

Oxymatrine attenuates pulmonary fibrosis via APE1-mediated regulation of the PINK1/Parkin pathway

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Abstract. Pulmonary fibrosis (PF) is a chronic, progressive lung disease characterized by impaired gas exchange and respiratory difficulties, which can ultimately lead to respiratory failure and mortality. The present study explored the therapeutic effects and underlying mechanisms of oxymatrine (OMT) in an 8-week-old C57BL/6 mouse model of bleomycin-induced PF. The results demonstrated that OMT alleviated lung tissue damage, inflammation and collagen deposition, while promoting autophagy and restoring mitochondrial function. OMT achieved these effects by upregulating apurinic/apyrimidinic endonuclease-1 (APE1) and activating the PTEN-induced kinase 1 (PINK1)/Parkin pathway, both of which are key for mitochondrial autophagy. Furthermore, Lewis lung carcinoma mouse lung cancer cells were transduced with an adeno-associated virus containing small interfering RNA *APE1* and a negative control, and the silencing efficiency was validated by reverse transcription-quantitative PCR and western blotting. The results revealed a significant reduction in APE1 expression in the *APE1* knockdown group compared with that in the negative control knockdown group. Immunohistochemistry and immunofluorescence confirmed that OMT increased the expression of APE1, PINK1 and Parkin while reducing markers of fibrosis, such as α -smooth muscle actin and collagen type I α 1. However, silencing *APE1* or inhibiting mitochondrial autophagy with mitochondrial division inhibitor-1 reversed the

beneficial effects of OMT, suggesting a key role for APE1 and the PINK1/Parkin pathway in its mechanism of action. These findings provide insights into the antifibrotic potential of OMT and highlight its potential as a basis for the development of new therapies for PF.

Introduction

Pulmonary fibrosis (PF) is a chronic, progressive lung disease of the pulmonary interstitium, characterized by continuous damage that leads to impaired gas exchange and breathing difficulties, ultimately resulting in respiratory failure and mortality (1). The etiology of PF is multifactorial, including allergens, chemicals, radiation and particulates (2). A previous epidemiological study indicated that idiopathic PF (IPF) is the fastest progressing and most lethal type of all fibrotic diseases, with a median survival time of 3.5 years for affected patients (3). Acute exacerbation is a notable concern in patients with progressive PF, leading to poor survival outcomes, with an increased risk of mortality following acute exacerbation (4). Additionally, a meta-analysis has highlighted the high incidence of acute exacerbations in patients with IPF, particularly within the first 2-3 years of diagnosis (5). Furthermore, although inhaled corticosteroids have been studied in chronic airway diseases, their use has been associated with an increased risk of IPF, particularly at higher doses (6). Current treatment modalities include antifibrotic drugs such as pirfenidone and nintedanib, which can only slow disease progression and cannot reverse the established fibrosis (7). In PF, inflammatory cytokines produced by lung epithelial cells can trigger the transformation of fibroblasts into myofibroblasts, leading to excessive production and deposition of collagen at sites of lung injury, a pathophysiological outcome of chronic lung damage (8). These changes are associated with oxidative stress and inflammatory processes (9,10). During these processes, cells must effectively respond to DNA damage and cellular stress, where apurinic/apyrimidinic endonuclease-1 (APE1), a multifunctional protein, serves a key role in DNA repair and cellular stress response regulation (11). APE1 is not only involved in base excision repair of DNA but also regulates various transcription factors through its redox activity, influencing inflammation and cellular stress responses. Studies have revealed that increased APE1 expression in non-small cell lung cancer promotes disease progression (12), as

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Abbreviations: PF, pulmonary fibrosis; OMT, oxymatrine; APE1, apurinic/apyrimidinic endonuclease-1; PINK1, PTEN-induced kinase 1; LC-3, microtubule-associated proteins 1A/1B light chain 3; COL1a1; collagen type I α 1; α -SMA, α -smooth muscle actin; TOM20, mitochondrial import receptor subunit TOM20 homolog; mdivi-1, mitochondrial division inhibitor-1; AAV, adeno-associated virus; siAPE1, small interfering RNA APE1

Key words: PF, OMT, APE1, PINK1/Parkin pathway, mitophagy

demonstrated by its association with poor prognosis and tumor growth (13,14). However, the expression and role of *APE1* in PF remain unclear.

Oxymatrine (OMT), the main active component of the traditional Chinese medicine *Sophora flavescens*, is known for its antitumor, antiviral and anti-inflammatory properties (15). A study demonstrated that OMT effectively protects against organ damage and disease progression, including in the brain, heart, liver, kidney and gastrointestinal tract, by regulating inflammation, oxidative stress, apoptosis and fibrosis (16). Feng *et al* (17) revealed that OMT, by activating the PI3K/AKT signaling pathway, can alleviate TGF- β 1-mediated mitochondrial apoptosis in alveolar epithelial cells, thereby demonstrating potential protective effects in the treatment of PF. In recent years, increasing attention has been paid to the role of mitochondrial function in PF therapy (18-20). Methods that promote mitochondrial biogenesis and transfer have been revealed to restore mitochondrial homeostasis and mitophagy in experimental models, effectively slowing the progression of PF (21). In lung diseases, the PTEN-induced kinase 1 (PINK1)/Parkin pathway influences mitophagy, mitochondrial biogenesis and dynamic equilibrium by regulating mitochondrial quality, serving a key role in the development of respiratory-related diseases, such as chronic obstructive pulmonary disease, lung cancer and IPF (22). Liang *et al* (23) revealed that OMT exerts its anticancer activity by inhibiting leucine-rich pentatricopeptide repeat-containing protein, promoting Parkin mitochondrial translocation and activating mitophagy. To the best of our knowledge, it has not yet been reported whether OMT exerts its therapeutic effects on PF through PINK1/Parkin pathway-mediated mitophagy. Therefore, the present study aimed to investigate the effects of OMT on the *APE1* and PINK1/Parkin pathways in an animal model of PF, which is important for further elucidating the therapeutic targets and pharmacological mechanisms of OMT in PF.

Materials and methods

Ethics approval. All animal experiments carried out in the present study were approved by the Ethics Committee of Hainan General Hospital [approval no. Med-Eth-Re (2024)718; Haikou, China]. All experimental procedures were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (24) and adhered to the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines (25) to ensure rigorous and transparent reporting of animal research.

Experimental design. Male C57BL/6 mice (age, 8 weeks; weight, 20-25 g) were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. A total of 42 mice were used in the present study. The experimental mice were housed in a standard animal facility at 22-24°C and a humidity of 50-60%, under a 12-h light/dark cycle, and with *ad libitum* access to food and water. After 1 week of acclimation, the C57BL/6 mice were randomly divided into seven groups based on body weight and identification numbers (n=6 mice/group). OMT (cat. no. SM4048; Beyotime Institute of Biotechnology) was prepared by dissolving 3 mg OMT in 1.13 ml DMSO to obtain

a 10 mM OMT solution. Bleomycin (cat. no. ST1450; Beyotime Institute of Biotechnology) was prepared by dissolving bleomycin in deionized water to a concentration of 20 mg/ml for later use. Mice were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (35 mg/kg) for 10-15 min. The limbs and head were secured, and under direct visualization using a headlamp, an epidural catheter with a connector was inserted into the trachea. Subsequently, 0.25 ml of 0.4% bleomycin solution (5 mg/kg) was administered intratracheally, while the normal group received an equal volume of normal saline. Immediately after the injection, the animals were rotated upright to ensure uniform distribution of the agent within the lungs (26). Based on previous studies demonstrating the efficacy of OMT in various animal models of pulmonary diseases (27,28), the doses of 10 and 40 mg/kg were selected for the present study. The OMT treatment groups (OMT) received OMT solutions at doses of 10 and 40 mg/kg daily via gavage for 21 days. The N-acetylcysteine-treated group (NAC), serving as the positive control, received NAC (cat. no. A7250; MilliporeSigma) at a dose of 150 mg/kg daily via intraperitoneal injection for 21 days following model induction. The *APE1* (GenBank ID, NM_009687.2) silencing treatment group (OMT + KD-*APE1*) received a tail vein injection of 1×10^{11} vector genomes of adeno-associated virus (AAV) containing small interfering RNA (siRNA) *APE1* (AAV-si*APE1*) containing the siRNA sequence: Sense: 5'-CGGGTGATTGTGGCTGAATTT-3' and antisense: 5'-AAATTCAGCCACAATCACCCG-3' (Shanghai GeneChem Co., Ltd.). The mitophagy inhibitor mitochondrial division inhibitor (mdivi)-1 treatment group (OMT + mdivi-1) was administered mdivi-1 (cat. no. M0199; MilliporeSigma) at a dose of 10 mg/kg via gavage twice weekly. On day 21, the mice were euthanized by intraperitoneal injection of an overdose of sodium pentobarbital (100 mg/kg), and death was verified by cessation of heartbeat and respiration (29). The average time under anesthesia before euthanasia was 5-7 min. Animal health and behavior were monitored daily throughout the experiment for signs of distress, abnormal posture, reduced activity, fur condition and body weight. Lung tissues from each mouse (six tissues per group) were collected for subsequent analyses.

H&E staining. Immediately after harvesting the lung tissues from the mice, the tissues were fixed in 10% neutral formalin for 24 h at room temperature. Post-fixation, the tissues were dehydrated using a graded ethanol series (80, 90, 95 and 100%) for 2 h each, followed by clearing with xylene. The tissues were then embedded in paraffin using a heated paraffin embedding system at 60-65°C (cat. no. G1150H; Leica Biosystems). Once the paraffin had solidified, tissue sections were cut at a thickness of 4 μ m using a paraffin microtome (cat. no. RM2235; Leica Biosystems) and floated on a water bath at 40-45°C. The sections were then mounted onto slides and dried on a slide warmer at 60-65°C (cat. no. HI1220; Leica Biosystems) for 15 min. Following deparaffinization in xylene at room temperature and rehydration through a graded ethanol series to water, the sections were washed with distilled water. The nuclei were stained with Harris hematoxylin (cat. no. MD911477; MDL) for 5 min at room temperature, differentiated with 1% hydrochloric acid alcohol and then blued with 0.6% ammonia water for 2 min at room temperature. The cytoplasm was counterstained

with eosin for 3 min at room temperature. Finally, the sections were dehydrated through 95% and absolute ethanol, cleared in xylene and mounted with neutral balsam (cat. no. MD911683; MDL). The stained sections were then examined and analyzed under a light microscope (Leica DM500; Leica Microsystems GmbH). Alveolitis and inflammation were semi-quantitatively assessed as the lung injury score, graded on a scale of 0 to 3 according to the Szapiel scoring system (30), as follows: 0, normal; 1, mild; 2, moderate; and 3, severe. For each mouse, three sections were examined, and 10 random fields per section were evaluated under a light microscope in a blinded manner. The median score was calculated and used for statistical analysis.

Masson's trichrome staining. Paraffin-embedded tissue sections were deparaffinized and rehydrated using standard protocols (31), and then subjected to Masson's trichrome staining according to the instructions provided with the Masson staining kit (cat. no. C0189M; Beyotime Institute of Biotechnology). The sections were first stained with Weigert's iron hematoxylin for 5 min at room temperature, followed by differentiation with acidic ethanol and then rinsed with water. The sections were subsequently blued with Masson bluing solution. Next, the sections were stained with Biebrich scarlet-acid fuchsin solution for 10 min at room temperature, followed by washing with phosphomolybdic acid solution for 2 min and staining with aniline blue solution for 2 min at room temperature. After each staining step, the sections were rinsed with a weak acid working solution (distilled water: weak acid solution, 2:1) for 1 min. Following rapid dehydration with 95% ethanol, the sections were cleared in xylene three times, each for 2 min and finally mounted with neutral balsam. The stained sections were then examined and analyzed under a light microscope (Leica DM500; Leica Microsystems GmbH).

TUNEL staining. TUNEL staining was carried out using a commercial kit (cat. no. 11684817910; Roche Diagnostics) according to the manufacturer's instructions. After deparaffinization and hydration, the sections were treated with proteinase K (20 µg/ml) at room temperature for 15 min. The sections were then washed with PBS and incubated with the TUNEL reaction mixture at 37°C for 1 h. Subsequently, the sections were developed using a DAB staining kit (cat. no. ZLI-9017; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.). The stained sections were observed in five fields of view under a light microscope (Leica DM500; Leica Microsystems GmbH).

Immunohistochemistry. In the immunohistochemistry experiments, paraffin-embedded tissue sections were heated at 60°C for 1 h, followed by deparaffinization in xylene (xylene I for 10 min; xylene II for 10 min) at room temperature, rehydration through a graded ethanol series (100% ethanol for 3 min; 95% ethanol for 3 min; 85% ethanol for 3 min and 75% ethanol for 3 min), and washing with PBS for 3 min. Antigen retrieval was carried out using 0.1 mol/l citrate buffer (pH 6.0) with microwave heating for 10 min. To quench endogenous peroxidase activity, the sections were incubated with 3% H₂O₂ at room temperature for 10 min. Non-specific binding was blocked by incubating the sections with 5% BSA blocking solution

(cat. no. C05-06002; Bioss) at room temperature for 30 min. The sections were then incubated overnight at 4°C with the following primary antibodies: α-smooth muscle actin (α-SMA; 1:1,500; cat. no. 14395-1-AP; Proteintech Group, Inc.), collagen type I α 1 (COL1a1; 1:5,000; cat. no. 67288-1-Ig Proteintech Group, Inc.), APE1 (1:2,000; cat. no. ab189474; Abcam), PINK1 (1:1,000; cat. no. 23274-1-AP; Proteintech Group, Inc.) and Parkin (1:500; cat. no. 14060-1-AP; Proteintech Group, Inc.). The next day, after washing with PBS, the sections were incubated with a biotinylated secondary antibody (1:200; cat. no. BA1000-1.5; Vector Laboratories, Inc.; Maravai LifeSciences) at room temperature for 1 h and streptavidin-HRP conjugate (cat. no. SAP-9100; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) at room temperature for 30 min. The color was developed using a DAB staining kit (cat. no. ZLI-9017; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.), and the sections were counterstained with hematoxylin. The sections were then dehydrated, cleared in xylene and mounted with neutral balsam. The stained sections were then examined and analyzed under a light microscope (Leica DM500; Leica Microsystems GmbH).

Immunofluorescence. After deparaffinization and rehydration, sections were subjected to antigen retrieval by heating in 0.1 mol/l citrate buffer (pH 6.0) in a microwave for 10 min. Endogenous peroxidase activity was quenched by incubating the sections with 3% H₂O₂ at room temperature for 10 min. Subsequently, the sections were blocked with 5% BSA blocking solution at room temperature for 30 min. The sections were then incubated overnight at 4°C with the following primary antibodies: Microtubule-associated proteins 1A/1B light chain 3 (LC-3; 1:200; cat. no. 14600-1-AP; Proteintech Group, Inc.) and mitochondrial import receptor subunit TOM20 homolog (TOM20; 1:200; cat. no. 11802-1-AP; Proteintech Group, Inc.). The next day, after washing with PBS, the sections were incubated at room temperature for 1 h with Alexa Fluor 488-conjugated (1:500; cat. no. A-11008; Invitrogen, Thermo Fisher Scientific, Inc.) and Alexa Fluor 594-conjugated (1:500; cat. no. A-11012; Invitrogen, Thermo Fisher Scientific, Inc.) secondary antibodies. The sections were then counterstained with DAPI (cat. no. D9542; MilliporeSigma) for 5 min at room temperature, washed with PBS and mounted with antifade mounting medium (cat. no. P36930; Thermo Fisher Scientific, Inc.). Fluorescence staining results were observed using a fluorescence microscope (DMI8; Leica Microsystems). Images were captured with a confocal microscope (Zeiss LSM 880; Leica Microsystems) to analyze the expression and colocalization of LC-3 and TOM20. Semi-quantitative analysis was carried out using ImageJ software (version 1.53; National Institutes of Health).

Cell culture and transduction. Lewis lung carcinoma (LLC) mouse lung cancer cells (cat. no. CL-0140; CVCL_4358; Wuhan Pricella Biotechnology Co., Ltd.) were cultured in RPMI-1640 medium (cat. no. 11875-093; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (cat. no. 10099-141; Gibco; Thermo Fisher Scientific, Inc.) and 1% penicillin-streptomycin solution (cat. no. 15140-122; Gibco; Thermo Fisher Scientific, Inc.) at 37°C in a humidified incubator with 5% CO₂. Ready-to-use

recombinant lentiviral particles (third-generation system) encoding an siRNA targeting mouse *APE1* (KD-*APE1*; vector GV493; Shanghai GeneChem Co., Ltd.) which carries the same siRNA sequence as that used for *in vivo* AAV delivery but with a lentiviral backbone optimized for *in vitro* transduction, and the matched negative control lentivirus (NC-KD; Shanghai GeneChem Co., Ltd.) were supplied by the manufacturer. According to the supplier's documentation, the lentiviral particles were produced by Shanghai GeneChem Co., Ltd. using 293T cells by co-transfecting 10 μ g GV493-si*APE1*, 7.5 μ g psPAX2 (packaging) and 2.5 μ g pMD2.G (envelope) per 10 cm dish with Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) at 37°C for 6 h. LLC cells were seeded at 3×10^5 cells/well in 6-well plates and when they reached ~70% confluence, they were transduced for 24 h at 37°C with KD-*APE1* or NC-KD lentivirus at a multiplicity of infection of 100 in the presence of 8 μ g/ml polybrene. After transduction, the medium was replaced with fresh complete medium. The cells were then incubated for an additional 48 h before subsequent experiments. For the generation of stable cell lines, cells were selected with 2 μ g/ml puromycin for 5 days and maintained in 1 μ g/ml puromycin. The siRNA sequences were as follows: si*APE1*, sense 5'-CGGGTGATTGTGGCTGAATTT-3' and antisense 5'-AAATTCAGCCACAATCACCCG-3'; NC-KD, sense: 5'-TTCTCCGAACGAGTCACGT-3' and antisense: 5'-ACG TGACTCGTTCGGAGAA-3'. The transduction efficiency was assessed by reverse transcription-quantitative PCR (RT-qPCR) and western blotting.

RT-qPCR. Total RNA was isolated from transfected LLC cells using TRIzol® reagent (cat. no. 15596026; Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. cDNA synthesis was carried out in a single step at 42°C for 60 min using the PrimeScript™ RT Reagent Kit (cat. no. 6110A; Takara Bio, Inc.). The cDNA was then subjected to qPCR amplification with SYBR Green PCR Master Mix (cat. no. 4367659; Thermo Fisher Scientific, Inc.) on a StepOnePlus™ Real-Time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.). The primers for *APE1* amplification were: Forward, 5'-GGGAAGAAC CCAAGTCGGAG-3' and reverse, 5'-TTGCCACTGGGT GAGGTTTT-3'. The expression levels of *APE1* mRNA was measured using the following thermocycling conditions: 95°C for 10 min, followed by 40 cycles at 95°C for 15 sec and 60°C for 1 min, and were normalized to GAPDH as an internal control, using the following GAPDH primers: Forward, 5'-TTGCAGTGGCAAAGTGGAGA-3' and reverse, 5'-ACTGTGCCGTGAATTTGCC-3'. The relative mRNA expression levels of *APE1* were calculated using the $2^{-\Delta\Delta C_q}$ method (32). Data were analyzed using StepOnePlus software (version 2.3; Applied Biosystems; Thermo Fisher Scientific, Inc.).

Western blotting. Total protein was extracted from lung tissue and LLC cells using RIPA buffer (cat. no. 89900; Thermo Fisher Scientific, Inc.) and quantified using the bicinchoninic acid assay. Equal amounts of protein samples (50 μ g protein/lane) were separated by SDS-PAGE on 10% polyacrylamide gels and transferred onto polyvinylidene difluoride

membranes. The membranes were blocked with 5% non-fat milk at room temperature for 1 h, followed by incubation with primary antibodies against APE1 (1:1,000; cat. no. ab189474; Abcam), PINK1 (1:1,000; cat. no. ab216144; Abcam) and Parkin (1:2,000; cat. no. ab77924; Abcam) overnight at 4°C. The following day, the membranes were washed with PBS and incubated with HRP-conjugated goat anti-mouse IgG(H+L) secondary antibody (1:5,000; cat. no. SA00001-1; Proteintech Group, Inc) or goat anti-rabbit IgG (H+L) secondary antibodies (1:5,000; cat. no. SA00001-2; Proteintech Group, Inc.) at room temperature for 1 h. Protein bands were visualized using an ECL reagent (ECL Plus; cat. no. RPN2232; Cytiva). GAPDH (1:5,000; cat. no. 60004-1-Ig; Proteintech Group, Inc.) was used as the internal control. Bands were imaged and were semi-quantified using ImageJ software (version 1.53; National Institutes of Health).

Statistical analysis. Statistical analysis was carried out using GraphPad Prism 8 software (Dotmatics). All data are presented as the mean $\pm$ standard deviation. Comparisons between groups were made using one-way ANOVA, followed by Tukey's post hoc test to determine differences between individual groups. Lung injury scores are presented as the median (interquartile range) and were analyzed using the Kruskal-Wallis test with Dunn's multiple-comparisons post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

OMT treatment reverses bleomycin-induced PF. A schematic diagram illustrating the treatment protocol is shown in Fig. 1A. H&E staining revealed that the lung tissue in the normal group had an intact and clear structure (Fig. 1B). The alveolar walls were thin and continuous with no notable infiltration of inflammatory cells in the alveoli. By contrast, the lung tissue of mice in the bleomycin-induced PF model group exhibited diffuse consolidation, thickened alveolar walls, disrupted alveolar septa and substantial infiltration of inflammatory cells in the alveoli, with numerous fibroblasts present in the lung interstitium. The lung injury score was significantly increased in the model group compared with that in the normal group ($P < 0.001$; Fig. 1G). After treatment with 40 mg/kg OMT or NAC, the alveolar structure appeared relatively normal, with thin and continuous alveolar walls and minimal inflammatory infiltration. The lung injury scores were significantly decreased compared with that in the model group (40 mg/kg: $P < 0.05$; NAC: $P < 0.05$).

Masson's trichrome staining revealed that the lung tissue structure in both the normal and OMT groups was clear, with no marked inflammatory cell infiltration or deposition of blue collagen fibers (Fig. 1C). In the model group, the lung tissue structure was disordered, with evident inflammatory cell infiltration in the lung interstitium and notably increased deposition of blue collagen fibers. Following treatment with 10 and 40 mg/kg OMT, the subepithelial deposition of blue collagen fibers was markedly reduced. Quantification analysis confirmed a significant reduction in collagen deposition in the 10 and 40 mg/kg OMT, and NAC groups compared with that in the model group ($P < 0.001$, Fig. 1H).

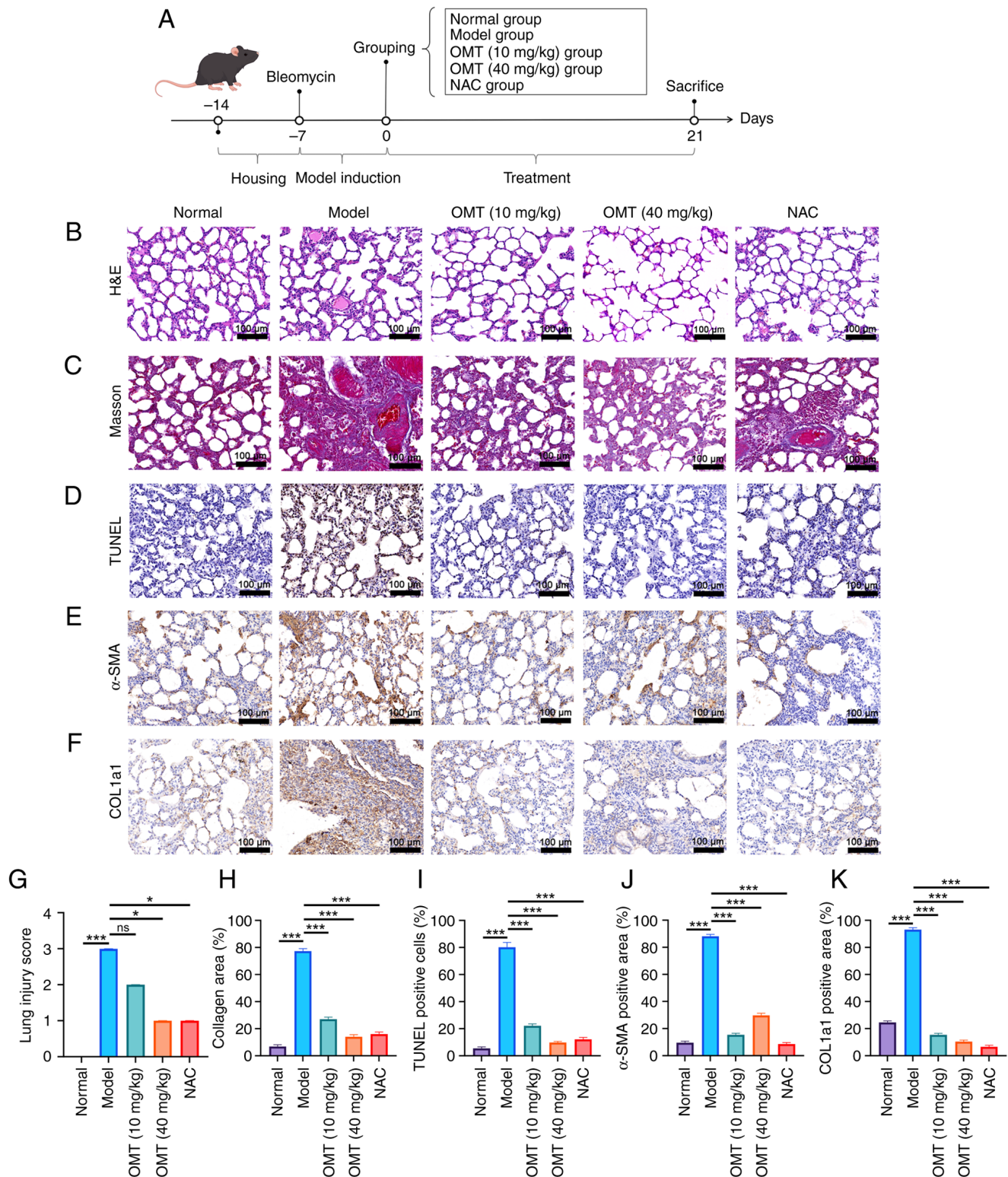


Figure 1. OMT treatment reverses bleomycin-induced PF. (A) Schematic diagram of the treatment protocol. (B) H&E staining was used to observe and evaluate lung tissue structure and morphology. (C) Masson's trichrome staining was performed to assess PF. (D) TUNEL staining was used to detect apoptosis. IHC staining was conducted to detect the expression levels of (E) α -SMA and (F) COL1a1. Semi-quantitative analysis of (G) H&E, (H) Masson's, (I) TUNEL, and IHC staining of (J) α -SMA and (K) COL1a1. Scale bar, 100 μ m. (G) Data are presented as median and IQR, and statistical significance was analyzed using the Kruskal-Wallis test. (H-K) Data are presented as the mean $\pm$ SD, and statistical significance was analyzed using one-way ANOVA; n=6. *P<0.05, ***P<0.001. The treatment schematic was designed using Figdraw (<https://www.figdraw.com/>). PF, pulmonary fibrosis; OMT, oxymatrine; NAC, N-acetylcysteine; α -SMA, α -smooth muscle actin; COL1a1, collagen type I α 1; IHC, immunohistochemical.

TUNEL staining demonstrated that, compared with the normal group, the model group had an increased number of positive cells (brown), which were widely distributed,

indicating an increased level of apoptosis (Fig. 1D). After treatment with 10 and 40 mg/kg OMT, the number of positive cells in the lung tissue was significantly reduced compared

with that in the model group ($P < 0.001$; Fig. 1I), suggesting that OMT may help reverse PF through its anti-apoptotic effects.

α -SMA and COL1a1 are specific markers for myofibroblasts and fibroblasts, respectively. Positive signals appear as brown or tan precipitates observable under a microscope (33). Observation of immunohistochemistry data revealed that, compared with the normal group, the expression of α -SMA (brown) and COL1a1 (brown) was increased in the lung tissue of the model group, indicating successful construction of the PF model (Fig. 1E and F). After treatment with 10 and 40 mg/kg OMT or NAC, the expression of α -SMA and COL1a1 in the lung tissue was reduced. Semi-quantitative analysis confirmed that the positive areas of α -SMA and COL1a1 were significantly decreased in the OMT and NAC groups compared with those in the model group ($P < 0.001$; Fig. 1J and K). These findings suggested that OMT may have an inhibitory effect on the progression of PF.

OMT mitigates PF by activating the APE1 and PINK1/Parkin pathways, and promoting autophagy and mitochondrial function restoration. Transduction efficiency was first assessed in LLC cells by RT-qPCR and western blotting; compared with in the NC-KD group, the KD-APE1 group exhibited significantly reduced expression of APE1 ($P < 0.001$; Fig. 2A and B), verifying the successful silencing of APE1. Observation of immunohistochemistry data indicated that the expression of APE1, PINK1 and Parkin was reduced in the model group compared with that in the normal group (Fig. 2C). Treatment with OMT resulted in an increase in the expression of these proteins, with the highest levels observed in the 40 mg/kg group; however, silencing APE1 or treating cells with the mitochondrial autophagy inhibitor mdivi-1 led to a significant reduction in the expression levels of APE1, PINK1 and Parkin compared with those in the 40 mg/kg OMT group ($P < 0.001$; Fig. 2D-F).

Analysis of western blotting data (Fig. 3A) revealed that compared with in the normal group, the expression levels of APE1 ($P < 0.01$; Fig. 3B), PINK1 ($P < 0.001$; Fig. 3C) and Parkin ($P < 0.001$; Fig. 3D) were significantly decreased in the model group. By contrast, the expression levels of APE1, PINK1 and Parkin were significantly increased in the 10 mg/kg OMT, 40 mg/kg OMT and NAC groups compared with those in the model group ($P < 0.001$). Furthermore, compared with in the 40 mg/kg OMT group, the expression levels of APE1 ($P < 0.01$), PINK1 ($P < 0.001$) and Parkin ($P < 0.001$) were significantly decreased in both the OMT + KD-APE1 and OMT + mdivi-1 groups. Immunofluorescence observation demonstrated enhanced expression and colocalization of LC-3 and TOM20 in the OMT groups, indicating improved mitochondrial autophagy (Fig. 3E). The percentage of the yellow fluorescent colocalization area of LC-3 and TOM20 relative to the entire field was significantly higher in the 10 and 40 mg/kg OMT groups compared with that in the model group ($P < 0.001$). However, in the OMT + KD-APE1 and OMT + mdivi-1 groups, the percentage of the yellow fluorescent colocalization area was significantly lower compared with in the 40 mg/kg OMT group ($P < 0.001$; Fig. 3F). These results suggested that OMT may exert its antifibrotic effects by promoting mitochondrial autophagy through the APE1 and PINK1/Parkin pathways.

Silencing APE1 reverses the antifibrotic effects of OMT on bleomycin-induced PF. A schematic diagram depicting the extended treatment protocol including the 40 mg/kg OMT and KD-APE1 intervention group is presented in Fig. 4A. H&E staining revealed that in the OMT treatment groups (10 and 40 mg/kg), the alveolar walls were thinner and more continuous compared with the model group, with a marked reduction in inflammatory cell infiltration (Fig. 4B). However, in the OMT + KD-APE1 treatment group, the mice exhibited pathological changes similar to those in the model group. Semi-quantitative analysis showed that lung injury score was significantly reduced by 40 mg/kg OMT or NAC treatment compared with that in the model group ($P < 0.05$), whereas no significant difference was observed in the 10 mg/kg OMT group. Furthermore, the lung injury score was significantly increased again after APE1 knockdown compared with that in the 40 mg/kg OMT group ($P < 0.05$; Fig. 4G). Masson's trichrome staining revealed that OMT treatment considerably reduced collagen deposition compared with in the model group, but in the OMT + KD-APE1 group, collagen deposition increased again, resembling that of the model group (Fig. 4C and H). TUNEL staining demonstrated that OMT treatment reduced apoptosis (Fig. 4D), as evidenced by significantly fewer TUNEL-positive cells in the OMT treatment groups compared with in the model group (Fig. 4I). Immunohistochemistry further demonstrated that the expression of α -SMA and COL1a1 was lower in the OMT treatment groups compared with in the model group (Fig. 4E and F), indicating its potential antifibrotic effects. However, the OMT + KD-APE1 group showed a significantly increased expression of α -SMA and COL1a1 compared with in the 40 mg/kg OMT group ($P < 0.001$; Fig. 4J and K), indicating that silencing APE1 reversed the antifibrotic effects of OMT.

Mitochondrial autophagy inhibitor reverses the antifibrotic effects of OMT on bleomycin-induced PF. A schematic diagram outlining the modified treatment protocol including the 40 mg/kg OMT and mdivi-1 treatment group is shown in Fig. 5A. H&E staining revealed that the OMT + mdivi-1 treatment group exhibited pathological characteristics similar to the model group, including consolidation, thickened alveolar walls and disrupted septa (Fig. 5B). In contrast to the protective effects observed with OMT treatment alone, mdivi-1 appeared to negate these benefits. Semi-quantitative analysis showed that the lung injury score was significantly increased in the OMT + mdivi-1 group compared with that in the 40 mg/kg OMT group ($P < 0.05$; Fig. 5G).

Masson's trichrome staining demonstrated that in the OMT + mdivi-1 treatment group, collagen deposition was increased, resembling that in the model group (Fig. 5C). Quantitative analysis confirmed significantly higher collagen levels in the OMT + mdivi-1 group compared with in the 40 mg/kg OMT group ($P < 0.001$; Fig. 5H). Additionally, in the OMT + mdivi-1 treatment group, the number of TUNEL-positive cells (brown) was significantly increased compared with that in the 40 mg/kg OMT group ($P < 0.001$; Fig. 5D and I), indicating an increase in

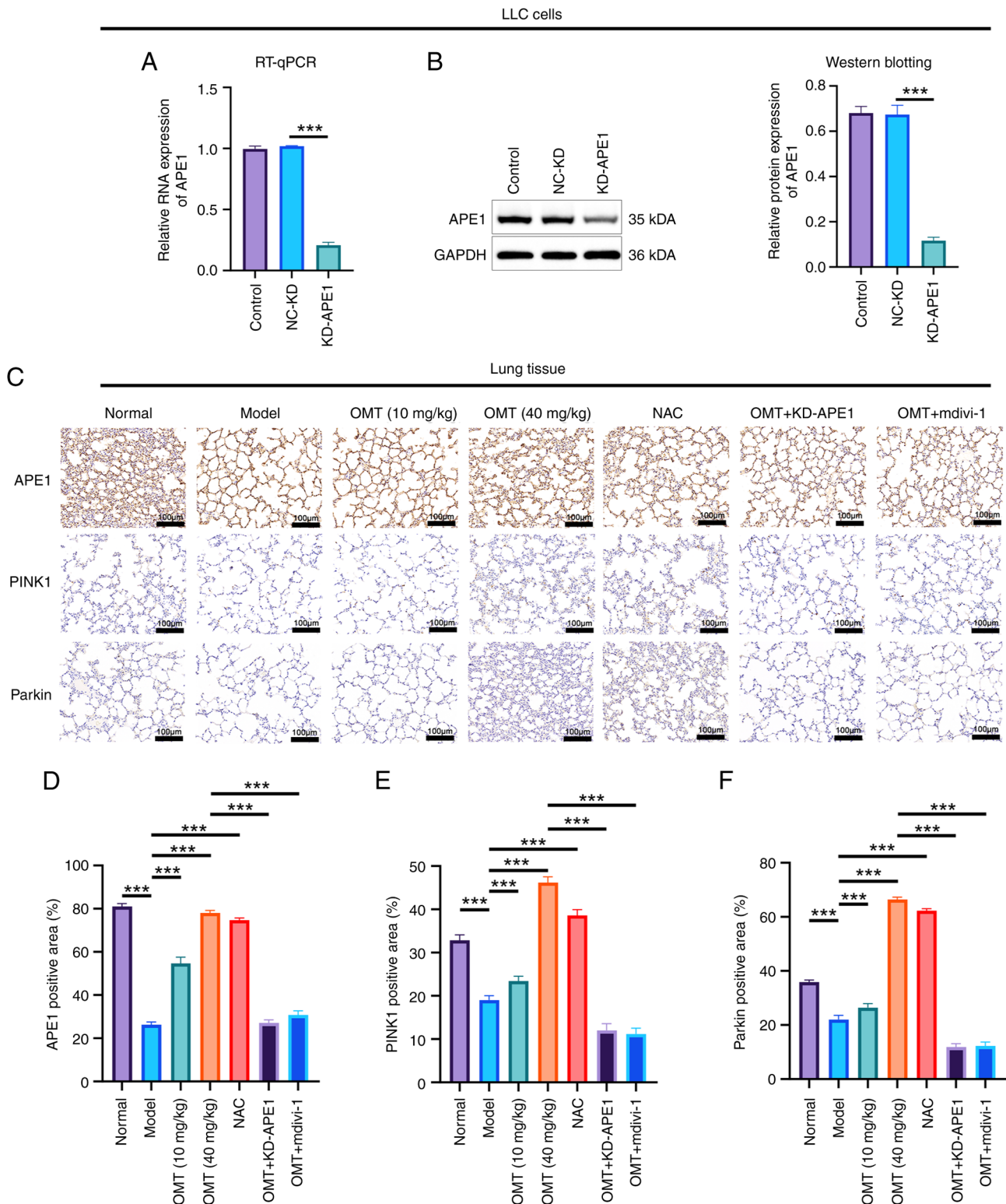


Figure 2. OMT mitigates PF by activating the APE1 and PINK1/Parkin pathways, and promoting autophagy and mitochondrial function restoration. (A) RT-qPCR of the relative expression of *APE1* mRNA following *APE1* silencing in LLC cells. (B) Western blotting of the relative expression of APE1 protein following *APE1* silencing in LLC cells. (C) IHC staining of APE1, PINK1 and Parkin expression in the lung tissue of C57BL/6 mice (scale bar, 100 μ m). Semi-quantitative analysis of (D) APE1, (E) PINK1 and (F) Parkin IHC staining. Data are presented as the mean $\pm$ SD. Statistical significance was analyzed using one-way ANOVA. *** P <0.001. OMT, oxymatrine; NAC, N-acetylcysteine; APE1, apurinic/apyrimidinic endonuclease-1; PINK1, PTEN-induced kinase 1; KD, knockdown; mdivi-1 mitophagy inhibitor mitochondrial division inhibitor-1, RT-qPCR, reverse transcription-quantitative PCR; LC-3, microtubule-associated proteins 1A/1B light chain 3; TOM20, outer membrane translocase 20; NC, negative control; IHC, immunohistochemical.

apoptosis. Immunohistochemistry results also revealed that the expression levels of α -SMA and COL1a1 were significantly increased in the OMT + mdivi-1 group

compared with those in the 40 mg/kg OMT group (P <0.001; Fig. 5E, F, J and K), indicating that mdivi-1 counteracted the effects of OMT.

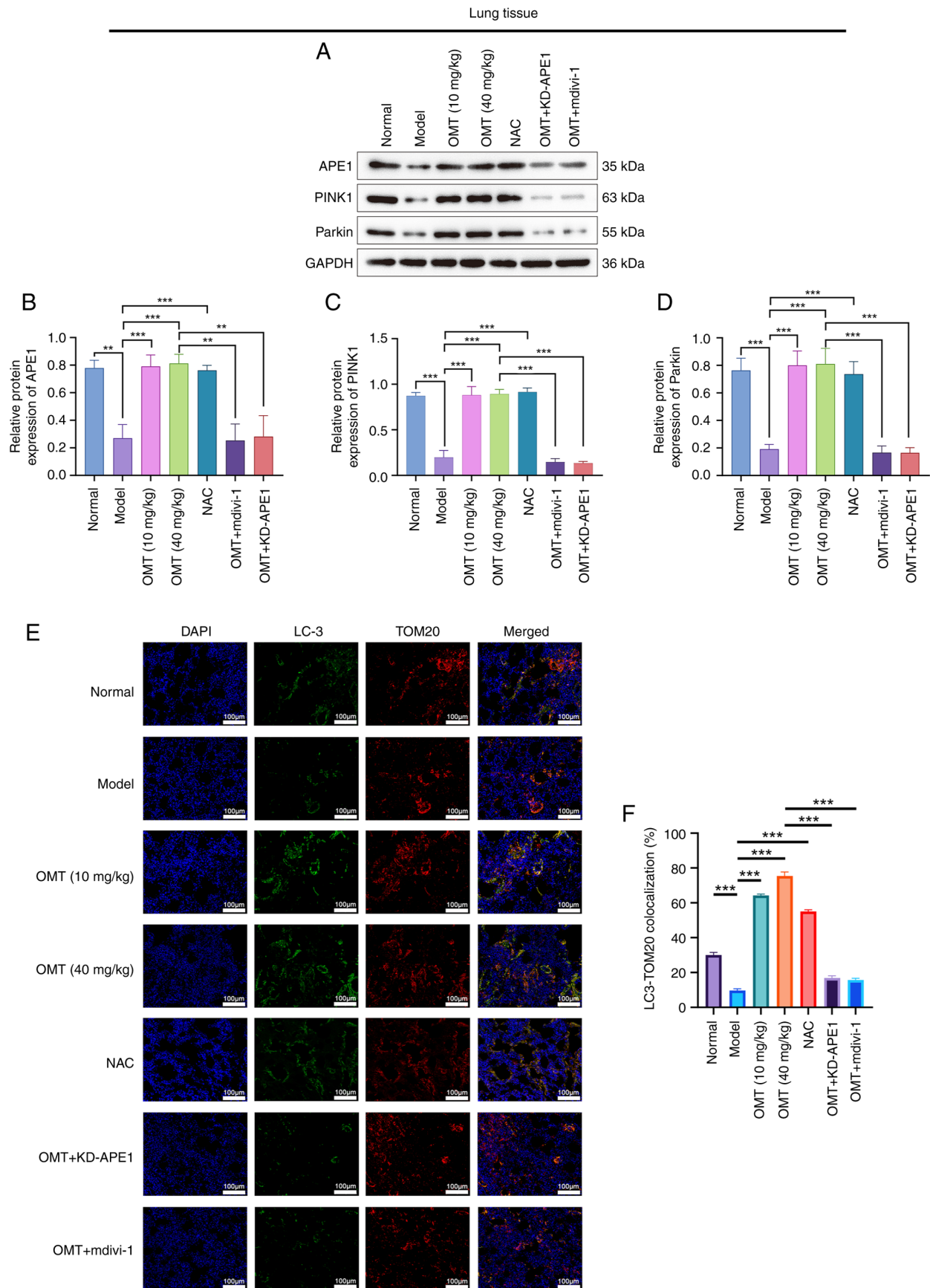


Figure 3. OMT regulates APE1 and mitophagy-related proteins and promotes LC3/TOM20 colocalization. (A) Western blotting of the relative expression levels of APE1, PINK1 and Parkin proteins in the lung tissue of C57BL/6 mice. Semi-quantitative analysis of (B) APE1, (C) PINK1 and (D) Parkin. (E) Immunofluorescence analysis of LC-3 and TOM20 expression and colocalization in the lung tissue of C57BL/6 mice (scale bar, 100 μ m). (F) Percentage of the yellow fluorescent colocalization area of LC-3 and TOM20. Data are presented as the mean $\pm$ SD. Statistical significance was analyzed using one-way ANOVA; n=6. **P<0.01, ***P<0.001. OMT, oxymatrine; NAC, N-acetylcysteine; APE1, apurinic/apyrimidinic endonuclease-1; PINK1, PTEN-induced kinase 1; KD, knockdown; mdivi-1 mitophagy inhibitor mitochondrial division inhibitor-1.

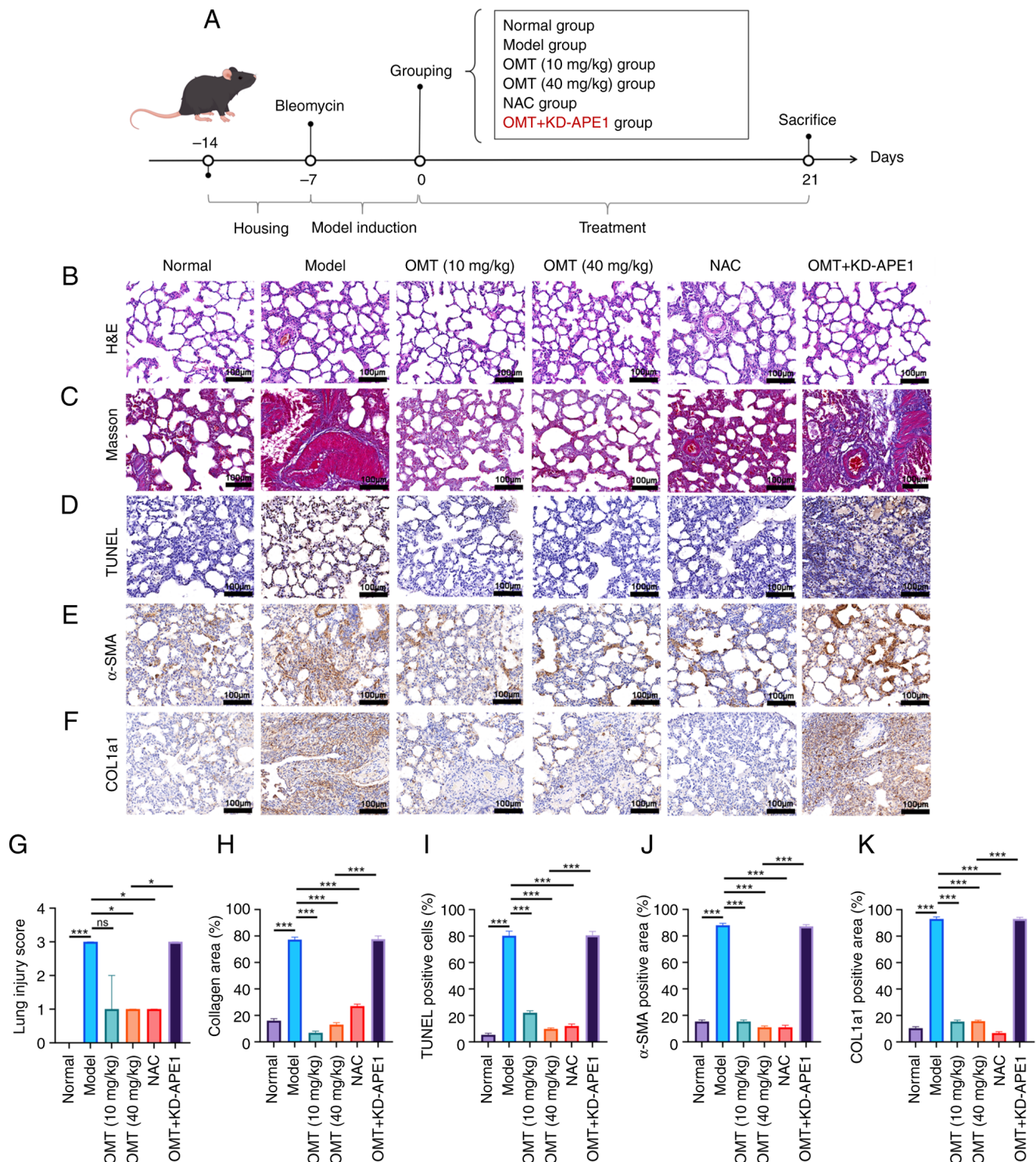


Figure 4. Silencing *APE1* reverses the antifibrotic effects of OMT on bleomycin-induced PF. (A) Schematic diagram of the treatment protocol incorporating the OMT + KD-*APE1* intervention group. (B) H&E staining was used to observe and evaluate lung tissue structure and morphology. (C) Masson's trichrome staining was performed to assess PF. (D) TUNEL staining was used to detect apoptosis. Immunohistochemical staining was conducted to detect the expression levels of (E) α -SMA and (F) COL1a1. Semi-quantitative analysis of (G) H&E, (H) Masson's, (I) TUNEL, and IHC staining of (J) α -SMA and (K) COL1a1. Scale bar, 100 μ m. (G) Data are presented as the median and IQR, and statistical significance was analyzed using the Kruskal-Wallis test. (H-K) Data are presented as the mean $\pm$ SD, and statistical significance was analyzed using one-way ANOVA; n=6. *P<0.05, ***P<0.001. The treatment schematic was designed using Figdraw (<https://www.figdraw.com/>). PF, pulmonary fibrosis; OMT, oxymatrine; NAC, N-acetylcysteine; α -SMA, α -smooth muscle actin; COL1a1, collagen type I α 1; KD, knockdown; APE1, apurinic/apyrimidinic endonuclease-1; IHC, immunohistochemical.

Discussion

PF, a progressive and fatal interstitial lung disease, is characterized by persistent inflammation of the alveolar walls

and collagen deposition, leading to structural destruction and functional loss of lung tissue. Myofibroblasts carry out a key role in the pathogenesis of PF by secreting large amounts of extracellular matrix (ECM) proteins, leading to decreased

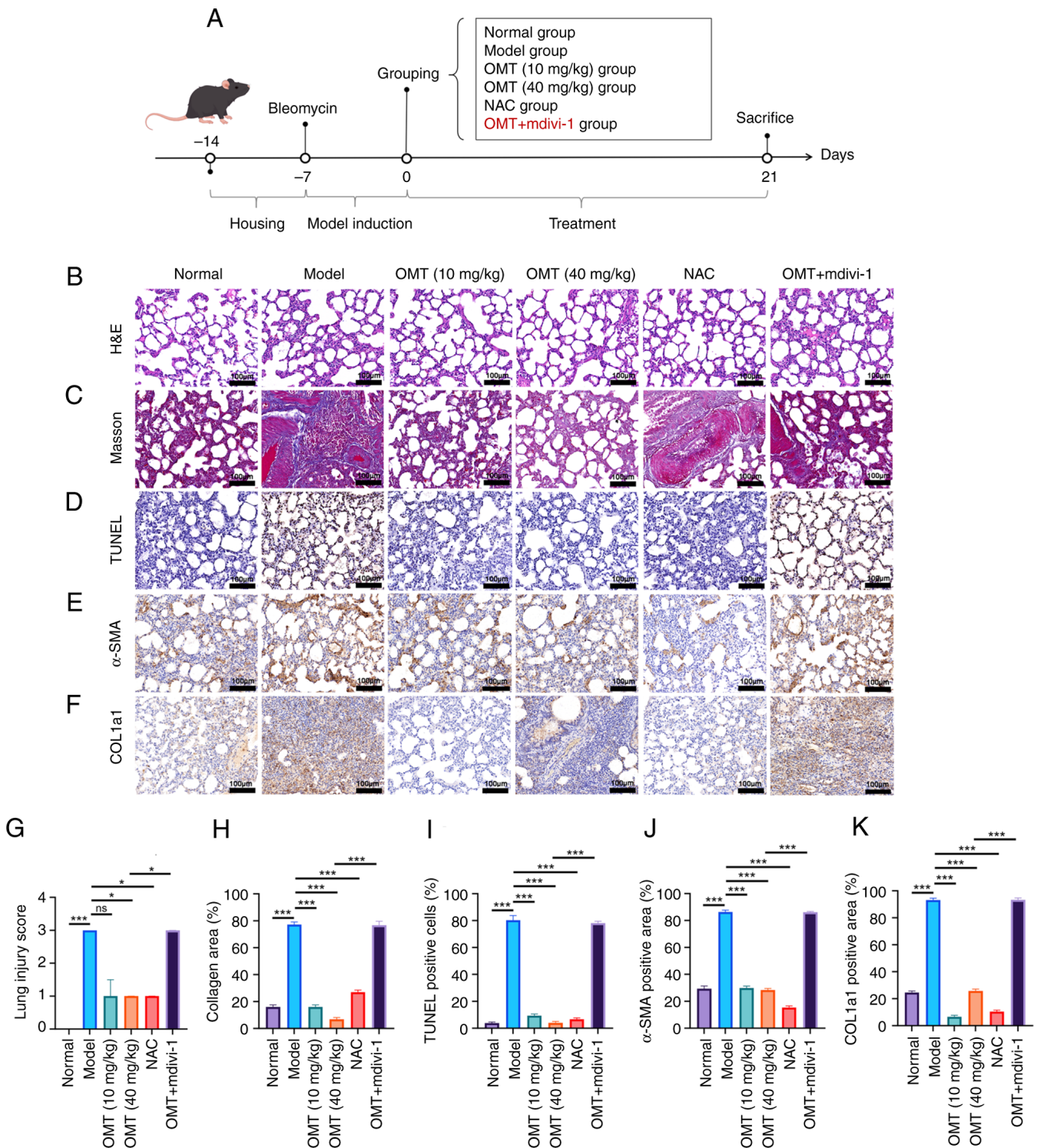


Figure 5. Mitochondrial autophagy inhibitors reverse the effects of OMT against bleomycin-induced PF. (A) Schematic diagram of the treatment protocol incorporating the OMT + mdivi-1 intervention group. (B) H&E staining was used to observe and evaluate lung tissue structure and morphology. (C) Masson's trichrome staining was performed to assess PF. (D) TUNEL staining was used to detect apoptosis. IHC staining was conducted to detect the expression levels of (E) α -SMA and (F) COL1a1. Semi-quantitative analysis of (G) H&E, (H) Masson's, (I) TUNEL, and IHC staining of (J) α -SMA and (K) COL1a1. Scale bar, 100 μ m. (G) Data are presented as the median and IQR, and statistical significance was analyzed using the Kruskal-Wallis test. (H-K) Data are presented as the mean $\pm$ SD, statistical significance was analyzed using one-way ANOVA; n=6. *P<0.05, ***P<0.001. The treatment schematic was designed using Figdraw (<https://www.figdraw.com/>). PF, pulmonary fibrosis; OMT, oxymatrine; NAC, N-acetylcysteine; α -SMA, α -smooth muscle actin; COL1a1, collagen type I α 1; KD, knockdown; APE1, apurinic/apyrimidinic endonuclease-1; IHC, immunohistochemical.

lung elasticity and function (33). α -SMA and COL1a1 are indicators of fibroblast activation and ECM deposition, and are commonly used as biomarkers for detecting PF (34). Andugulapati *et al* (35) revealed that fibrosis-related genes

(TGF- β 1, α -SMA, Twist-1, SNAIL, COL3A1 and CTGF) are upregulated in LL29 and diseased (IPF) human lung fibroblast cells (both lung fibroblasts from patient with IPF). Bleomycin, an antineoplastic drug, induces PF by promoting

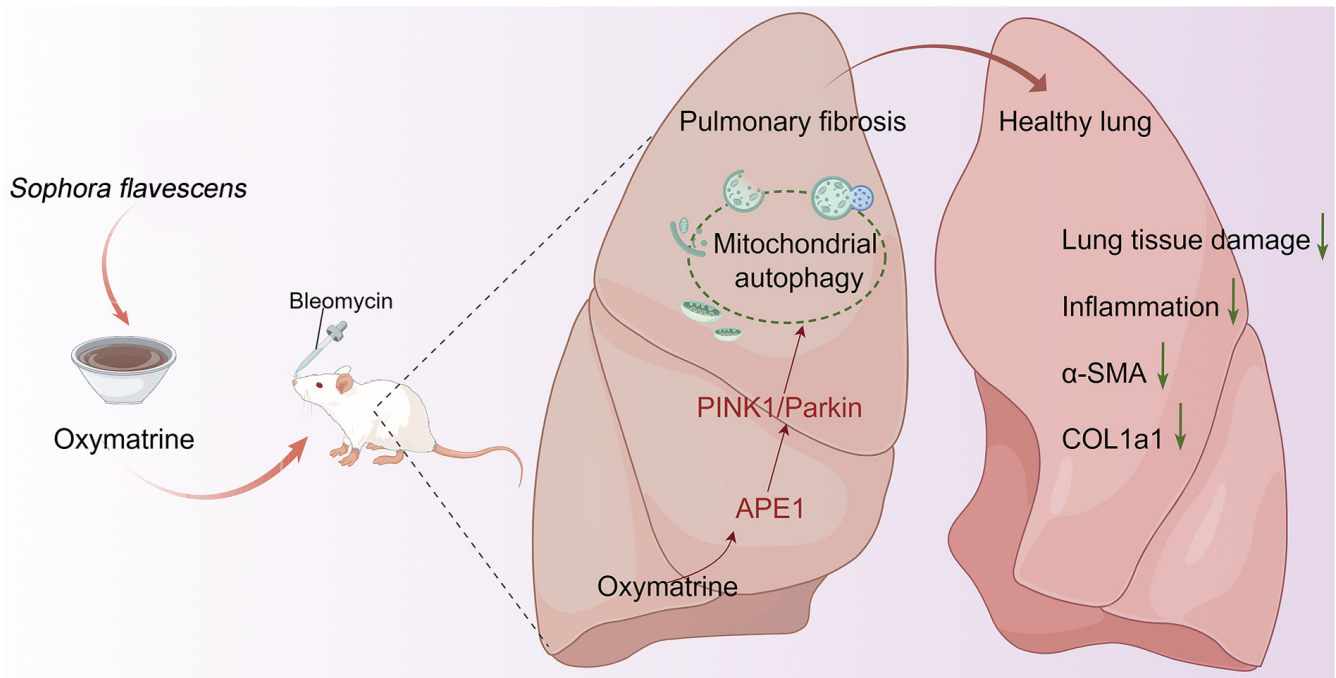


Figure 6. Mechanism by which oxymatrine alleviates pulmonary fibrosis by upregulating APE1, activating the PINK1/Parkin pathway and promoting mitochondrial autophagy. The mechanism diagram was self-designed by the authors using Figdraw (<https://www.figdraw.com/>). α -SMA, α -smooth muscle actin; COL1a1, collagen type I α 1; APE1, apurinic/aprimidinic endonuclease-1; PINK1, PTEN-induced kinase 1.

fibroblast proliferation and increasing ECM production (36). Increased alveolar septal thickening and excessive alveolar space collapse, accompanied by fibroblast proliferation, have previously been observed in a bleomycin-induced PF model, whereas biochanin-A was shown to reverse this phenomenon (35). The present study revealed consistent results using various types of staining. Additionally, TUNEL staining revealed an increase in positive cells in the lung tissue of bleomycin-treated mice, indicating elevated levels of apoptosis. OMT, an alkaloid extracted from *Sophora flavescens*, has demonstrated potential therapeutic effects in hepatic and renal fibrotic diseases (37,38). Studies have revealed that OMT is a natural compound that regulates the autophagy network in various contexts (39,40). In scar repair research, OMT has been shown to reduce the activity and collagen metabolism of human scar fibroblasts by inhibiting autophagy, markedly downregulating the expression of COL1 and α -SMA (41). In the present study, OMT treatment reduced the elevated levels of α -SMA and COL1a1 induced by bleomycin and improved lung injury and tissue apoptosis.

Bleomycin treatment stimulates differentiation, proliferation, ECM production and collagen deposition in lung tissue through classical pathways such as the PI3K/Akt signaling pathway (42,43). In the present study, following bleomycin-induced PF in mice, the expression of PINK1 and Parkin was significantly decreased and LC-3-labeled autophagosomes were reduced. During mitochondrial damage, PINK1 accumulates on the outer mitochondrial membrane, forming high molecular weight complexes with the TOM complex. Subsequently, PINK1 phosphorylates ubiquitin, recruiting and activating Parkin. This process targets damaged mitochondria for autophagosomes, which are then degraded and recycled by the autophagy mechanisms of the cell (44,45). The

PINK1/Parkin pathway is key for maintaining cellular energy homeostasis and responding to cellular stress (46). Bleomycin interferes with this pathway, inhibiting mitophagy and potentially exacerbating PF. OMT treatment significantly enhanced the expression of PINK1 and Parkin, as detected by western blotting. However, the antifibrotic effects of OMT were reversed upon treatment with the mitophagy inhibitor mdivi-1, suggesting that mitophagy may be an important mechanism underlying the antifibrotic action of OMT.

APE1, a multifunctional protein, serves a key role in DNA repair and transcriptional regulation. Its primary functions include repairing DNA damage during base excision repair and regulating transcription factors through redox activity (47). APE1 exerts a protective effect in acute lung injury by inhibiting ferroptosis and promoting autophagy through nuclear factor erythroid 2-related factor 2 regulation. Intervention in APE1 activity can considerably alleviate liver injury, reduce oxidative stress and promote the expression of protective proteins (48). Furthermore, APE1 has a key role in maintaining mitochondrial function. The absence of APE1 leads to the accumulation of damaged mitochondrial mRNA, thereby affecting mitochondrial protein translation and ultimately reducing mitochondrial respiratory function (11). Li *et al* (49) demonstrated that APE1 overexpression can enhance mitophagy, promoting cisplatin resistance in lung cancer cells through Parkin-dependent mitophagy. Tang *et al* (50) reported similar findings, demonstrating that APE1 overexpression protects against cardiac ischemia/reperfusion injury by inducing PINK1/Parkin-mediated mitophagy. In the present study, APE1 expression was considerably decreased following bleomycin-induced PF in mice, whereas OMT treatment markedly enhanced APE1 expression. However, silencing APE1 led to a pronounced decrease in PINK1 and Parkin expression,

reduced LC-3-labeled autophagosomes and reversed the anti-fibrotic effects of OMT. The potential mechanistic pathway is illustrated in Fig. 6. Thus, APE1 may be a key target for the antifibrotic effects of OMT, exerting a protective effect against PF by regulating mitochondrial function through the PINK1/Parkin pathway.

Despite these promising findings, the current study has several limitations. Firstly, it primarily relies on animal models, which may not fully recapitulate the pathological mechanisms and therapeutic responses of human diseases. Moreover, the dose-response relationship and potential side effects of OMT require further investigation and validation before clinical application. Additionally, a negative control group for *APE1* silencing was not included in the animal experiments, which limits the ability to fully exclude potential effects from the vector used.

In conclusion, the present study not only demonstrated the efficacy of OMT against bleomycin-induced PF in mice but also preliminarily revealed its potential mechanism of action through the APE1-mediated PINK1/Parkin pathway. Future studies should focus on exploring the clinical application potential and safety of OMT, aiming to provide more therapeutic options for patients with PF.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

WX conceived and designed the study, carried out the investigation, administered the project, validated the results and wrote the main manuscript text. TX and BZ conducted the formal analysis, validated the results and prepared Figures 1-3. JZ and LZ performed data collection, statistical analysis and visualization using GraphPad Prism 8, and prepared Figures 4 and 5. YZ interpreted the data and prepared Figure 6. YD conceived and supervised the study, acquired funding and contributed to writing, reviewing and editing the manuscript. WX and YD confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The animal experiments were approved by the Ethics Committee of Hainan General Hospital (approval no. Med-Eth-Re [2024]718). The present study also adhered to the ARRIVE guidelines.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Koudstaal T, Funke-Chambour M, Kreuter M, Molyneaux PL and Wijsenbeek MS: Pulmonary fibrosis: From pathogenesis to clinical decision-making. *Trends Mol Med* 29: 1076-1087, 2023.
- Ishida Y, Kuninaka Y, Mukaida N and Kondo T: Immune mechanisms of pulmonary fibrosis with bleomycin. *Int J Mol Sci* 24: 3149, 2023.
- Selvarajah B, Platé M and Chambers RC: Pulmonary fibrosis: Emerging diagnostic and therapeutic strategies. *Mol Aspects Med* 94: 101227, 2023.
- Kim MJ, Yang J and Song JW: Acute exacerbation of progressive pulmonary fibrosis: incidence and outcomes. *Respir Res* 25: 415, 2024.
- Wang Y, Ji Z, Xu B, Li S and Xie Y: The incidence of acute exacerbation of idiopathic pulmonary fibrosis: A systematic review and Meta-analysis. *Sci Rep* 14: 21080, 2024.
- Lee H and Yoon HY: Association between inhaled corticosteroids and incidence of idiopathic pulmonary fibrosis: Nationwide population-based study. *BMJ Open Respir Res* 12: e002566, 2025.
- Aggarwal K, Arora S and Nagpal K: Pulmonary fibrosis: Unveiling the pathogenesis, exploring therapeutic targets, and advancements in drug delivery strategies. *AAPS PharmSciTech* 24: 152, 2023.
- Diwan R, Bhatt HN, Beaven E and Nurunnabi M: Emerging delivery approaches for targeted pulmonary fibrosis treatment. *Adv Drug Deliv Rev* 204: 115147, 2024.
- Otoupalova E, Smith S, Cheng G and Thannickal VJ: Oxidative stress in pulmonary fibrosis. *Compr Physiol* 10: 509-547, 2020.
- Mattoo H, Bangari DS, Cummings S, Humulock Z, Habel D, Xu EY, Pate N, Resnick R, Savova V, Qian G, *et al*: Molecular features and stages of pulmonary fibrosis driven by type 2 inflammation. *Am J Respir Cell Mol Biol* 69: 404-421, 2023.
- Barchiesi A, Bazzani V, Jabczynska A, Borowski LS, Oeljeklaus S, Warscheid B, Chacinska A, Szczesny RJ and Vascotto C: DNA Repair protein APE1 degrades dysfunctional abasic mRNA in mitochondria affecting oxidative phosphorylation. *J Mol Biol* 433: 167125, 2021.
- Peng L, Liu Y, Chen J, Cheng M, Wu Y, Chen M, Zhong Y, Shen D, Chen L and Ye X: APEX1 regulates alternative splicing of key tumorigenesis genes in non-small-cell lung cancer. *BMC Med Genomics* 15: 147, 2022.
- Long K, Gu L, Li L, Zhang Z, Li E, Zhang Y, He L, Pan F, Guo Z and Hu Z: Small-molecule inhibition of APE1 induces apoptosis, pyroptosis, and necroptosis in non-small cell lung cancer. *Cell Death Dis* 12: 503, 2021.
- Zhang Z, Lin Y, Pan X and Chen S: Inhibition of Non-small cell lung cancer metastasis by knocking down APE1 through regulating myeloid-derived suppressor cells-induced immune disorders. *Aging (Albany NY)* 16: 10435-10445, 2024.
- Huan DQ, Hop NQ and Son NT: Oxymatrine: A current overview of its health benefits. *Fitoterapia* 168: 105565, 2023.
- Lan X, Zhao J, Zhang Y, Chen Y, Liu Y and Xu F: Oxymatrine exerts organ- and tissue-protective effects by regulating inflammation, oxidative stress, apoptosis, and fibrosis: From bench to bedside. *Pharmacol Res* 151: 104541, 2020.
- Feng T, Duan R, Zheng P, Qiu J, Li Q and Li W: Oxymatrine inhibits TGF- β 1-mediated mitochondrial apoptotic signaling in alveolar epithelial cells via activation of PI3K/AKT signaling. *Exp Ther Med* 25: 198, 2023.
- Pokharel MD, Garcia-Flores A, Marciano D, Franco MC, Fineman JR, Aggarwal S, Wang T and Black SM: Mitochondrial network dynamics in pulmonary disease: Bridging the gap between inflammation, oxidative stress, and bioenergetics. *Redox Biol* 70: 103049, 2024.
- Tong Z, Du X, Zhou Y, Jing F, Ma J, Feng Y, Lou S, Wang Q and Dong Z: Drp1-mediated mitochondrial fission promotes pulmonary fibrosis progression through the regulation of lipid metabolic reprogramming by ROS/HIF-1 α . *Cell Signal* 117: 111075, 2024.

20. Li H, Dai X, Zhou J, Wang Y, Zhang S, Guo J, Shen L, Yan H and Jiang H: Mitochondrial dynamics in pulmonary disease: Implications for the potential therapeutics. *J Cell Physiol* 239: e31370, 2024.
21. Huang T, Lin R, Su Y, Sun H, Zheng X, Zhang J, Lu X, Zhao B, Jiang X, Huang L, *et al*: Efficient intervention for pulmonary fibrosis via mitochondrial transfer promoted by mitochondrial biogenesis. *Nat Commun* 14: 5781, 2023.
22. Liu J, Wang J, Xiong A, Zhang L, Zhang Y, Liu Y, Xiong Y, Li G and He X: Mitochondrial quality control in lung diseases: Current research and future directions. *Front Physiol* 14: 1236651, 2023.
23. Liang L, Sun W, Wei X, Wang L, Ruan H, Zhang J, Li S, Zhao B, Li M, Cai Z and Huang J: Oxymatrine suppresses colorectal cancer progression by inhibiting NLRP3 inflammasome activation through mitophagy induction in vitro and in vivo. *Phytother Res* 37: 3342-3362, 2023.
24. National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals: The National Academies Collection: Reports funded by National Institutes of Health. In: *Guide for the Care and Use of Laboratory Animals*. National Academies Press, National Academy of Sciences, Washington, DC, 2011.
25. Percie du Sert N, Hurst V, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, *et al*: The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol* 18: e3000410, 2020.
26. Guo B, Liu W, Ji X, Xi B, Meng X, Xie W, Sun Y, Zhang M, Liu P, Zhang W, *et al*: CSF3 aggravates acute exacerbation of pulmonary fibrosis by disrupting alveolar epithelial barrier integrity. *Int Immunopharmacol* 135: 112322, 2024.
27. Peng J, Wang Q, Guo M, Liu C, Chen X, Tao L, Zhang K and Shen X: Development of inhalable Chitosan-Coated oxymatrine liposomes to alleviate RSV-Infected Mice. *Int J Mol Sci* 23: 15909, 2022.
28. Deng W, Zhang Y, Fang P, Shi H and Yang S: Silencing lncRNA Snhg6 mitigates bleomycin-induced pulmonary fibrosis in mice via miR-26a-5p/TGF- β 1-smads axis. *Environ Toxicol* 37: 2375-2387, 2022.
29. Pang D and Laferriere C: Review of intraperitoneal injection of sodium pentobarbital as a method of euthanasia in laboratory rodents. *J Am Assoc Lab Anim Sci* 59: 346, 2020.
30. Shariati S, Kalantar H, Pashmforoosh M, Mansouri E and Khodayar MJ: Epicatechin protective effects on bleomycin-induced pulmonary oxidative stress and fibrosis in mice. *Biomed Pharmacother* 114: 108776, 2019.
31. Dong L, Wang Y, Zheng T, Pu Y, Ma Y, Qi X, Zhang W, Xue F, Shan Z, Liu J, *et al*: Hypoxic hUCMSC-derived extracellular vesicles attenuate allergic airway inflammation and airway remodeling in chronic asthma mice. *Stem Cell Res Ther* 12: 4, 2021.
32. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408, 2001.
33. Chen G, Li J, Liu H, Zhou H, Liu M, Liang D, Meng Z, Gan H, Wu Z, Zhu X, *et al*: Cepharanthine ameliorates pulmonary fibrosis by inhibiting the NF- κ B/NLRP3 pathway, fibroblast-to-myofibroblast transition and inflammation. *Molecules* 28: 753, 2023.
34. Zhang J, Zhang Y, Chen Q, Qi Y and Zhang X: The XPO1 inhibitor selinexor ameliorates bleomycin-induced pulmonary fibrosis in mice via GBP5/NLRP3 inflammasome signaling. *Int Immunopharmacol* 130: 111734, 2024.
35. Andugulapati SB, Gourishetti K, Tirunavalli SK, Shaikh TB and Sistla R: Biochanin-A ameliorates pulmonary fibrosis by suppressing the TGF- β mediated EMT, myofibroblasts differentiation and collagen deposition in in vitro and in vivo systems. *Phytotherapy* 78: 153298, 2020.
36. Hamidi N, Feizi F, Azadmehr A, Zabihi E, Khafri S, Zarei-Behjani Z and Babazadeh Z: Disulfiram ameliorates bleomycin induced pulmonary inflammation and fibrosis in rats. *Biotech Histochem* 98: 584-592, 2023.
37. Yang Y, Sun M, Li W, Liu C, Jiang Z, Gu P, Li J, Wang W, You R, Ba Q, *et al*: Rebalancing TGF- β /Smad7 signaling via Compound kushen injection in hepatic stellate cells protects against liver fibrosis and hepatocarcinogenesis. *Clin Transl Med* 11: e410, 2021.
38. Xiao Y, Peng C, Xiao Y, Liang D, Yuan Z, Li Z, Shi M, Wang Y, Zhang F and Guo B: Oxymatrine inhibits Twist-mediated renal tubulointerstitial fibrosis by upregulating Id2 expression. *Front Physiol* 11: 599, 2020.
39. Lu J, Bian J, Wang Y, Zhao Y, Zhao X, Wang G and Yang J: Oxymatrine protects articular chondrocytes from IL-1 β -induced damage through autophagy activation via AKT/mTOR signaling pathway inhibition. *J Orthop Surg Res* 19: 178, 2024.
40. Maharajan N, Lee CM, Vijayakumar KA and Cho GW: Oxymatrine improves oxidative Stress-induced senescence in HT22 cells and mice via the activation of AMP-activated protein kinase. *Antioxidants (Basel)* 12: 2078, 2023.
41. Deng X, Zhao F, Zhao D, Zhang Q, Zhu Y, Chen Q, Qiang L, Xie N, Ma J, Pan X, *et al*: Oxymatrine promotes hypertrophic scar repair through reduced human scar fibroblast viability, collagen and induced apoptosis via autophagy inhibition. *Int Wound J* 19: 1221-1231, 2022.
42. Pan L, Cheng Y, Yang W, Wu X, Zhu H, Hu M, Zhang Y and Zhang M: Nintedanib ameliorates Bleomycin-induced pulmonary fibrosis, inflammation, apoptosis, and oxidative stress by modulating PI3K/Akt/mTOR pathway in mice. *Inflammation* 46: 1531-1542, 2023.
43. Xu Y, Wang X, Han D, Wang J, Luo Z, Jin T, Shi C, Zhou X, Lin L and Shan J: Revealing the mechanism of Jiegeng decoction attenuates bleomycin-induced pulmonary fibrosis via PI3K/Akt signaling pathway based on lipidomics and transcriptomics. *Phytotherapy* 102: 154207, 2022.
44. Wang S, Long H, Hou L, Feng B, Ma Z, Wu Y, Zeng Y, Cai J, Zhang DW and Zhao G: The mitophagy pathway and its implications in human diseases. *Signal Transduct Target Ther* 8: 304, 2023.
45. Eldeeb MA, Bayne AN, Fallahi A, Goiran T, MacDougall EJ, Soumbasis A, Zorca CE, Tabah JJ, Thomas RA, Karpilovsky N, *et al*: Tom20 gates PINK1 activity and mediates its tethering of the TOM and TIM23 translocases upon mitochondrial stress. *Proc Natl Acad Sci USA* 121: e2313540121, 2024.
46. Poole LP and Macleod KF: Mitophagy in tumorigenesis and metastasis. *Cell Mol Life Sci* 78: 3817-3851, 2021.
47. Li J, Zhao H, McMahon A and Yan S: APE1 assembles biomolecular condensates to promote the ATR-Chk1 DNA damage response in nucleolus. *Nucleic Acids Res* 50: 10503-10525, 2022.
48. Diao J, Fan H, Zhang J, Fu X, Liao R, Zhao P, Huang W, Huang S, Liao H, Yu J, *et al*: Activation of APE1 modulates Nrf2 protected against acute liver injury by inhibit hepatocyte ferroptosis and promote hepatocyte autophagy. *Int Immunopharmacol* 128: 111529, 2024.
49. Li Z, Wang Y, Wu L, Dong Y, Zhang J, Chen F, Xie W, Huang J and Lu N: Apurinic endonuclease 1 promotes the cisplatin resistance of lung cancer cells by inducing Parkin-mediated mitophagy. *Oncol Rep* 42: 2245-2254, 2019.
50. Tang W, Lin D, Chen M, Li Z, Zhang W, Hu W and Li F: PTEN-mediated mitophagy and APE1 overexpression protects against cardiac hypoxia/reoxygenation injury. *In Vitro Cell Dev Biol Anim* 55: 741-748, 2019.



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