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Effect of Peroxiredoxin 1 on the biological function of airway epithelial cells and epithelial-mesenchymal transition

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Abstract: Peroxiredoxin 1 (PRDX1) participates in tumor cell proliferation, apoptosis, migration, invasion, and the epithelial-to-mesenchymal transition (EMT). This study aimed to investigate the effect of PRDX1 on the EMT of airway epithelial cells stimulated with lipopolysaccharide (LPS) and transforming growth factor-beta 1 (TGF-β1). PRDX1 overexpression significantly increased the proliferation and migration of human bronchial epithelial (BEAS-2B) cells, reduced cell apoptosis ($p < 0.01$), and induced EMT and collagen deposition by upregulating the expression of the matrix metallopeptidase (MMP)2, MMP9, α-smooth muscle actin (α-SMA), N-cadherin, vimentin and twist proteins and inhibiting E-cadherin expression ($p < 0.05$). PRDX1 overexpression promoted TGF-β1-mediated inhibition of cell proliferation and migration and significantly enhanced the TGF-β1-induced EMT and collagen synthesis ($p < 0.05$). Knockdown of PRDX1 inhibited cell proliferation, migration, EMT, and collagen synthesis ($p < 0.01$), reversed LPS-mediated inhibition of cell proliferation and migration, and significantly suppressed LPS-induced EMT and collagen synthesis ($p < 0.01$). The result indicating that PRDX1 may be involved in LPS/TGF-1-induced EMT and collagen synthesis in human bronchial epithelial cells.

Introduction

Peroxiredoxin 1 (PRDX1) is proposed to play an important role in the pathogenesis of respiratory diseases such as acute respiratory distress syndrome, acute lung injury, and lung cancer (Chang *et al.*, 2001; Liu *et al.*, 2014). PRDX1 expression is significantly increased in lung tissues with acute injury and promotes airway inflammation through the activation of NF-κB signaling (Liu *et al.*, 2014). PRDX1 also plays a central role in maintaining cell homeostasis and responses by eliminating various reactive oxygen species. For instance, airway epithelial cells lacking PRDX1 exhibit increased oxidative stress in response to respiratory syncytial virus infection (Chang *et al.*, 2001). Inhibition of the synthesis of the antioxidant proteins PRDX1 and catalase leads to oxidative stress, which induces occupational asthma, an inflammatory disease characterized by airway remodeling (Kim *et al.*, 2010). PRDX1 is also a major 2-Cys protein of the peroxiredoxin family of antioxidant enzymes and plays an important role in regulating cell growth, differentiation, and apoptosis (Chenbo, Xiaobo and Guoqiu, 2016; Mark *et al.*, 2018). For example, PRDX1 directly binds to nuclear p53 or transcription factors such as c-Myc, NF-κB, and AR to influence the biological activity of genes, thereby inducing or suppressing cell death (Chenbo, Xiaobo and Guoqiu, 2016). In lung cancer, oral squamous cell carcinoma, and head and neck squamous cell carcinoma, PRDX1 overexpression promotes tumor growth and metastasis by activating the epithelial-to-mesenchymal transition (EMT) and NF-κB or Wnt/β-catenin signaling (Niu *et al.*, 2016; Song *et al.*, 2020; Jiang *et al.*, 2019). PRDX1 plays an antiapoptotic role through direct or indirect interactions with several reactive oxygen species-dependent effectors, including ASK1, p66Shc, GSTpi/JNK, and c-Abl kinase (Ding et al., 2016). When regulating cell differentiation, PRDX1 represses the transformation of activated fibroblasts into cancer-associated fibroblasts through JNK inhibition (Jezierska-Drutel *et al.*, 2019). In summary, many genes participate in the formation of a complete PRDX1 signaling network to regulate cellular biological functions.

PRDX1 is released by various cells, such as airway epithelial cells, human embryonic kidney cells, and monocytic cells, upon exposure to inflammatory stimuli, such as lipopolysaccharide (LPS) and TNF-α (Liu *et al.*, 2013; Mullen *et al.*, 2015; Liu *et al.*, 2014). *In vitro* experiments have also confirmed that PRDX1 is involved in the regulation of LPS-induced injury. For example, LPS + elastase-induced chronic

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obstructive pulmonary disease results in the infiltration of inflammatory cells into the lungs and increased levels of proinflammatory cytokines and PRDX-1 (Singla *et al.*, 2020). PRDX1 deficiency strongly protects mice from LPS-induced death and the inflammatory response (Ying *et al.*, 2020). It is an enzyme that reduces oxidative stress. It alleviates LPS-induced acute lung injury by reducing oxidative stress and the activity of the P38/JNK signaling pathway (Ying *et al.*, 2020). However, overexpression of PRDX1 in acute lung injury enhances the LPS-induced increase in the levels of the proinflammatory cytokines IL-6, IL-8, and TNF-α (Liu *et al.*, 2014). In addition, PRDX1 knockout enhances LPS-induced liver apoptosis by increasing levels of the cleaved caspase-3 protein, resulting in high lethality after the LPS challenge (Sun *et al.*, 2018). LPS induces cell damage and apoptosis and affects cellular EMT and matrix remodeling. In human peritoneal mesothelial cell lines, LPS induces EMT and fibrosis by upregulating α-smooth muscle actin (α-SMA), vimentin, MMP2/9, and collagen 1/3 and reducing E-cadherin expression (Shao *et al.*, 2019). PRDX1 also plays an important role in the EMT and matrix remodeling. Loss of PRDX1 increases collagen remodeling associated with breast cancer progression (Attaran *et al.*, 2021) and induces the expression of the metastasis-associated genes MMP1 and MMP2 (Yang *et al.*, 2019). In head and neck squamous cell carcinoma and oral squamous cell carcinoma, PRDX1 overexpression promotes EMT and migration by inducing Vimentin expression and inhibiting E-cadherin expression (Jiang *et al.*, 2019; Niu *et al.*, 2016). Overall, LPS induces PRDX1 expression, which modulates the LPS-induced EMT and collagen remodeling.

The EMT allows epithelial cells to assume a mesenchymal cell phenotype and is a therapeutic target for several persistent inflammatory airway diseases related to tissue remodeling (Park *et al.*, 2016; Lee *et al.*, 2017). Transforming growth factor (TGF)-β1 is a multifunctional growth factor that regulates cell functions such as proliferation, differentiation, and migration and is involved in airway disease-related remodeling (Schleimer, 2017). TGF-β1 stimulates the expression of the EMT markers, including E-cadherin, fibronectin, vimentin, and α-SMA in airway cells and induces migration (Lee *et al.*, 2017). In human conjunctival fibroblasts, TGF-β1 induced cell proliferation, collagen 1/3 synthesis, and MMP 2/9 expression (Sun *et al.*, 2020). PRDX1 knockdown significantly inhibits the TGF-β1-induced EMT and A549 cell migration, whereas its overexpression enhances these processes (Ha *et al.*, 2012). PRDX1 may be a key factor regulating the EMT and cell migration induced by TGF-β1.

Although PRDX1 has been implicated in inflammatory processes and tumor progression (e.g., cell growth, apoptosis, migration, EMT, and extracellular matrix synthesis), little is known about its role in the EMT and airway remodeling during inflammatory airway diseases. This study aimed to explore the role of PRDX1 in the acute injury of airway epithelial cells and extracellular matrix remodeling. Based on the results, PRDX1 knockdown improves the LPS- or TGF-β1-induced EMT and collagen synthesis in airway epithelial cells. Collectively, these findings provide new insight into the potential role of PRDX1 as a therapeutic target for inflammatory airway diseases.

Materials and Methods

Cell culture

Human bronchial epithelial cells (BEAS-2B) were purchased from the Shanghai Institute of Biochemistry and Cell Biology, Chinese Academy of Science (Shanghai, China) and cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM; Gibco®, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (FBS; PAN Biotech, Germany), 1% 100 U/mL penicillin (MedChemExpress, USA) and 100 μg/mL streptomycin (MedChemExpress, USA). Cells were cultured in a humidified 5% CO_2 incubator (SANYO, Japan) at 37°C.

Cell transfection and treatment

The PRDX1 overexpression plasmid pcDNA3.1-PRDX1, the knockdown plasmid pLVX-shRNA2-Puro-PRDX1, and the corresponding negative control plasmid (NC) were constructed by Zolgene Biotechnology (Fuzhou, China). Specifically, the sh-PRDX1, sh-NC, and PRDX1 sequences were amplified using the following primers: sh-PRDX1 5′-CACCGCACCATTGCTCAGGATTATGCGAACATAATC-CTGAGCAATGGTGC-3′ (forward) and 5′-AAAAGCAC-CATTG CTCAGGATTATGTTCGCATAATCCTGAGCAA-TGGTGC-3′ (reverse), sh-NC 5′-GATCCGATCAATACTAT-TCATCAATTC AAGAGATTGATGAATAGTATTGATCT-TTTTG-3′, and PRDX1 5'-CGGGATCCCGATGTCTTCA-GGAAATGCTAA-3′ (forward) and 5′-CCCTCGAGGG-TCACTTCTGCTTGGAGAAAT-3′ (reverse). Then, the DNA products were digested with the BamHI and EcoRI restriction enzymes (Fermentas, Lithuania) and inserted into the pLVX-shRNA2-Puro or pcDNA3.1 vector.

Before transfection, 5×10^5 BEAS-2B cells/well were inoculated into 6-well plates (Thermo Fisher Scientific, USA). When the cells grew to a density of approximately 60%–70%, transfection was performed along with Lipofectamine 2000 (Invitrogen, USA). After 6 h, DMEM containing 10% FBS was added to cells. After 24 h, the efficiency of PRDX1 knockdown and overexpression was analyzed by quantitative real-time polymerase chain reaction (qPCR) and western blotting. After transfection for 24 h, BEAS-2B cells were treated with 10 μg/mL LPS (Sigma–Aldrich, St. Louis, Missouri, USA) for 24 h or 10 ng/mL recombinant human TGF-β1 (PeproTech, Rock Hill, New Jersey, USA) (Liu *et al.*, 2014; Sun *et al.*, 2020). All experiments were repeated three times.

Quantitative real-time polymerase chain reaction

The cells were collected, and total RNA was extracted with TRIzol reagent (Invitrogen, USA). Reverse transcription was performed according to the manufacturer's instructions for the PCR nucleotide mix (Promega, USA). Then, 2.0 μL of cDNAs, 2.0 μL of primers, and 8.5 μL of sterile water were mixed with 12.5 μL of SYBR Green qPCR master mix (A6001, Promega, USA) to make a final volume of 25 μL. Subsequently, the mixture was analyzed using an ABI 7500 PCR system (ABI, USA) to assess mRNA expression. The results were calculated using the $2^{-\Delta\Delta Ct}$ method. The qPCR conditions were as follows: 95°C for 5 min, followed by 40 cycles of 95°C for 10 s, 60°C for 30 s, 72°C for 30 s, and

95°C for 15 s. The primers were designed and synthesized by Sangon Biotech (Shanghai, China). The PRDX1 primer sequences were 5′-CCCAACTTCAAAGCCACAGC-3′ (forward) and 5′-AAGCAATGATCTCCGTGGGG-3′ (reverse). The GAPDH primers were 5′-GTCATCCCTGAGCTGAACGG-3′ (forward) and 5′-CCACCTGGTGCTCAGTGTAG-3′ (reverse).

CCK-8 assay

Cell viability was determined using the CCK-8 kit (Dojindo, China). BEAS-2B cells (1×10^5 cells/mL) were seeded in 96-well plates(Thermo Fisher Scientific, USA). The cells were incubated for 0 h, 24 h, 48 h, or 72 h at 37°C with 5% CO_2. Approximately 100 μL of 10% CCK-8 reagent were added to each well of the plate. After incubation for 1 h, the optical density was detected at 450 nm using a microplate reader (DNM-9602, Beijing, China), and the reference wavelength was 650 nm.

Flow cytometry

The cells were harvested and washed with precooled PBS. The apoptosis ratio was detected using an Annexin V-FITC/PI kit (Solarbio, China). For this experiment, 100 μL of 1 × binding buffer and 5 μL of Annexin V-FITC were added to the cells and incubated at room temperature for 15 min. Then, 5 μL of PI was added and incubated at room temperature for 5 min in the dark. The number of apoptotic cells in each group was analyzed using a flow cytometer (Beckman Coulter, USA).

Wound healing assay

A total of 3×10^5 cells were cultured for 24 h in six-well plates. Subsequently, the Ibili-cell plug-in (Corning, USA) was removed. Then, the cells were cultured in a serum-free medium for 24 h and examined under an inverted microscope at 100 × magnification to measure the wound migration area with ImageJ software (National Institutes of Health, USA).

Western blotting

Protein samples were extracted from BEAS-2B cells with RIPA buffer containing PMSF (Beyotime, China). The total protein concentration was determined using a PC0020-500 BCA protein assay kit (Solarbio Technology Co., Ltd., Beijing, China). The proteins were separated on a 10% SDS-polyacrylamide gel and transferred to a pure nitrocellulose membrane (BioTrace, USA). The membrane was blocked with 5% bovine serum albumin (Beyotime, China) for 2 h and incubated with primary antibodies against PRDX1 (ab15571, 1:1000, 22 kDa), collagen 1 (ab34710, 1:1000, 130 kDa), collagen 3 (ab184993, 1:1000, 150 kDa), matrix metallopeptidase2 (MMP2, ab97779, 1:500, 74 kDa), MMP9 (ab38898, 1:1000, 92 kDa), Twist (ab175430, 1:1000, 21 kDa), α-SMA (ab5694, 1:500, 42 kDa), N-cadherin (ab76011, 1:5000, 100 kDa), glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (ab8245, 1:5000, 36 kDa) (Abcam, Cambridge, UK), E-cadherin (3195S, 1:1000, 135 kDa), and vimentin (5741S, 1:1000, 57 kDa) (Cell Signaling Technology, USA) at 4°C overnight. The blots were then incubated with an HRP-conjugated secondary antibody (1:5000, Santa Cruz, USA) for 2 h. Subsequently, the chemiluminescence signal was detected using an electrochemiluminescence kit (Thermo Fisher Scientific, USA). The results were obtained with a Versa DocTM imaging system (Peiqing Technology Co., Ltd., Shanghai, China), analyzed using ImageJ software, and normalized to a housekeeping protein to determine the gray value of the target protein.

Statistical analysis

All statistical analyses were conducted using SPSS 20.0 statistical software. The data are presented as the means ± standard deviations. The paired *t*-test was used for comparisons between the two groups. One-way ANOVA with the LSD post hoc test was performed for comparisons between more than two groups. $p < 0.05$ was considered statistically significant.

Results

Peroxiredoxin1-mediated promotion of the proliferation, migration, epithelial-mesenchymal transition, and collagen synthesis in BEAS-2B cells

Compared with the corresponding control group, transfection of pLVX-shRNA2-Puro-PRDX1 significantly inhibited the expression of the PRDX1 mRNA and protein, while transfection of pcDNA3.1-PRDX1 significantly increased PRDX1 mRNA ($p < 0.01$, Fig. 1A) and protein levels ($p < 0.001$, Fig. 1B). PRDX1 knockdown significantly inhibited cell proliferation ($p < 0.01$, Fig. 1C) and migration ($p < 0.01$, Fig. 1E) and significantly increased cell apoptosis ($p < 0.001$, Fig. 1D); however, PRDX1 overexpression exerted the opposite effects.

The western blot results showed that PRDX1 upregulated the expression of the EMT markers MMP2, MMP9, α-SMA, N-cadherin, vimentin, twist, and reduced collagen 1 and collagen 3 expression of the epithelial marker E-cadherin ($p < 0.05$, Fig. 1F). Downregulation of PRDX1 level inhibited the EMT and collagen biosynthesis ($p < 0.01$, Fig. 1F).

Peroxiredoxin1 enhanced lipopolysaccharide-mediated changes in BEAS-2B cell proliferation, migration, epithelial-mesenchymal transition, and collagen synthesis

LPS treatment significantly inhibited cell proliferation ($p < 0.001$, Fig. 2A) and migration ($p < 0.001$, Fig. 2C) and significantly increased cell apoptosis ($p < 0.001$, Fig. 2B). PRDX1 knockdown significantly reversed LPS-mediated inhibition of cell proliferation and migration ($p < 0.05$) and enhanced LPS-induced cell apoptosis ($p < 0.01$). However, PRDX1 overexpression rescued LPS-induced inhibition of cell proliferation and migration ($p < 0.05$) and suppressed LPS-induced cell apoptosis ($p < 0.01$).

Furthermore, LPS significantly led to an increase in the expression of the EMT markers MMP2, MMP9, α-SMA, N-cadherin, vimentin, and twist and the synthesis of collagen 1 and collagen 3 but decreased E-cadherin expression ($p < 0.05$, Fig. 2D). PRDX1 knockdown inhibited LPS-induced EMT marker expression and collagen synthesis, while PRDX1 overexpression enhanced the LPS-induced expression of these proteins ($p < 0.05$, Fig. 2D).

Peroxiredoxin1 enhanced TGF-β1-induced BEAS-2B cell proliferation, migration, epithelial-mesenchymal transition, and collagen synthesis

Cell proliferation ($p < 0.001$, Fig. 3A) and migration ($p < 0.05$, Fig. 3C) were significantly increased by TGF-β1, and cell apoptosis was significantly reduced ($p < 0.01$, Fig. 3B).

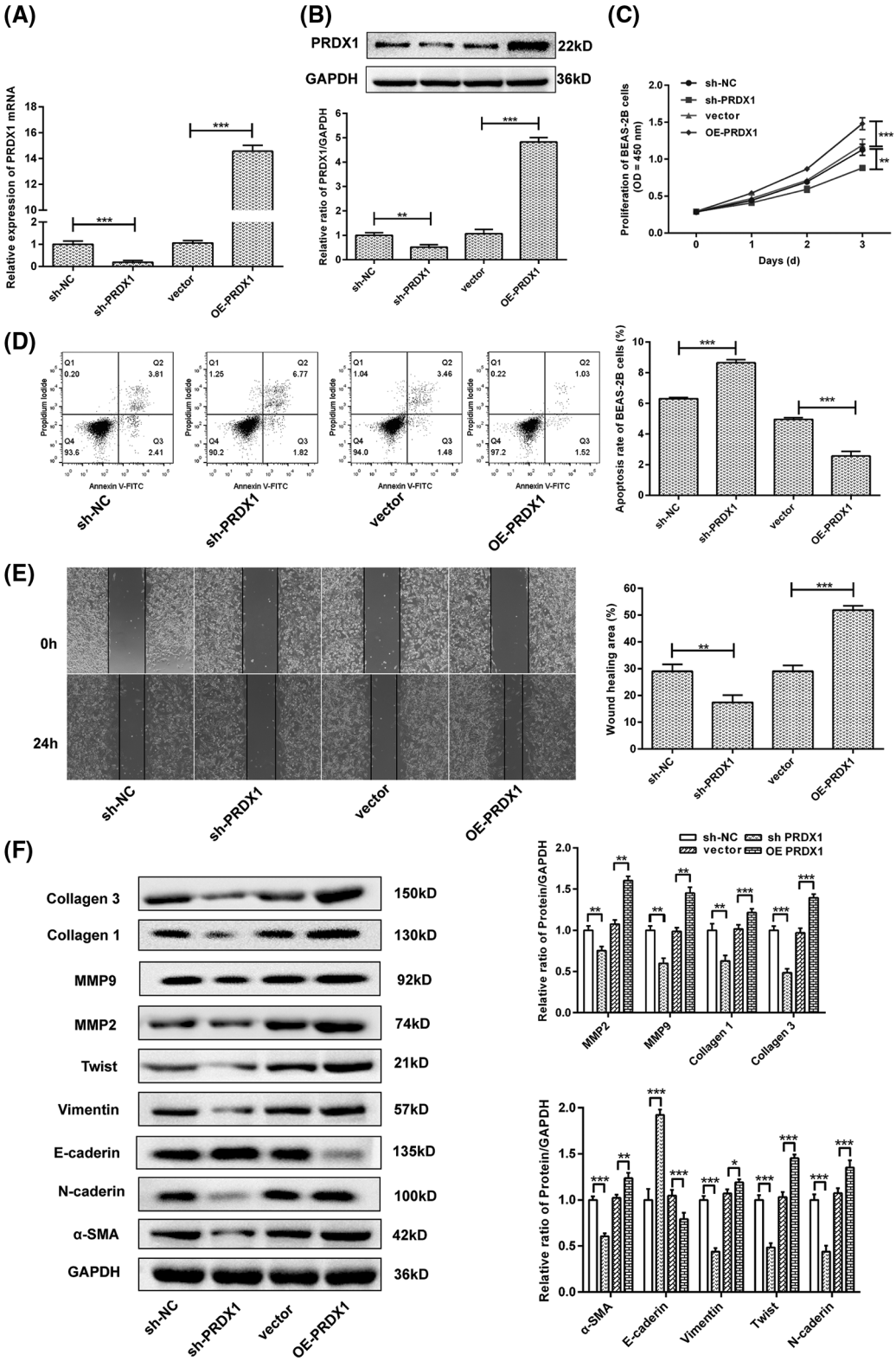

FIGURE 1. PRDX1 promoted the proliferation, migration, epithelial-mesenchymal transition (EMT), and collagen synthesis of BEAS-2B cells. PRDX1 mRNA (A) and protein (B) expression after transfection; PRDX1-mediated regulation of proliferation (C), apoptosis (D), and migration (E) of BESA-2B cells. (F) PRDX1 altered the expression of the EMT markers MMP2, MMP9, α-SMA, E-cadherin, N-cadherin, vimentin, and twist, and the synthesis of collagen 1 and collagen 3. All data are presented as the means ± standard deviations (n = 3). Compared with the NC group, $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. sh, short hairpin; OE, overexpression; NC, negative control; OD, optical density; EMT: epithelial-mesenchymal transition; MMP: matrix metallopeptidase; α-SMA: alpha-smooth muscle actin; PRDX1: peroxiredoxin.

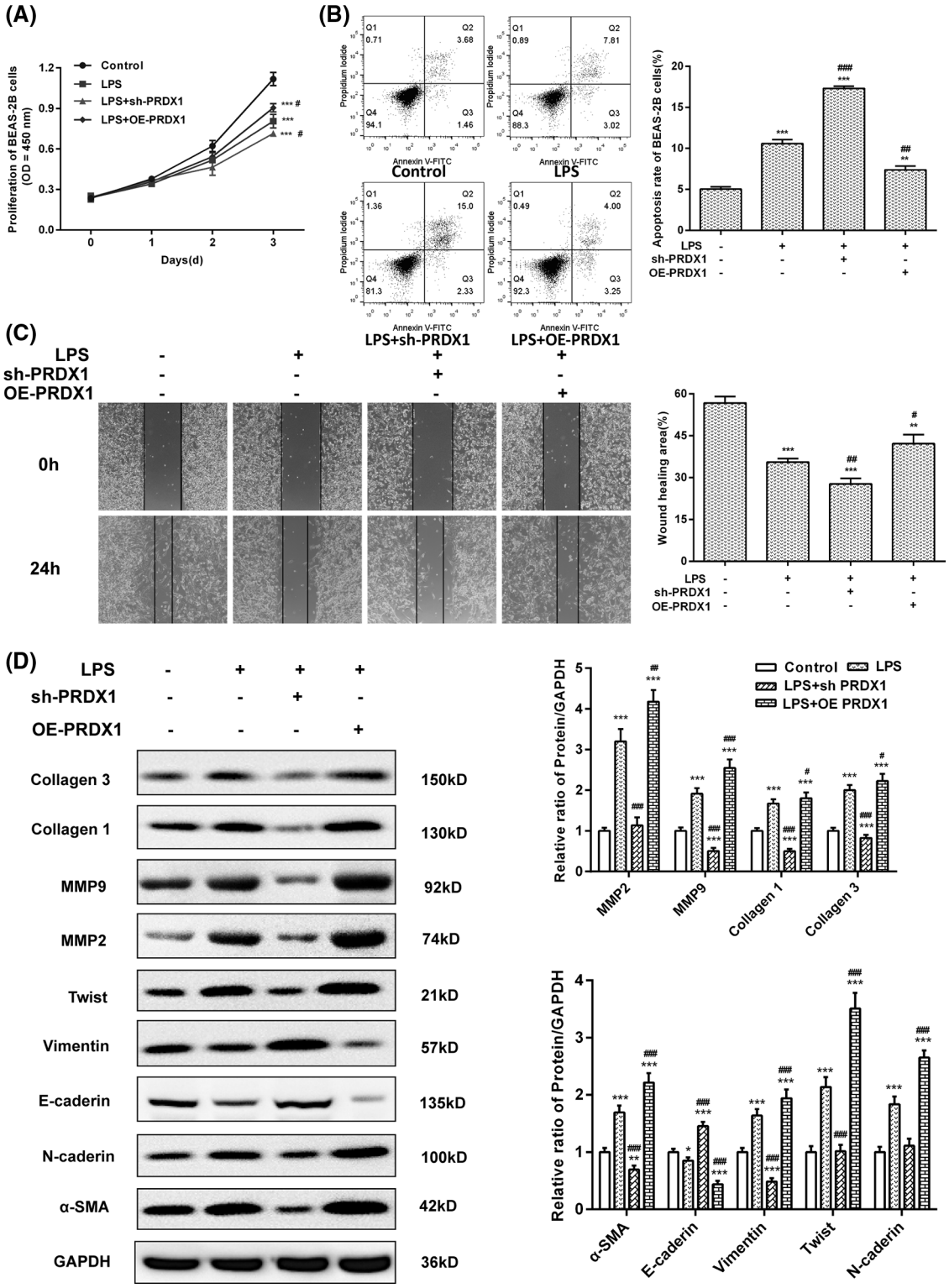

FIGURE 2. PRDX1 enhanced LPS-mediated changes in BEAS-2B cell proliferation, migration, EMT, and collagen synthesis. After transfection for 24 h, BEAS-2B cells were treated with 10 μg/mL LPS for 24 h. PRDX1 rescued LPS-mediated inhibition of cell proliferation (A), suppressed LPS-induced cell apoptosis (B), and reversed the LPS-induced suppression of cell migration (C). (D) PRDX1 enhanced the LPS-induced expression of the EMT markers MMP2, MMP9, α-SMA, N-cadherin, vimentin, and twist, and collagen 1/3 synthesis, and the LPS-mediated inhibition of E-cadherin expression. All data are presented as the means ± standard deviations (n = 3). Compared with the control group, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Compared with the LPS group, # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$. sh, short hairpin; OE, overexpression; NC, negative control; OD, optical density; PRDX1: Peroxiredoxin1; LPS: lipopolysaccharide; EMT: epithelial-mesenchymal transition; MMP: matrix metallopeptidase; α-SMA: alpha smooth muscle actin.

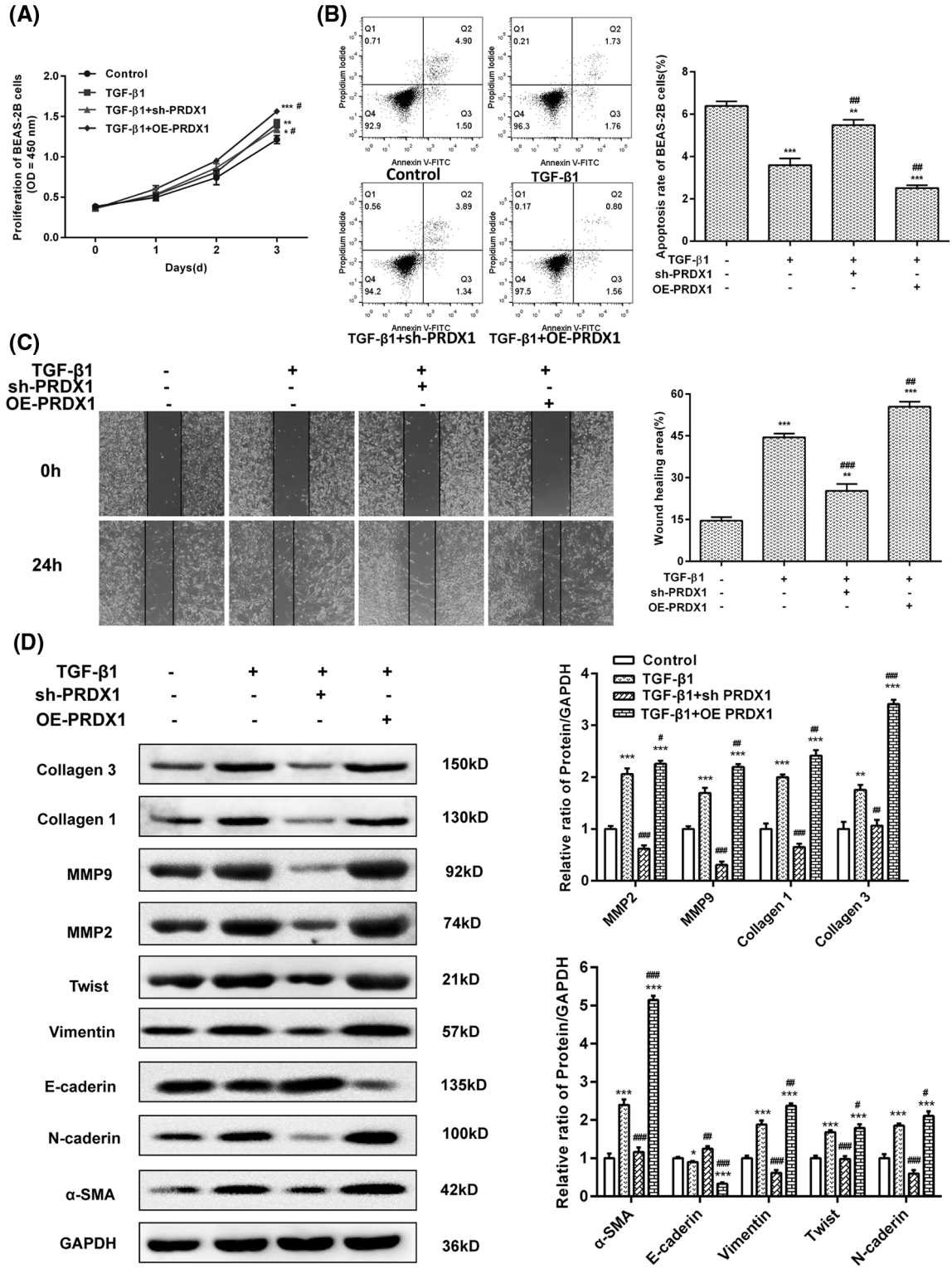

FIGURE 3. PRDX1 enhanced TGF-β1-induced BEAS-2B cell proliferation, migration, EMT, and collagen synthesis. After transfection, BEAS-2B cells were treated with 5 ng/mL recombinant human TGF-β1 for 24 h. PRDX1 enhanced TGF-β1-induced cell proliferation (A), TGF-β1-mediated inhibition of cell apoptosis (B), and TGF-β1-induced cell migration (C). (D) PRDX1 promoted the TGF-β1-induced expression of MMP2, MMP9, α-SMA, vimentin, twist, collagen 1, and collagen 3 and enhanced TGF-β1-mediated inhibition of E-cadherin expression. All of the data are presented as the means ± standard deviations (n = 3). Compared with the control group, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Compared with the TGF-β1 group, # $p < 0.05$, ## $p < 0.01$, and ### $p < 0.001$. sh, short hairpin; OE, overexpression; NC, negative control; OD, optical density; PRDX1: Peroxiredoxin1; EMT: epithelial-mesenchymal transition; MMP: matrix metallopeptidase; α-SMA: alpha-smooth muscle actin; TGF-β1: transforming growth factor β1.

Reduced expression of PRDX1 reversed TGF-β1-induced cell proliferation and migration and rescued TGF-β1-mediated inhibition of cell apoptosis ($p < 0.05$), while PRDX1 overexpression enhanced TGF-β1-mediated effects ($p < 0.05$).

Next, TGF-β1 treatment significantly increased the expression of the EMT markers MMP2, MMP9, α-SMA, N-cadherin, vimentin and twist, and the synthesis of collagen 1 and collagen 3 but reduced the level of E-cadherin ($p < 0.05$, Fig. 3D). PRDX1 knockdown inhibited TGF-β1-induced EMT and collagen synthesis, but increased PRDX1 level promoted the effect of TGF-β1 ($p < 0.05$).

Discussion

PRDX1 is a key protein that regulates many physiological processes for the development of various respiratory diseases, cancers, septic shock, and other diseases (Mq *et al.*, 2019; Sieńko *et al.*, 2019). The respiratory epithelium forms an important barrier against inhaled pollutants and microorganisms, and its barrier function is often compromised during inflammatory airway diseases (Olson *et al.*, 2011). LPS induces airway-centered inflammation and airway fibrosis and remodeling (Takahagi *et al.*, 2020). According to recent studies, LPS induces EMT in BEAS-2B cells and mouse models, which are characterized by a decrease in E-cadherin levels, increased levels of α-SMA, vimentin, MMP2, and MMP9 proteins, and inflammatory response, leading to extracellular matrix deposition and lung fibrosis (Chen *et al.*, 2020). TGF-β1 is a key growth factor involved in inducing inflammatory processes and EMT. TGF-β1 is upregulated in the airway epithelium of individuals with chronic obstructive pulmonary disease and induces the EMT in BEAS-2B cells (Zhu *et al.*, 2021). In this study, PRDX1 was found to be involved in regulating the LPS- and TGF-β1-induced proliferation, EMT, and collagen synthesis in airway epithelial cells.

In the present study, PRDX1 induced BEAS-2B cell proliferation and migration and inhibited cell apoptosis, consistent with the role in cervical cancer (Lu *et al.*, 2020). Overexpression of PRDX1 significantly promoted proliferation by upregulating Nanog and proliferating cell nuclear antigen, inhibited cell apoptosis by increasing B-cell lymphoma 2 (Bcl-2) expression, and downregulating Bcl-2-associated X protein, and promoted cell migration by upregulating Snail and MMP-9 expression and downregulating E-cadherin expression (Lu *et al.*, 2020). A lowered expression of PRDX1 inhibited cell proliferation and migration through the inhibition of the p38 mitogen-activated protein kinase (MAPK) signaling pathway (Cong *et al.*, 2018). PRDX1 forms a heterodimer with p38α MAPK-14, stabilizing phospho-p38α to amplify hepatocyte growth factor-mediated signaling and stimulate actin cytoskeleton dynamics that promote cell migration (Wirthschaft *et al.*, 2018). After the disruption of intact epithelial cells, airway epithelial cells migrate first to replace damaged cells (Mccormack *et al.*, 2013). The airway epithelial cells migrating at the wound edge acquire an EMT-like phenotype, manifested by a loss of E-cadherin expression and increased vimentin and α-SMA levels (Mccormack *et al.*, 2013). Then, airway epithelial cells may transform into myofibroblasts through the EMT to promote matrix remodeling and cell migration (Minor and Proud, 2017). Approximately 30% of fibroblasts/myofibroblasts detected during airway remodeling are derived from the EMT (Johnson *et al.*, 2011). Myofibroblasts are highly active profibrotic cells that secrete excessive extracellular matrix (ECM). The expression of α-SMA is a characteristic of myofibroblasts. The number of α-SMA+ myofibroblasts increases in subjects with chronic obstructive pulmonary disease, which might lead to increased collagen deposition, EMT activity in epithelial cells, and decreased lung function (Eapen *et al.*, 2021). The role of the MMP family has been implicated in the EMT. In particular, MMP-2/9 inhibit E-cadherin expression and increase N-cadherin and vimentin levels by upregulating epidermal growth factor receptor, protein kinase B (AKT), and extracellular-signal-regulated kinase (ERK)1/2 in airway epithelial cells (Agraval and Yadav, 2019). In cystic fibrosis, twist is considered the driver of the EMT in endogenous airway tissue and primary cells (Quaresma *et al.*, 2020). Here, we found that PRDX1 increased the expression of MMP 2 and MMP 9, induced the expression of the mesenchymal markers α-SMA, vimentin, twist, and N-cadherin, and inhibited the expression of the epithelial marker, E-cadherin *in vitro*. The increased α-SMA expression indicated that airway cells transformed from an epithelial to a myofibroblast phenotype, which promoted ECM collagen 1 and collagen 3 secretions. These results are similar to those reported in previous studies, namely, PRDX1-mediated changes in the expression of EMT markers to induce the EMT and upregulation of MMP 2/9 to increase the invasion and migration of cancer cells (Li *et al.*, 2018; Jiang *et al.*, 2019).

LPS and TGF-β1 are both inducers of EMT and fibrosis (Lee *et al.*, 2017; Sun *et al.*, 2020). In the present study, LPS induced apoptosis in BEAS-2B cells and reduced cell proliferation and migration. This finding was consistent with LPS-mediated cellular activities (Pooladanda *et al.*, 2019; Sun *et al.*, 2018). LPS induces PRDX1 expression in airway epithelial cells and macrophagocytes (Bast *et al.*, 2010; Liu *et al.*, 2014). PRDX1, in turn, promotes acute lung injury (Liu *et al.*, 2014). Loss of PRDX1 protects against LPS-induced acute lung injury (Ying *et al.*, 2020). The effects of LPS on upregulating MMP 2/9 expression, inducing EMT, and promoting collagen deposition have been confirmed (Shao *et al.*, 2019). Our results show that PRDX1 not only enhanced the LPS-induced expression of EMT markers but also promoted LPS-induced MMP 2/9 expression and collagen production. Airway epithelial cells lost the expression of the epithelial protein E-cadherin, which is responsible for tight junctions, and gained the expression of the mesenchymal markers N-cadherin (promotes cell migration), vimentin (helps form the extracellular matrix), and twist (promotes fibroblast proliferation), leading to the disordered epithelial barrier (Nathan *et al.*, 2018). The EMT alters cellular characteristics, such as enhanced migration, invasion, and wound healing (Samy Lamouille and Derynck, 2014). In breast cancer, PRDX1 functions as a tumor suppressor to inhibit collagen remodeling (Attaran *et al.*, 2021). A lowered expression of PRDX1 promotes the production of reactive

oxygen species and activation of NF-κB in breast cancer, consequently inducing the expression of the metastasis-related genes C-X-C chemokine receptor 4, MMP1, and MMP2 (Yang *et al*., 2019). Thus, different functions of PRDX1 in airway epithelial cells and breast cancer cells may be derived from specific cell types or disease progression.

TGF-β1, a representative profibrotic factor, induces airway tissue remodeling by activating the EMT (Park *et al*., 2016; Lee *et al*., 2017). Herein, we found that TGF-β1 treatment induced the proliferation, migration, and EMT of airway epithelial cells and decreased the rate of apoptosis. This finding was consistent with those reported in previous studies (Park *et al*., 2016; Sun *et al*., 2020). In the present study, PRDX1 enhanced the effect of TGF-β1 on BEAS-2B cells. TGF-β1 promotes corneal cell proliferation and wound healing by inducing PRDX1 expression (Tchah *et al*., 2005). TGF-β1-mediated induction of PRDX1 secretion (Chang *et al*., 2006) might be one of the mechanisms by which TGF-β1 induces the EMT. In particular, PRDX1 overexpression enhances the TGF-β1-induced EMT and cell migration in airway epithelial cells by reducing E-cadherin and increasing N-cadherin expressions (Ha *et al*., 2012). PRDX1 knockdown promotes cell proliferation, migration, invasion, and EMT in nasopharyngeal carcinoma by activating the phosphoinositide 3-kinases/AKT/TNF receptor-associated factor −1 signaling (Xiao *et al*., 2020). In lung cancer, PRDX1 protects DNA from oxidative damage induced by the cigarette carcinogen NNK and inhibits tumor growth. This phenotypic discrepancy arises from distinct roles of PRDX1 in certain cell types and tumor stages.

Collectively, in this study, PRDX1 promoted the proliferation, migration, EMT, and collagen deposition of BESA-2B cells. PRDX1 overexpression enhanced the LPS- and TGF-β1-induced EMT and collagen synthesis. Based on these results, PRDX1 may be involved in LPS/TGF-β1-induces EMT and collagen synthesis, and it may represent a new target for the prevention and treatment of airway injury.

Limitations

However, the present study had a few limitations. First, the conclusion that PRDX1 enhanced the LPS- and TGF-β1-induced EMT and collagen synthesis is based on results obtained only from a human bronchial epithelial cell line (BEAS-2B). Although the role of PRDX1 in regulating the TGF-β1-induced EMT and fibrosis has been previously documented in A549 cells (Ha *et al*., 2012), further validation in other airway epithelial cell lines or primary cells is still needed. Second, in the present study, we could not construct an animal model to verify the role of PRDX1 in airway damage. While PRDX1 protects against LPS-induced acute lung injury (Liu *et al*., 2014; Ying *et al*., 2020) and liver injury (Sun *et al*., 2018) in mice by reducing oxidative stress and inflammation, there is no evidence that the PRDX1-regulated EMT and fibrosis affect airway injury *in vitro*. Therefore, more animal experiments are needed to determine the importance of PRDX1 as a treatment target for airway injury and its effects on the biological functions of other airway cells. Finally, although there were no significant differences in cell viability and changes in protein expression between cells transfected with si-NC and vector plasmids, some differences between the two groups should still be considered. Therefore, this conclusion must be confirmed in different cell lines.

Availability of Data and Materials: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Author Contribution: Ling Gu and Huo-gen Liu contributed to the study conception and design. Ling Gu, Yun-di Shi, Hai-lin Shu, Feng-ming Huang, Huo-gen Liu, Xin Wan, Ying Liu, and Zhen-bin Gong contributed to data acquisition, analysis, and interpretation. Ling Gu participated in drafting the manuscript, and Huo-gen Liu revised it critically for its intellectual content.

Ethics Approval: Not applicable.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present work.

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