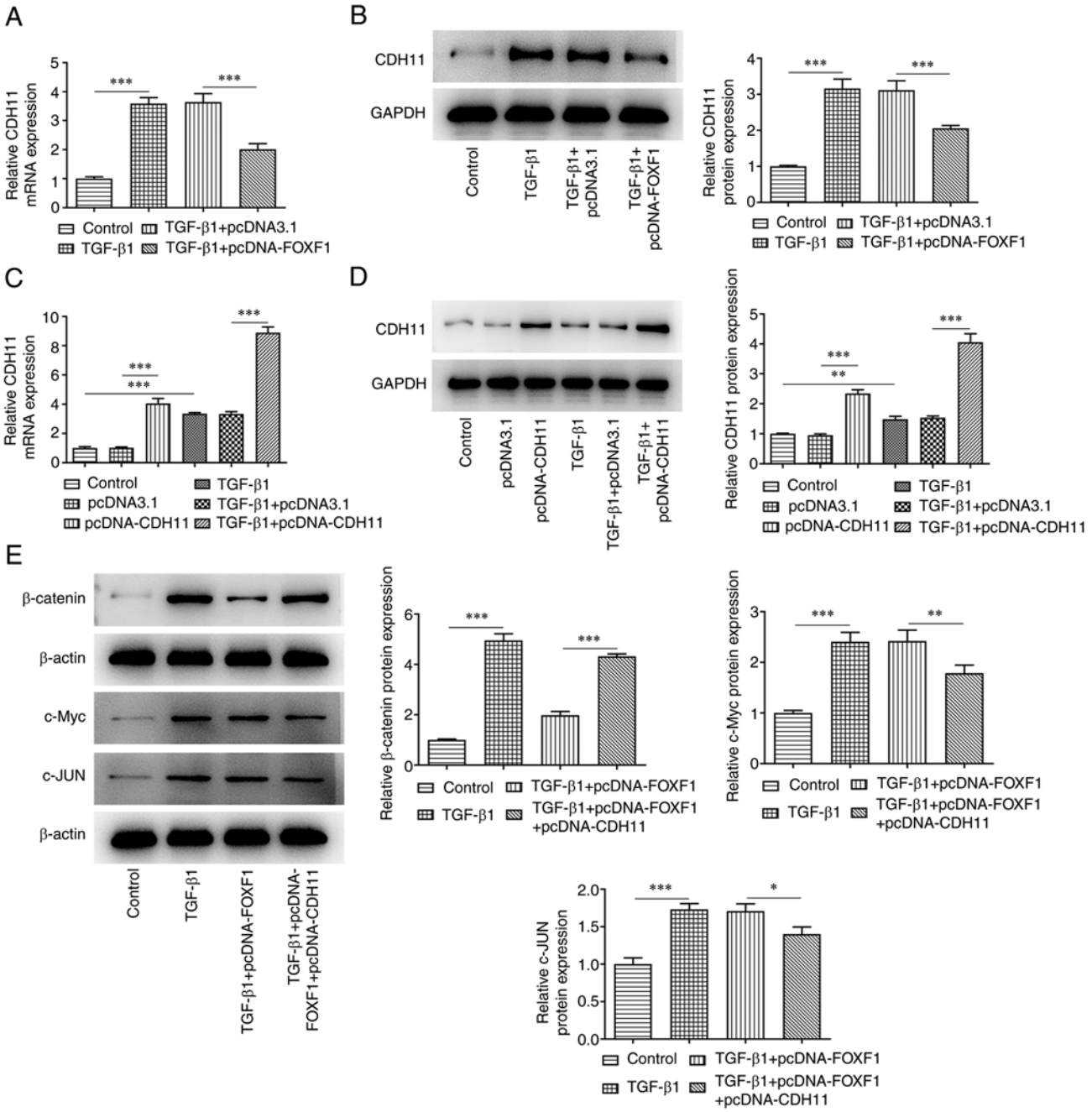

Figure 4. FOXF1 regulates the Wnt/β-catenin signaling by inhibition of CDH11. (A) mRNA and (B) protein expression level of CDH11 in TGF-β1-induced BEAS-2B cells transfected with pcDNA-FOXF1 were detected using RT-qPCR and western blotting, respectively. (C) mRNA and (D) protein expression level of CDH11 in TGF-β1-induced BEAS-2B cells transfected with pcDNA-CDH11 were detected using RT-qPCR and western blotting, respectively. (E) Levels of β-catenin, c-Myc and c-JUN in TGF-β1-induced BEAS-2B cells transfected with pcDNA-FOXF1 with or without pcDNA-CDH11. *P<0.05, **P<0.01, ***P<0.001. FOXF1, forkhead box F1; TGF-β1, transforming growth factor β1; CDH11, cadherin 11; RT-qPCR, reverse transcription-quantitative PCR.

attenuate TGF-β1-induced bronchial epithelial cell injury by inhibiting CDH11-mediated Wnt/β-catenin signaling.

FOXF1 plays an important role in regulating lung development and lung injury (22). A previous study has shown that FOXF1 stimulates pulmonary angiogenesis and alveolation during recovery from neonatal hyperoxia injury (23). In addition, FOXF1 maintains endothelial barrier function and prevents edema following lung injury (15). A previous study has shown that FOXF1 protects against BEAS-2B apoptosis and oxidative stress induced by paraquat (22). Therefore, it is speculated that FOXF1 is involved in the regulation of airway epithelial cell injury induced by asthma. In the present study, TGF-β1 treatment induced the downregulation of FOXF1 expression in BEAS-2B cells and FOXF1 overexpression reduced TGF-β1-induced apoptosis by increasing Bcl-2 expression, reducing the levels of Bax and cleaved PARP and inhibiting the release of inflammatory factors IL-6, TNF-α and IL-1β. In addition, FOXF1 overexpression suppressed the production of α-SMA, fibronectin and collagen IV to reduce TGF-β1-induced fibrosis; it also limited EMT by enhancing the expression of E-cadherin while decreasing the levels of N-cadherin and vimentin. These findings are in line with

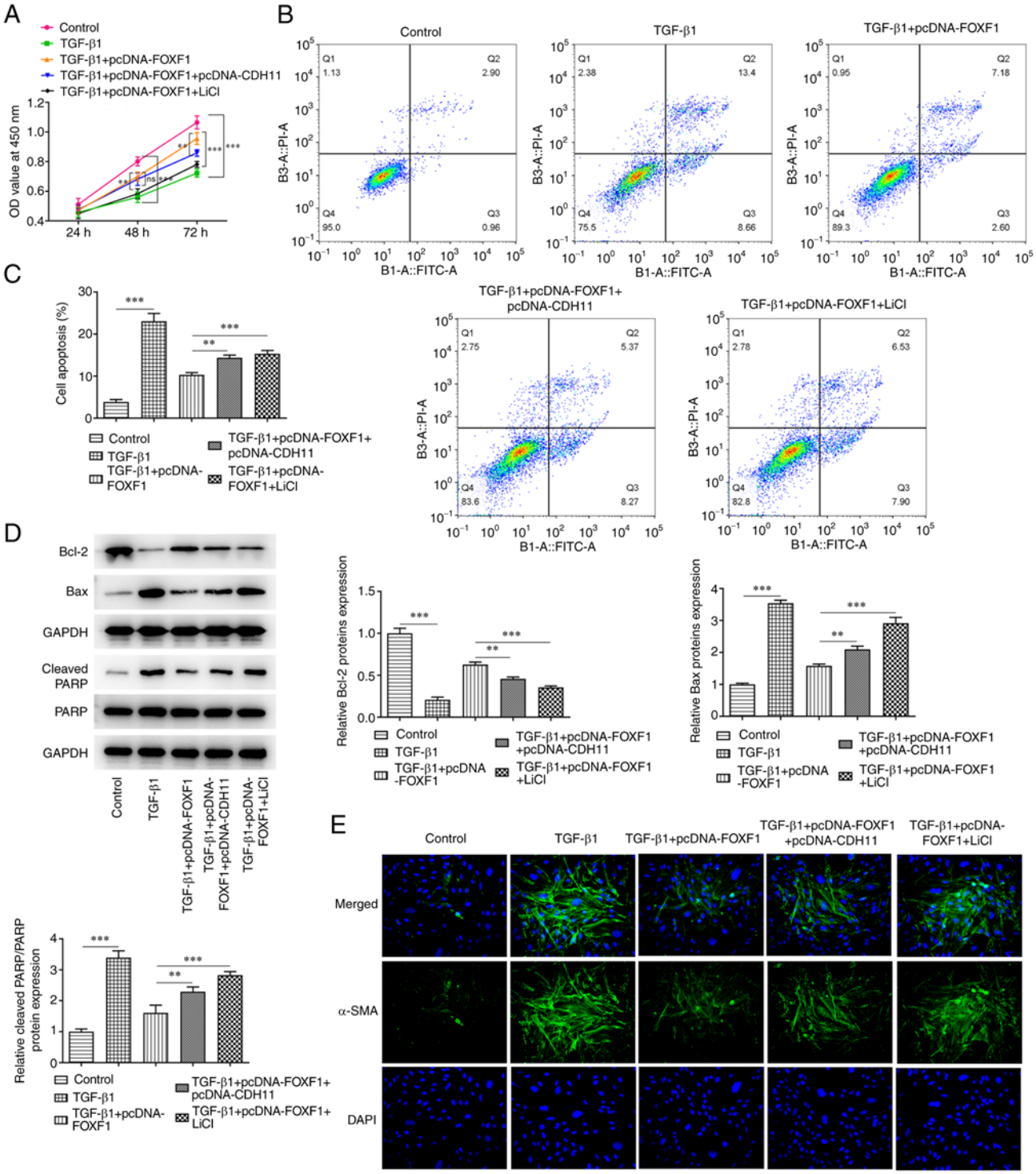

Figure 5. FOXF1 reduces TGF-β1-induced BEAS-2B cell injury by inhibiting CDH11-mediated Wnt/β-catenin signaling. (A) Cell Counting Kit-8 assay was used to detect cell proliferation. Flow cytometry was performed to (B) identify and (C) quantify apoptosis. (D) Western blotting was performed to detect the protein expression level of Bcl-2, Bax, PARP and cleaved PARP. (E) Immunofluorescence was used to detect the expression of α-SMA (magnification, 200x). **P<0.01, ***P<0.001. FOXF1, forkhead box F1; TGF-β1, transforming growth factor β1; OD, optical density; PI, propidium iodide; FITC-A, FITC-Annexin-V; PARP, poly(ADP ribose) polymerase; α-SMA, smooth muscle α-actin.

previous results demonstrating that the FOXF1 transcription factor can promote lung regeneration following partial pneumonectomy and can induce fetal lung mesenchymal cell proliferation and promote lung morphogenesis (24,25).

CDH11 is a member of the cadherin family of proteins, with its gene located on chromosome 16q22.1 (26). Previous studies have reported that CDH11 serves key roles in the occurrence and development of several diseases, including tumors and arthritis (27,28). Wang *et al* (29) demonstrated that microRNA-451a suppresses airway remodeling by regulating CDH11 in an allergic asthma model in neonatal mice. In addition, FOXF1 transcription factor has been demonstrated to inhibit CDH11 expression (30). It also inhibits pulmonary fibrosis by blocking CDH2-CDH11 cadherin conversion in

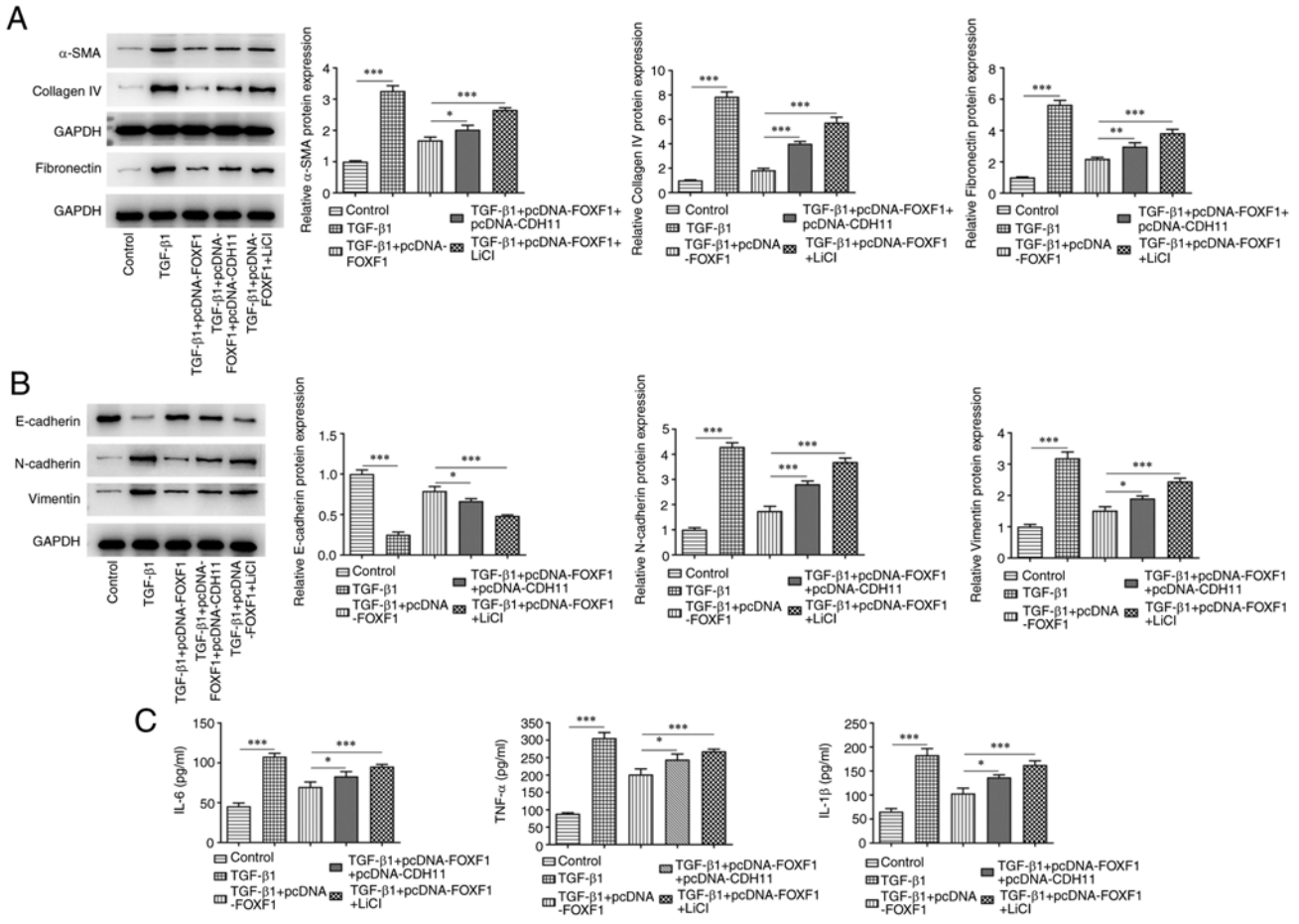

Figure 6. FOXF1 reduces TGF-β1-induced BEAS-2B cell fibrosis, epithelial-mesenchymal transition and inflammation by inhibiting CDH11-mediated Wnt/β-catenin signaling. (A) Western blotting was performed to detect the protein levels of α-SMA, fibronectin and collagen IV. (B) Protein expression levels of E-cadherin, N-cadherin and vimentin were assessed using western blotting. (C) Levels of IL-6, TNF-α and IL-1β were detected using ELISA. *P<0.05, **P<0.01, ***P<0.001. FOXF1, forkhead box F1; TGF-β1, transforming growth factor β1; CDH11, cadherin 11; α-SMA, smooth muscle α actin; IL, interleukin; TNF-α, tumor necrosis factor-α.

myofibroblasts (30). In the current study, it was revealed that TGF-β1 treatment increased CDH11 expression in BEAS-2B cells, whereas FOXF1 overexpression downregulated CDH11 expression following TGF-β1 stimulation. Furthermore, FOXF1 ameliorated TGF-β1-triggered viability injury, apoptosis, fibrosis and EMT in BEAS-2B cells. CDH11 overexpression reversed the effects of FOXF1 overexpression in TGF-β1-treated BEAS-2B cells. These data indicated that FOXF1might function in TGF-β1-treated BEAS-2B cells by regulating CDH11.

CDH11 has been revealed to induce cancer cell apoptosis, suppress cell motility and invasion and inhibit cancer progression via the Wnt/β-catenin pathway (31). It has also been shown to be a key upstream regulator of the Wnt/β-catenin pathway (31). Dong *et al* (32) reported that upregulation of CDH11 in osteoarthritis activates the Wnt/β-catenin pathway. Huang *et al* (33) demonstrated that vitamin D alleviates airway remodeling in asthma by downregulating the expression levels of the Wnt/β-catenin pathway proteins Wnt5a and β-catenin. Furthermore, Yang *et al* (34) revealed that curcumin reduces lung inflammation in a mouse model of asthma via the Wnt/β-catenin signaling pathway. In the present study, TGF-β1 treatment significantly increased the expression levels of β-catenin, c-Myc and c-Jun in BEAS-2B cells. Also, the reduced β-catenin, c-Myc, and c-Jun expression levels imposed by FOXF1 in TGF-β1-treated BEAS-2B cells were reversed by CDH11. Moreover, FOXF1 ameliorated TGF-β1-triggered viability injury, apoptosis, fibrosis and EMT in BEAS-2B cells, which were restored by the activation of Wnt/β-catenin signaling, which suggested that the Wnt/β-catenin pathway regulated the biological response of TGF-β1-treated BEAS-2B cells via the subsequent regulation of FOXF1/CDH11.

In summary, the results of the present study suggested that FOXF1 overexpression increased BEAS-2B cell proliferation and repressed apoptosis and inflammation. In addition, overexpression of FOXF1 was demonstrated to reduce fibrosis and EMT in TGF-β1-treated BEAS-2B cells. These protective effects may rely on the regulation of the CDH11-mediated Wnt/β-catenin pathway, which may provide a novel fundamental insight into the pathogenesis of asthma and may be useful in developing therapeutic strategies for the treatment of pediatric asthma. Limitations of the present study included the use of only one cell line and the use of an *in vitro* experiment to explore the role of FOXF1. It is necessary to investigate these effects in an *in vivo* asthma model to support the conclusions of the current study.

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Availability of data and materials

All data generated and/or analyzed during this study are included in this published article.

Authors' contributions

QC and QT designed the study, drafted and revised the manuscript. XL, LL and LW analyzed the data and searched the literature. All authors performed the experiments. All authors read and approved the final manuscript. QC and QT confirm the authenticity of all the raw data.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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