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FBXW7 regulates epithelial barrier impairment in human bronchial epithelial cells *in vitro* by targeting apoptosis signal-regulating kinase1 via the p38 pathway

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Abstract: Bronchial asthma is a common chronic inflammatory disease characterized by airway hyperresponsiveness (AHR), inflammatory cell infiltration, and airway remodeling. F-box/WD repeat-containing protein 7 (FBXW7), an E3 ubiquitin ligase, is required for various endothelial functions, such as cell migration, inflammation, and endothelial integrity. This study aimed to investigate the role of FBXW7 in lipopolysaccharide (LPS)-induced epithelial barrier impairment in bronchial epithelial cells *in vitro*. By using lentivirus-based technology, FBXW7 was overexpressed or silenced (24 h) in human bronchial epithelial (16HBE) cells, which were treated with LPS or not (24 h). Immunoprecipitation (IP) detection and Western blot analysis were used to evaluate the interaction of target proteins. Cell permeability was measured using transepithelial electrical resistance and FITC dextran flux (48 h). IL-1β, IL-18 and TNF-α in cell supernatants were measured using ELISA (48 h). The results showed that LPS stimulation suppressed FBXW7 expression in a time- and dose-dependent manner. LPS exposure decreased cell proliferation, elevated IL-1β, IL-18 and TNF-α, increased epithelial permeability, and p38 phosphorylation. These LPS-induced changes were partly compromised by FBXW7 overexpression. Similar to LPS stimulation, FBXW7 knockdown increased epithelial permeability and levels of inflammatory cytokines and p38 phosphorylation, which were, in part, blocked by apoptosis signal-regulating kinase (ASK) 1 knockdown or p38 pathway inhibition. IP and Western blot analysis showed that FBXW7 interacted with ASK1. ASK1 expression was inversely associated with FBXW7 expression. FBXW7 overexpression markedly enhanced ASK1 ubiquitination. These data revealed that FBXW7 counter against inflammation and protects epithelial barrier integrity in bronchial epithelial cells by promoting ubiquitination-mediated degradation of ASK1 via the p38 pathway.

Abbreviations

AHR:	airway hyperresponsiveness
FBXW7:	F-box/WD repeat-containing protein 7
LPS:	lipopolysaccharide
IP:	Immunoprecipitation
ASK:	apoptosis signal-regulating kinase
TJs:	tight junctions
AJs:	adherens junctions
ZO:	zonula occludens
MAP3K5:	mitogen-activated protein kinase kinase kinase 5
IL-1β:	interleukin-1β
TNF-α:	tumor necrosis factor-α
JNK:	c-Jun N-terminal kinase

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Introduction

Bronchial asthma is a common chronic inflammatory disease characterized by airway hyperresponsiveness (AHR), inflammatory cell infiltration, and airway remodeling (Michalik *et al.*, 2018; Nanda and Wasan, 2020). Airway epithelium acts as a frontline defense against penetration of foreign substances and is implicated in regulating innate and adaptive immune responses in asthma (Mertens *et al.*, 2017). Airway epithelial barrier dysfunction is critical for the onset and progression of asthma (Georas and Rezaee, 2014; Gon and Hashimoto, 2018). Apical junctional complexes play important roles in maintaining airway epithelial integrity and are composed of tight junctions (TJs) and adherens junctions (AJs) (Tatsuta *et al.*, 2019). TJ proteins, such as zonula occludens (ZO) and claudin proteins, and AJ proteins, such as E-cadherin, have been demonstrated to be important components of the junctional complexes (Coopman and Djiane, 2016).

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Airway inflammation is central to current asthma management. Lipopolysaccharide (LPS) may induce IL-8 or neutrophil inflammation in the airways of patients with severe asthma. A study has related LPS levels to airway neutrophils or IL-8 in the bronchoalveolar lavage fluid of asthmatic children (Hauk *et al.*, 2008). Moreover, the bronchoalveolar lavage fluid LPS and genes related to the activation of LPS signaling were higher in corticosteroid-resistant asthma (Goleva *et al.*, 2008). 16HBE cell line (originally a cystic fibrosis cell line) is usually used for *in vitro* models of bronchial asthma (Yin *et al.*, 2019; Lan *et al.*, 2020). This cell line is readily accessible and easy to manipulate and is widely used to analyze pathways related to barrier function in response to environmental agents (Vinhas *et al.*, 2011; Heijink *et al.*, 2012). However, such immortalized cell line is not "normal," and they do not possess the underlying genetic or epigenetic features, which contribute to the disease phenotypes of asthma patients (Blume and Davies, 2013).

Apoptosis signal-regulating kinase (ASK) 1, namely, mitogen-activated protein kinase kinase kinase 5 (MAP3K5), belongs to the MAPKKK family, playing an essential role in a wide range of diseases, such as inflammatory diseases and cancer (Fujisawa, 2017). There is evidence that ASK1 is involved in AHR sensitivity and cytokine production in mice models with bronchial asthma stimulated by ovalbumin and may serve as a promising therapeutic target of asthma (Takada *et al.*, 2013). ASK1 inhibition exerts a protective effect against hyperoxia-induced lung injury by suppressing the production of interleukin (IL)-1β and tumor necrosis factor (TNF)-α, recruitment of immune cells, and cell apoptosis (Fukumoto *et al.*, 2016). ASK1 is involved in activating various signaling pathways governing cell apoptosis, growth, and differentiation, such as c-Jun N-terminal kinase (JNK) and p38 pathways (Cheon *et al.*, 2018). P38 signaling pathway has been considered as a classical inflammatory pathway (Yeung *et al.*, 2018). In addition, various pathways, such as NF-κB, HIF-1, and β-catenin, have been reported to be involved in the occurring and progression of bronchial asthma (Jia *et al.*, 2019; Yuan *et al.*, 2019; Sun *et al.*, 2020). For instance, JAX2 can prevent bronchial asthma by inhibiting MAPK/NF-kB inflammatory signaling (Yuan *et al.*, 2019). β-catenin pathway regulates asthma airway remodeling by modulating c-myc and cyclin D1 via the p38 MAPK-dependent pathway (Jia *et al.*, 2019).

F-box/WD repeat-containing protein 7 (FBXW7) is a subunit of E3 ubiquitin ligase complex for recognizing substrate and has been established to be a tumor suppressor in the regulation of a wide range of oncoproteins responsible for ubiquitination and proteasome degradation (Yeh *et al.*, 2018; Lan and Sun, 2019). FBXW7 is required for various endothelial functions, such as cell migration, inflammation, and endothelial integrity (Wang *et al.*, 2013; Pronk *et al.*, 2019). In a preliminary analysis, we found that FBXW7 was a predicted E3 ligase of ASK1 using UbiBrowser software. We postulate that FBXW7/ASK1 may be involved in the pathophysiology of bronchial asthma. 16HBE cell line (originally a cystic fibrosis cell line) is usually used for *in vitro* models of bronchial asthma (Yin *et al.*, 2019; Lan *et al.*, 2020). To test this postulation, acute lung injury in bronchial epithelial (16HBE) cells was caused by LPS. FBXW7 was overexpressed or interfered, to investigate its effect on cell proliferation, the release of inflammatory cytokines, and cell permeability and unravel the underlying molecular mechanisms.

Materials and Methods

Cell culture and treatment

We cultured 16HBE airway epithelial cells, which was purchased from the Cell Bank of the Chinese Academy of Science Shanghai, China, in DMEM medium (Hyclone, SH30243.01; Logan, UT, US) supplemented with 10% FBS (GIBCO, 16000e044; Carlsbad, CA, USA) and 1% P/S (Solarbio, P1400, Beijing, China) in an incubator (37°C, 5% CO_2). The cells were treated with 0, 1.25, 2.5, or 5 μg/mL LPS (derived from *Escherichia coli* O55:B5, HY-D1056, MedChemExpress) for 0, 12, 24, or 48 h.

Plasmid construction

For FBXW7 overexpression (oeFBXW7), the coding sequence of FBXW7 (NM_033632.3) was synthesized and integrated into pLVX-Puro. The following primers were used: FBXW7-F: 5' -CGGAATTCATGAATCAGGAACTGCTCTCTGTG-3' (*Eco*RI); FBXW7-R: 5'-CGGGATCCTCACTTCATGT-CCACATCAAAGTC-3' (*Bam*HI).

The FBXW7 interference (shFBXW7) sequences integrated into pLKO.1-puro for FBXW7 silencing were as follows: shFBXW7-1 (721-739), CCATGCAAAGTCTCAGAAT; shFBXW7-2 (1195-1213), GGTTTCATACACAGTCCAT; shFBXW7-3 (1669-1687), GCAGTCCGCTGTG-TTCAAT.

Cell transfection

When 16HBE cells grow to 60–70% confluence in 6-well plates, the cells were transfected with oeFBXW7 (MOI = 5, 5 μL) and empty plasmids (vector, MOI = 5, 5 μL), or shFBXW7-1, shFBXW7-2, shFBXW7-3 (MOI = 5, 5 μL) and shNC (MOI = 5, 5 μL) using Lipofectamine 2000 reagent (Invitrogen, CA, USA) according to the manufacturer' instructions. After 24-h transfection, serum-free medium was replaced by the medium containing 10% FBS for 48-h culture.

Cell Counting Kit-8 (CCK-8) assay

CCK-8 assay was performed with a Cell Proliferation and Cytotoxicity Assay Kit (SAB, CP002; USA). Briefly, 100 mL of 16HBE cells suspension (2×10^3 cells) was added to each well of a 96-well plate. The cells were transfected with oeFBXW7 or empty plasmid (vector). After 24 h-culture, the cells were treated with LPS or vehicle. CCK-8 solution (100 μL) was added to each well for 1 h incubation, and then the absorbance at 450 nm, indicating cell ability, was measured using a microplate reader (E8051, Promega).

Quantitative real-time PCR (qRT-PCR)

Briefly, RNA samples were reverse transcribed to cDNA using RevertAid First Strand cDNA Synthesis Kit (Fermentas, Hanover, MD, USA) and amplified with SYBR Green qPCR Master Mixes (#K0223, Thermo Fisher, Rockford, IL, USA) according to the standard protocol. The primer sequences, listed in Suppl. Tab. 1, were applied in the present study.

Enzyme-linked immunosorbent assay

Briefly, the cells were collected and centrifuged at 2000×*g* for 20 min following the required treatments. According to the manufacturer's manual, IL-1β, IL-18, and TNF-α levels in supernatants were detected using ELISA Kits (Abcam, Cambridge, MA, USA).

Immunoprecipitation (IP) detection and Western blot analysis

IP and western blot analysis was conducted as previously described (Yang *et al*., 2017b; Zhang *et al*., 2019b). Protein A/G Plus-Agarose beads from Santa Cruz Biotechnology Inc (CA, USA) were used. Anti-FBXW7 (ab109617, Abcam) and anti-ASK1 (#8662, CST) antibodies were applied for IP detection. Anti-FBXW7 (ab105752), anti-ZO-1 (ab190085), anti-E-cadherin (ab40772), anti-p-p38 (ab195049), anti-p38 (ab31828) from Abcam, and anti-ASK1 (#3762), anti-GAPDH (#5174), and HRP-conjugated secondary antibodies from Cell Signaling Technology (MA, USA), were used for western blot analysis. The protein blots were estimated using a chemiluminescent imaging system (Tanon 5200, Shanghai, China).

Cell permeability assay

Cell permeability was measured using two methods: transepithelial electrical resistance (TEER) and FITC dextran flux (Ryu *et al*., 2018). TEER was measured using a voltohmmeter (MillicellERS-2, Millipore). The resistance value was calculated as follows: TEER [Ω•cm^2] = (R1 − R2) [Ω] × Effective membrane area [cm^2], followed by calculation of electric resistance per unit area (R1: experimental group; R2: blank group). For dextran flux measurement, 10 kD FITC-conjugated dextran (sc-263323, Santa Cruz) was placed in the upper chamber of the transwell and incubated for 5 min, and then the supernatants from the lower chamber were collected to measure the base value. Later, the cells were supplemented with culture medium and incubated for 2 h. The supernatants from the lower chamber were collected to detect FITC fluorescence intensity using a microplate reader (E8051, Promega). The permeability (concentrations) was calculated according to the standard curve.

Statistical analysis

GraphPad Prism 7.0 software (San Diego, CA, USA) was used for all statistical analysis. Each experiment was performed in triplicate. Data were presented as mean value ± SD. One-way analysis of variance (ANOVA) with Tukey's *post hoc* tests was applied for comparison of mean values between groups. p-value < 0.05 was considered statistically significant.

Results

FBXW7 was lowly expressed in LPS stimulation-caused acute lung injury in human bronchial epithelial cells

Q-PCR and western blot showed that FBXW7 was lowly expressed in peripheral blood of patients with asthma (Figs. 1A, 1B). In order to investigate the impact of LPS on FBXW7 expression in 16HBE cells, the cells were stimulated by LPS at different concentrations (0, 1.25, 2.5, or 5 μg/mL) for different time durations (0, 12, 24, or 48 h). Expression of mRNA and protein of FBXW7 were remarkably decreased in response to the exposure of 2.5 or 5 μg/mL LPS for 24 h (p < 0.05, Figs. 1C–1D). Stimulation of 2.5 μg/mL LPS for 12, 24, or 48 h resulted in significant decreases in mRNA and protein of FBXW7 (Figs. 1E, 1F). These observations suggest that LPS stimulation inhibits FBXW7 expression in 16HBE cells in a dose- and time-dependent manner. Treatment of 2.5 μg/mL LPS for 24 h was selected to be used in further analysis.

Overexpression of FBXW7 reversed LPS stimulation-caused cell proliferation inhibition, release of inflammatory cytokines and increased cell permeability

16HBE cells were transfected with oeFBXW7 to overexpress FBXW7 or empty vector prior to LPS stimulation (2.5 μg/mL, 24 h). As shown in Figs. 2A, 2B, FBXW7 was successfully overexpressed at mRNA and protein level in16HBE cells (p < 0.001). LPS exposure exerted a suppressive effect on cell proliferation (p < 0.01), which was partly restored by FBXW7 overexpression (p < 0.001, Fig. 2C). IL-1β, IL-18, and TNF-αwere obviously elevated in supernatants of the cells on exposure to LPS stimulation (p < 0.001, Figs. 2D–2F). And the LPS-induced release of IL-1β, IL-18, and TNF-α was partly compromised by FBXW7 overexpression (p < 0.01, Figs. 2D–2F). Moreover, to detect the effects of LPS and FBXW7 overexpression on cell permeability, TEER, FITC dextran flux, and expression of ZO-1 and E-cadherin were examined. LPS stimulation led to a precipitous decrease in TEER (p < 0.001), a remarkable increase in FITC dextran flux (p < 0.001), and obvious up-regulation of ZO-1 and E-cadherin at mRNA (p < 0.001) and protein level, which were all significantly suppressed by overexpressing FBXW7 (p < 0.01, Figs. 2G–2J). FBXW7 up-regulation alleviated the p38 phosphorylation stimulated by LPS as well (Fig. 2J). These results show that FBXW7 overexpression significantly reverses the impacts of LPS stimulation on cell proliferation, the release of inflammatory factors, cell permeability, and p38 pathway activation in 16HBE cells.

The interaction between FBXW7 and ASK1

FBXW7 was predicted to be an E3 ligase of ASK1 (MAP3K5) using UbiBrowser software. We tested whether ASK1 expression was regulated by FBXW7. 16HBE cells were transfected with oeFBXW7 or shFBXW7 to overexpress or silence FBXW7. FBXW7 overexpression resulted in a down-regulation of ASK1 protein, while FBXW7 knockdown caused an up-regulation ofASK1 protein (Fig. 3B). In sharp contrast, ASK1 mRNA expression was little affected by overexpressing or silencing FBXW7 (Fig. 3A). Besides, Western blot and IP analysis found interactions between FBXW7 and ASK1 (Fig. 3C). Furthermore, the ubiquitination of ASK is little after treatment of LPS (2.5 μg/mL), while FBXW7 overexpression dramatically promoted ASK1 ubiquitination in the LPS (2.5 μg/mL)-stimulated 16HBE cells co-treated with proteasome inhibitor MG132 or not (Fig. 3D). It implies thatFBXW7 interacts with ASK1 and affects ASK1 expression through modulating ubiquitination-mediated degradation of ASK1.

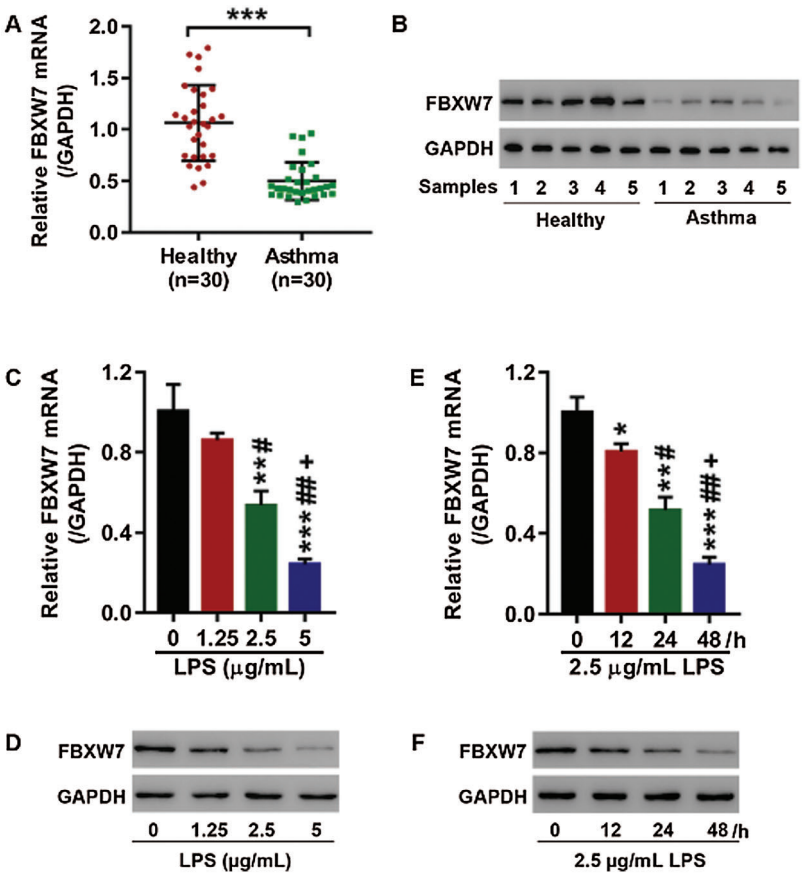

FIGURE 1. LPS suppresses FBXW7 expression in16HBEcells.
(A, B) the expression of FBXW7 mRNA and protein was detected by Q-PCR (A) and western blot (B). (C, D) different concentrations of LPS decreases FBXW7 expression at mRNA (C) and protein level (D). The cells are treated with 0, 1.25, 2.5, or 5 μg/mL LPS for 24 h. $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* 0 μg/mL LPS; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* 1.25 μg/mL LPS; $^{+}p < 0.05$ *vs.* 2.5 μg/mL LPS. (E, F) 2.5 μg/mL LPS decreases FBXW7 expression at mRNA (E) and protein level (F) in a time-dependent manner. The cells are exposed to 2.5 μg/mL LPS for 0, 12, 24, or 48 h. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* 0 h; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* 12 h; $^{+}p < 0.05$ *vs.* 24 h.

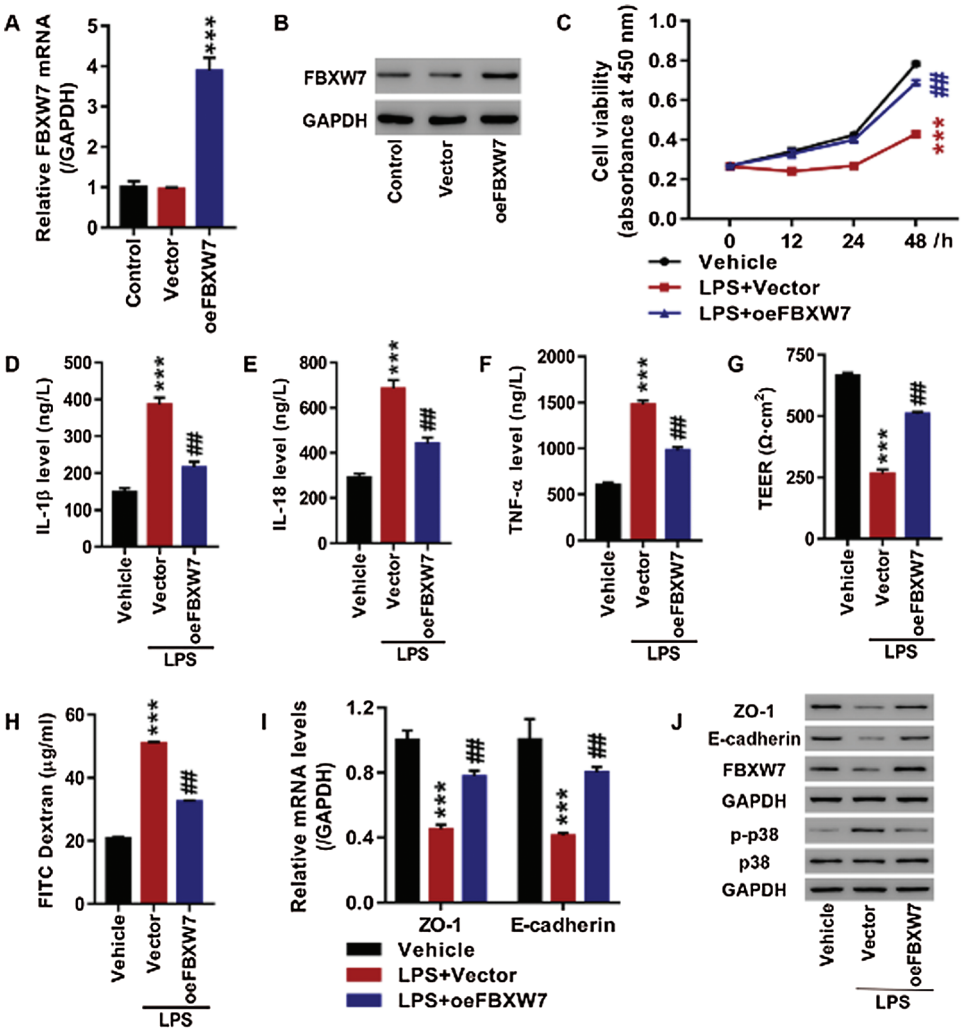

FIGURE 2. Up-regulation of FBXW7 significantly reverses the LPS-induced cell proliferation inhibition, release of inflammatory cytokines and increased cell permeability in 16HBE cells.
The cells are transfected with oeFBXW7 (MOI = 5) or vector for 24 h prior to 24-h exposure to LPS. (A, B) FBXW7 is overexpressed in 16HBE cells at mRNA and protein levels. The cells are transfected with oeFBXW7 (MOI = 5) or vector for 24 h prior to 24-h exposure to LPS. $^{***}p < 0.001$ *vs.* vector. (C) FBXW7 overexpression restores cell proliferation inhibition by LPS. (D–F) FBXW7 overexpression reverses the LPS-induced elevations of IL-1β (D), IL-18 (E) and TNF-α (F) in supernatants. (G-H) FBXW7 overexpression alleviates the decreased TEER and increased FITC dextran flux by LPS. (I) FBXW7 overexpression compromises the downregulation of ZO-1 and E-cadherin at mRNA level by LPS. (J) FBXW7 overexpression restores the downregulation of ZO-1 protein and E-cadherin protein and the phosphorylation of p38 protein by LPS. $^{***}p < 0.001$ *vs.* vehicle; $^{\#\#}p < 0.01$ *vs.* LPS + vector.

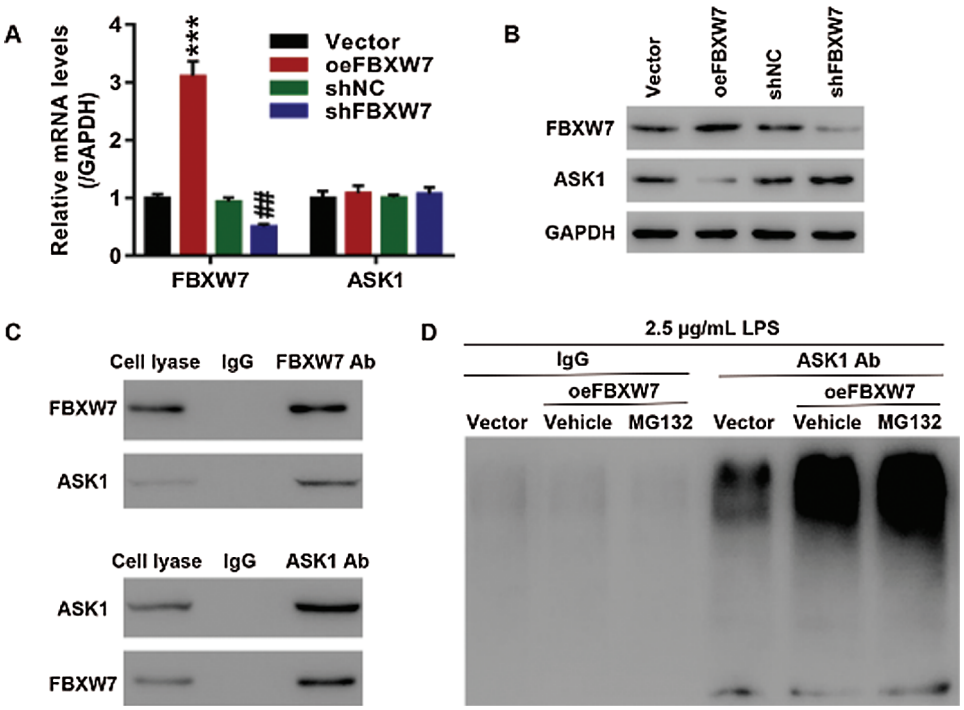

FIGURE 3. The interaction between FBXW7 and ASK1 in 16HBE cells. (A, B) FBXW7 expression is negatively associated with ASK1 expression at mRNA (A) and protein (B) level, $^{***}p < 0.001$ *vs.* vector; $^{\#\#}p < 0.01$ *vs.* shNC. (C) Results of immunoprecipitation and western blot show that FBXW7 interacts with ASK1. (D) FBXW7 overexpression, in combination with LPS, enhances ASK1 ubiquitination. ASK1 ubiquitination is detected by immunoprecipitation and Western blot. The cells are transfected with oeFBXW7 or shFBXW7 to overexpress or silence FBXW7 expression. The cells transfected with oeFBXW7 or vector are then treated with 2.5 μg/mL LPS or 10 μmol/L MG132, or in combination, for 24 h.

FBXW7 regulated cell permeability in 16HBE cells probably by targeting ASK1 via p38 pathway

We further explored the role of ASK1 and p38 pathways in FBXW7 regulating the release of inflammatory factors and cell permeability of 16HBE cells. As depicted in Figs. 4A, 4B, FBXW7 was successfully silenced at mRNA and protein level in the 16HBE cells transfected with shFBXW7-1, shFBXW7-2, or shFBXW7-3. FBXW7 silencing markedly elevated IL-1β, IL-18, and TNF-α levels in the supernatants ($p < 0.001$, Figs. 4C–4E), decreased TEER ($p < 0.001$, Fig. 4F), increased FITC dextran flux ($p < 0.001$, Fig. 4G), down-regulated ZO-1 and E-cadherin at mRNA and protein level ($p < 0.001$, Figs. 4H, 4I), and up-regulated ASK1 and p-p38 protein (Fig. 4I), which were similar to the LPS-induced effects described above. Furthermore, in order to determine whether ASK1 and p38 pathways mediate the FBXW7 silencing-induced effects on inflammation and cell permeability, the shFBXW7-transfected cells were transfected with shASK1 to down-regulate ASK1 or treated with SB203580 to deactivate the p38 pathway. Either ASK1 knockdown or treatment of SB203580 attenuated the effects of FBXW7 knockdown on the release of inflammatory factors and cell permeability (Figs. 4C–4I). These results suggest that FBXW7 silencing may up-regulate ASK1 and activate the p38 pathway, thereby enhancing the release of IL-1β, IL-18, and TNF-α and increasing cell permeability in 16HBE cells.

Discussion

Previous studies have demonstrated the key involvement of FBXW7 in cancers and metabolism, suggesting the clinical potential of anticancer therapies against FBXW7 (Cao *et al.*, 2016; Shimizu *et al.*, 2018). For example, through promoting epithelial–mesenchymal transition, FBXW7 acts as a driver of uterine carcinosarcoma (Cuevas *et al.*, 2019). It also reported that FBXW7-mediated STAT2 stability regulation plays an essential role in cell proliferation and cancer growth of melanoma (Lee *et al.*, 2020). Besides, it has been established that FBXW7 is a potent regulator of angiogenesis (Yang *et al.*, 2017a; Shao *et al.*, 2019). Nevertheless, the implication of FBXW7 in the pathogenesis of bronchial asthma has been rarely studied. Previous studies have revealed that administration of LPS could establish the model of asthma, and exacerbate the inflammation, oxidative stress in an experimental model of asthma, as well as increased epithelial barrier permeability (Aggarwal *et al.*, 2010; Camargo *et al.*, 2017; Yu and Chen, 2018). Consistent with these, in the current study, 16HBE cells were treated with LPS to cause acute lung injury *in vitro*. We found that LPS stimulation exerted a suppressive effect on FBXW7 expression and cell proliferation and a stimulatory effect on the release of inflammatory cytokines (IL-1β, IL-18, and TNF-α) and cell permeability.

Some studies report that FBXW7 counters against inflammation (Balamurugan *et al.*, 2013; Zhang *et al.*, 2019a). Moreover, overexpression of FBXW7 has been found to abate the LPS-induced septic liver injury by inhibiting apoptosis and inflammation (Zhou and Xia, 2020). Nonetheless, a recent study holds a different viewpoint that FBXW7 promotes inflammation by acting as a novel E3 ubiquitin ligase for IκBα to degrade IκBα and activate NF-κB in inflammatory bowel disease (Meng *et al.*, 2020). The current study showed that FBXW7 overexpression mitigated the LPS-induced release of IL-1β, IL-18, and TNF-α, whereas FBXW7 deficiency promoted the release of these inflammatory cytokines resembling LPS stimulation, supporting the protective role of FBXW7 against inflammation in bronchial epithelial cells. It can be speculated that FBXW7 has the potential to regulate inflammation positively and negatively through different mechanisms. These findings highlight the need for further

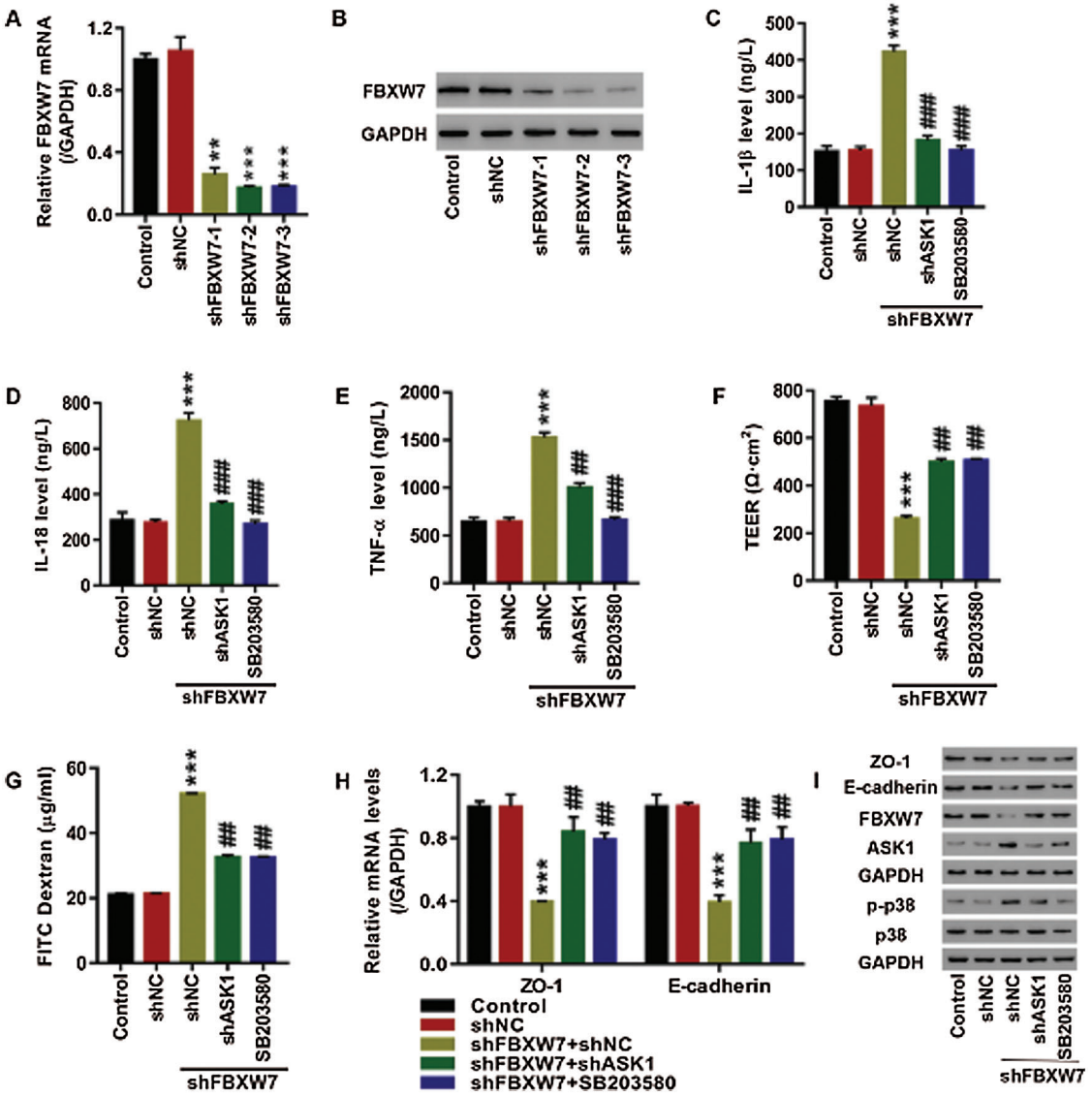

FIGURE 4. FBXW7 regulated cell permeability in 16HBE cells, probably by targeting ASK1 via the p38 pathway.
(A, B) FBXW7 is silenced at the mRNA and protein levels. Cells are transfected with shFBXW7-1, shFBXW7-2, shFBXW7-3 or shNC. (C–E) interfering ASK1 or inhibiting p38 pathway decreases the releases of IL-1β, IL-18 and TNF-α in supernatants induced by FBXW7 silencing. (F, G) interfering with ASK1 or inhibiting the p38 pathway counteracts the effects of FBXW7 knockdown on TEER and FITC dextran flux. (H, I) interfering ASK1 or inhibiting p38 pathway, significantly abolishes the downregulation of ZO-1 and E-cadherin at mRNA (H) and protein level (I) and the p38 phosphorylation (I) induced byFBXW7 knockdown. Cells are transfected with shFBXW7 or shNC for 24 h, followed by shASK1 transfection or treatment of p38 pathway inhibitor SB203580 for 24 h. $^{***}p < 0.001$ *vs.* shNC; $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ *vs.* shFBXW7 + shNC.

studies on the regulatory effect of FBXW7 on inflammation and the underlying molecular mechanisms.

FBXW7 has long been established as a key regulator of endothelial barrier function (Wang *et al.*, 2013). AJ protein E-cadherin and TJ protein ZO-1 are essential regulators of endothelial barrier functions and protect against tumor metastasis (Campbell *et al.*, 2017). FBXW7 suppresses metastasis of various cancers, such as gastric cancer and lung cancer, by up-regulating E-cadherin expression (Huang *et al.*, 2018; Zhang *et al.*, 2018). In this study, either LPS stimulation or FBXW7 knockdown increased epithelial cell permeability, as evidenced by the observations of decreased TEER, increased FITC dextran flux, down-regulated ZO-1 and E-cadherin expression. Moreover, FBXW7 overexpression significantly decreased epithelial cell permeability, compromising the effect of LPS on epithelial cell permeability. Collectively, it can be concluded that FBXW7 expression protects epithelial barrier integrity and function. In accordance with our results, a recent study showed that loss of FBXW7 increases contractility and permeability of umbilical vein endothelial cells by accumulating RhoB GTPase (Pronk *et al.*, 2019).

ASK1 is a key molecule in the ASK family that can be activated in response to a wide range of stressors and participates in the regulation of cellular processes, such as inflammation and cell apoptosis (Ryuno *et al.*, 2017). Ubiquitination is crucial for ASK1 activation, and the E3 ligase FBXW5 has been revealed to be a critical activator of ASK1 ubiquitination (Bai *et al.*, 2019). Our study suggests that FBXW7 overexpression promotes ASK1 ubiquitination, thus down-regulating ASK1 protein expression. Moreover, Western blot and IP analysis provide convincing evidence in support of FBXW7-ASK1 interaction. FBXW7 may be

another activator of ASK1 ubiquitination. As far as we know, this is the first study uncovering associations of FBXW7 with ASK1. ASK1/2 signaling stimulates the inflammatory response, increases vascular endothelial permeability, promotes macrophages infiltration, and facilitates tumor metastasis (Tartey *et al*., 2018; Najafi *et al*., 2019). Consistently, the study found that interfering with ASK1 could partly abolish the FBXW7 silencing-induced elevations of inflammatory cytokines and increased cell permeability. It implies that FBXW7 may suppress inflammation and decrease bronchial epithelial permeability by down-regulating ASK1 via promoting ubiquitination-mediated ASK1 degradation.

P38 pathway is an important pathway regulating inflammatory response, and down-regulating the p38 pathway protects against inflammation (Zhou *et al*., 2017; Wu *et al*., 2018). Increasing evidence has pointed out that the p38 signaling pathway is involved in regulating asthmatic airway remodeling, the primary characteristic of asthma (Cao *et al*., 2018; Jia *et al*., 2019). P38 activation mediates the airway epithelial barrier dysfunction and asthma induced by toluene diisocyanate (Sulaiman *et al*., 2018). Similarly, in this study, inhibiting the p38 pathway alleviated inflammation and the increased epithelial permeability caused by FBXW7 silencing. Moreover, FBXW7 overexpression abrogated p38 pathway activation, indicating that FBXW7 may suppress inflammation and protects epithelial barrier integrity via the p38 pathway. ASK1 functions as an upstream regulator of the p38 pathway (Tesch *et al*., 2016). It can be speculated that FBXW7 may regulate inflammation and epithelial barrier integrity through regulating ASK1 via the p38 pathway.

However, there are also some limitations in the present research. One of the limitations is the lack of *in vivo* experiments in the current study. The next steps of our study are that need to be undertaken is looking at FBXW7 regulation on *in vivo* experiments. FBXW7 overexpression and KO mice would be highly informative to acute lung injury. Another is that only one cell line was used in our study. If possible, some other cell lines will be used to enrich our experimental content in the future. Although further investigations are needed, this study identifies a potential therapeutic approach for bronchial asthma.

Conclusions

In summary, this study demonstrates that FBXW7 suppresses the inflammatory response and decreases cell permeability in bronchial epithelial cells through boosting ubiquitination-mediated degradation of ASK1 via the p38 pathway. It also provides evidence that FBXW7 interacts with ASK1. This study not only stresses the relevance of FBXW7 as a potential therapeutic target in bronchial asthma but also mentions other pathologies for which FBXW7 might prove to be a potential target, which expands our understanding of the physiological contribution of FBXW7 to bronchial asthma, supporting the pursuit of FBXW7 as a potential therapeutic target against bronchial asthma.

Availability of Data and Materials: All data generated or analyzed during this study are included in this published article.

Authors' Contributions: XYZ conceived and designed the study. JRS, WL, and YD performed the experiments and collected and analyzed the data. JK wrote the manuscript. XYZ read and approved the final manuscript. All authors read and approved the final manuscript and agree to be accountable for all aspects of the research in ensuring that the accuracy or integrity of any part of the work is appropriately investigated and resolved.

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Conflicts of Interest: The authors declare that they have no competing interests.

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SUPPLEMENTARY TABLE 1

The primer sequences

Name	Primer sequences
FBXW7	Primer-F: 5'-CTCTATGTGCTTTCATTCC-3' Primer-R: 5'-CCTCTTCTTTGCATTTCTC-3'
ZO-1	Primer-F: 5'-TACCTCTTGAGCCTTGAAC-3' Primer-R: 5'-TAGGGCACAGCATTGTATC-3'
E-cadherin	Primer-F: 5'-GAGAACGCATTGCCACATACAC-3' Primer-R: 5'-AAGAGCACCTTCCATGACAGAC-3'
ASK1	Primer-F: 5'-GAGGCCAAGGCATTCATAC-3' Primer-R: 5'-ATCTCTTTCTCCGCAGGAC-3'
GAPDH	Primer-F: 5'-AATCCCATCACCATCTTC-3' Primer-R: 5'-AGGCTGTTGTCATACTTC-3'