Tartrate-resistant acid phosphatase 5 serves as a viable target against pulmonary fibrosis by modulating β -catenin signaling

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Abstract

Idiopathic pulmonary fibrosis (IPF) is a fatal interstitial lung disease with limited therapeutic options. Tartrate-resistant acid phosphatase 5 (ACP5) performs a variety of functions. However, its role in IPF remains unclear. Here, we demonstrated that the levels of ACP5 were increased in IPF patient samples and mice with bleomycin (BLM)-induced pulmonary fibrosis. In particular, higher levels of ACP5 were noted in the sera of IPF patients with a diffusing capacity of the lungs for carbon monoxide (DLCO) less than 40% of the predicted value. Additionally, *Acp5* deficiency protected mice from BLM-induced lung injury and fibrosis and reduced the differentiation and proliferation of fibroblasts. Mechanistic studies revealed that *Acp5* was upregulated by TGF- β 1 in a TGF β R1/Smad3-dependent manner, after which *Acp5* dephosphorylated p- β -catenin at Ser33 and Thr41, inhibiting the degradation of β -catenin and subsequently enhancing β -catenin signaling in the nucleus, which promoted the differentiation and proliferation of fibroblast. Notably, the treatment of mice with BLM-induced fibrosis with *Acp5* siRNA-loaded liposomes robustly reversed lung fibrosis. Collectively, these data indicate that *Acp5* plays an essential role in the initiation and progression of pulmonary fibrosis; therefore, strategies aimed at silencing *Acp5* could be novel therapeutic approaches against pulmonary fibrosis in a clinical setting.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a fatal interstitial lung disease characterized by the deposition of excessive extracellular matrix (ECM), destruction of the lung parenchyma and a pattern of usual interstitial pneumonia (UIP) by radiology and histopathology¹. Although the development of new antifibrotic agents (pirfenidone and nintedanib) has improved patient wellbeing, the incidence and mortality rate of IPF have barely improved, resulting in a median survival time following diagnosis of only 3 to 5 years^{2,3}. Therefore, exploration of the pathogenesis of IPF and the discovery of new therapeutic methods for all IPF patients are urgently needed.

According to the current paradigm, the main pathological features of IPF include epithelial injury⁴, the recruitment of inflammatory cells⁵, the aberrant differentiation and proliferation of fibroblasts⁶ and the persistence of apoptosis-resistant myofibroblasts⁷ in fibrotic lesions. Resident lung fibroblast-derived myofibroblasts are the major contributors to the processes of ECM deposition and tissue distortion in IPF^{8,9}. Under stimulation with fibrotic factors, such as transforming growth factor- β (TGF- β)¹⁰, platelet-derived growth factor (PDGF)¹¹ and connective tissue growth factor (CTGF)¹², resident fibroblasts in the lung lesion transform into myofibroblasts, which are characterized by a spindle or stellate morphology with α -smooth muscle actin (α -SMA) stress fibers coupled with a hypersecretion phenotype due to which they produce copious amounts of fibrillary ECM proteins, such as collagen and fibronectin.

Tartrate-Resistant Acid Phosphatase 5 (ACP5), also named purple acid phosphatase, which maps to chromosome 19p13.2, contains a binuclear iron center¹³. Previous studies, including ours, have demonstrated that ACP5 plays a critical role in the pathogenesis of tumors, such as lung

adenocarcinoma¹⁴, colorectal cancer¹⁵, breast cancer¹⁶ and hepatocellular carcinoma¹⁷. Given that IPF and cancer share several pathogenic pathways^{18,19}, we hypothesize that ACP5 is involved in the pathogenesis of IPF.

To assess the feasibility of this hypothesis, we first detected the expression of ACP5 in IPF patients and mice with bleomycin (BLM)-induced pulmonary fibrosis. Notably, the levels of ACP5 in the sera of IPF patients were positively correlated with disease severity. Furthermore, the loss of *Acp5* significantly protected mice from BLM-induced lung injury and fibrosis, accompanied by a marked reduction in myofibroblast accumulation in the lung. Mechanistically, *Acp5*, a kind of phosphatase, selectively bound and dephosphorylated β -catenin at Ser33 and Thr41 in the cytoplasm and then reduced the degradation of β -catenin, which enhanced the levels of β -catenin in the nucleus to promote fibroblast differentiation, proliferation and migration. Therefore, intratracheal administration of liposomes carrying *Acp5* siRNA significantly protected mice from BLM-induced pulmonary fibrosis. Collectively, our data support the notion that ACP5 plays a critical role in the progression of pulmonary fibrosis; therefore, strategies aimed at silencing *ACP5* could be viable therapies against pulmonary fibrosis in clinical settings.

Material And Methods

Human samples

Sera from patients with IPF (n = 20) and control subjects (n = 13), lung explant material from IPF patients (n = 3) and resected para-carcinoma lung tissues from non-small-cell lung cancer (NSCLC) patients (n = 3) were collected at Tongji Hospital with informed consent. An IPF diagnosis was made according to consensus diagnostic criteria from the European Respiratory Society (ERS)/American Thoracic Society (ATS)²⁰. The experiments were approved by the Human Assurance Committee of Tongji Hospital. Clinical data from IPF patients and control subjects are provided in table 1.

Measurement of Serum ACP5 Levels

To detect ACP5 in the sera of the IPF patients and control subjects, an ACP5 ELISA kit (Hycult Biotechnology, Uden, The Netherlands) was used in accordance with the manufacturer's protocol. Briefly, whole-blood samples originating from IPF patients and control subjects were centrifuged at 3000 rpm for 15 minutes. Then, the serum samples were frozen at -80°C. Each sample was assayed in duplicate. ELISA plates were scanned on a microtiter plate reader (ELx800, BioTek Instruments, Inc., Winooski, VT) at 450 nm. Levels of ACP5 were calculated according to a standard curve.

Immunofluorescence staining

Cryosections of lung tissues from IPF patients and mice with the onset of BLM-induced pulmonary fibrosis were used for immunofluorescence staining performed as previously reported²¹. The primary antibodies used for staining were as follows: mouse anti-ACP5 (Abnova, Taipei, China, 1: 100), rabbit anti-ACP5 (Proteintech, Wuhan, China, 1:100), rabbit anti-FSP1 (Proteintech, Wuhan, China, 1:100), mouse

anti- α -SMA (Cell Signaling Technology, MA, USA, 1:100), rabbit anti- β -catenin (Cell Signaling Technology, MA, USA, 1:100), and rabbit anti-phospho-smad3 (Cell Signaling Technology, MA, USA, 1:100). Alexa 488-conjugated anti-mouse and Alexa 594-conjugated anti-rabbit (Abbkine, CA, USA, 1:400) were used as fluorescent secondary antibodies, and nuclei were counterstained with 4'-6-diamidino-2-phenylindole (DAPI). Images were obtained with a fluorescence microscope (Olympus, Shinjuku, Japan).

Animals

Acp5 knockout mice (*Acp5*^{-/-} mice, C57BL/6 background) and wild-type mice (WT, C57BL/6 background) were purchased from GemPharmatech Co., Ltd. (Nanjing, China). *Acp5* depletion was confirmed by genotyping of the tail blood DNA (Supplementary figure 1). Genotyping of *Acp5*^{-/-} mice was performed using the following primers: primers 1: forward primer, 5'-TGC TAC TGG TGT GTC TGT GGA ACT G-3' and reverse primer, 5'-TCA TGT TCA GCA GGA CCT TGC TAA-3' and primers 2: forward primer, 5'-TGC TAC TGG TGT GTC TGT GGA ACT G-3' and reverse primer, 5'-GAT CTC TTT GGC ATT GGC CAT TT-3'. All animals were housed in a specific pathogen-free animal facility at Tongji Hospital under a 12:12 hour light/dark photocycle and provided food and water ad libitum. All experimental procedures were approved by the Animal Care and Use Committee at Tongji Hospital.

BLM-mediated induction of pulmonary fibrosis

Pulmonary fibrosis was induced in male WT and *Acp5*^{-/-} mice (8–10 weeks old) as previously described with minor modifications²². Briefly, the mice were anesthetized with 1% pentobarbital sodium (60 mg/kg) and then intratracheally administered 2 U/kg BLM (Huirui, Shanghai, China) in 40 μ l of sterile saline. Mice administered the same volume of sterile saline served as controls. SiRNA-loaded liposomes were injected into the anesthetized animals via intratracheal injection on days 14 and 18 after BLM injection. The mice were euthanized on day 21 following BLM challenge to analyze pulmonary fibrosis.

Pathological staining and histopathologic assessment

The lungs were removed at 21 days after bleomycin or saline administration. Following fixation, the lungs were embedded in paraffin and sectioned. The sections were then stained with hematoxylin and eosin (H&E), Masson's trichrome stain and Sirius red as previously described²³. Fibrosis was scored on a scale of 0–8 using the Ashcroft scoring method²⁴. The severity of fibrotic changes in each histological section of the lung was assessed as the mean of the severity scores from the observed microscopic fields. Fifty randomly chosen regions from each mouse lung were graded, after which their scores were averaged, and the average scores are depicted in a graph at 200-fold magnification. Grading was performed in a blinded manner by three independent observers.

Hydroxyproline assay

Lung collagen deposition was assessed by measuring the hydroxyproline content of lung homogenates with a hydroxyproline assay kit (BioVision, CA, USA) as previously described²⁵. Briefly, lung tissues were

homogenized in ddH₂O, after which an equal volume of concentrated 12 N HCl was added to the tissues in a pressure-tight, capped Teflon vial and hydrolyzed at 120°C for 3 h. After centrifugation at 14000 rpm for 3 min, 10 µl of each hydrolyzed sample was transferred to a 96-well plate. In each well, 100 µl of chloramine T reagent was added to the sample, which was incubated at room temperature for 5 min, after which 100 µl of DMAB reagent was added. After incubation for 90 min at 60°C, the plates were read at 560 nm with a microtiter plate reader (ELx800, BioTek Instruments, Inc., Winooski, VT), and the hydroxyproline concentration in the sample was calculated from a standard curve and related to the amount of lung tissue used. The hydroxyproline contents in the lung tissues are given as µg of hydroxyproline per mg of lung tissue.

Cell culture and treatment

Primary human pulmonary fibroblasts (PHLFs) were isolated from para-carcinoma lung tissues resected from NSCLC patients, and primary mouse pulmonary fibroblasts (PMLFs) were isolated from the lung tissues of *Acp5*^{-/-} or WT mice as described previously^{24,26}. Briefly, fibroblasts were generated by mincing lung tissue into submillimeter-sized pieces, plated evenly in 100 mm plates containing 2 ml of medium, which was changed after 24 hours. Cells were cultured in DMEM containing 10% fetal bovine serum (FBS) and penicillin/streptomycin at 37°C and tested for mycoplasma regularly. PHLFs and PMLFs between passages 3 and 5 were used.

Cell transfection

Small interfering RNAs (siRNAs) specific for *ACP5* and a corresponding scrambled siRNA were purchased from RiboBio (Guangzhou, China) and then transiently transfected into PHLFs using Lipofectamine 3000 (Invitrogen, Shanghai, China) as previously reported²⁷. Briefly, PHLFs were seeded in 6-well or 96-well plates at 24 h before transfection. SiRNA transfection was performed when the cells reached 50-60% confluence. Transfection efficiency was monitored by RT-PCR or Western blotting at 48 h after transfection. The siRNA specific for *ACP5* targeted the following sequence in *ACP5* mRNA: 5'-GAC ACT ATG TGG CAA CTC A-3'.

ACP5-plasmid and a vector were purchased from GeneChem (Shanghai, China), and 2 µg of purified DNA was mixed with transfection reagent and applied to the cells. Forty-eight hours after transfection, the cells were analyzed by Western blotting.

Real-time PCR

Total RNA was isolated from lung tissues and fibroblasts with TRIzol reagent (Takara, Dalian, China) as previously reported²⁸. The RNA quantity and quality were measured using a NanoDrop 2000 spectrophotometer (Thermo Scientific, MA, USA). Complementary DNA synthesis was performed using an M-MLV reverse transcriptase kit (Invitrogen, CA, USA). Real-time PCR was performed on a CFX96 Real-time PCR detection system (Bio-Rad, CA, USA) using SYBR Green mix (Takara, Dalian, China) under the following conditions: 30 s at 95°C for initial denaturation, followed by 40 cycles of 95°C for 5 s and 60°C

for 30 s. The comparative Ct method was used to normalize the data for presentation as mean normalized transcript levels. The RNA levels of *Actb* were used to normalize the data. The following primer sequences were used: human *FIBRONECTIN* forward, 5'- GAT GTC CGA ACA GCT ATT TAC CA-3' and reverse, 5'- CGA CCA CAT AGG AAG TCC CAG-3'; human *COL1A1* forward, 5'- GAG GGC CAA GAC GAA GAC ATC-3' and reverse, 5'- CAG ATC ACG TCA TCG CAC AAC-3'; human α -SMA forward, 5'-GAC GCT GAA GTA TCC GAT AGA ACA CG-3' and reverse, 5'- CAC CAT CTC CAG AGT CCA GCA CAA T-3'; human *ACTB* forward, 5'-AGC GAG CAT CCC CCA AAG TT-3' and reverse, 5'-GGG CAC GAA GGC TCA TCA TT-3'; mouse *Acp5* forward, 5'-CCT GAG ATT TGT GGC TGT GG-3' and reverse, 5'-TCT TGT CGC TGG CAT CGT G-3'; mouse *Fibronectin* forward, 5'- GAT GTC CGA ACA GCT ATT TAC CA-3' and reverse, 5'-CCT TGC GAC TTC AGC CAC T-3'; mouse *Col1a1* forward, 5'-TAA GGG TCC CCA ATG GTG AGA-3' and reverse, 5'-GGG TCC CTC GAC TCC TAC AT-3'; mouse α -SMA forward, 5'-GGA CGT ACA ACT GGT ATT GTG C-3' and reverse, 5'-TCG GCA GTA GTC ACG AAG GA-3'; mouse β -catenin forward, 5'- TCC CAT CCA CGC AGT TTG AC-3' and reverse, 5'- TCC TCA TCG TTT AGC AGT TTT GT-3'; and mouse *Actb* forward, 5'-GCC ACA GCA CTC CAT CGA C-3' and reverse, 5'-GTC TCC GAT CTG GAA AAC GC-3'.

Western blotting

Lung tissues and fibroblasts were homogenized in RIPA lysis buffer (Beyotime, Shanghai, China). For subcellular fractionation, protein was extracted using NE-PERTM Nuclear and Cytoplasmic Extraction reagents (Thermo Fisher Scientific, USA). Western blotting was performed according to previously reported protocols²⁹. The primary antibodies used were anti-ACP5 (Gentex, CA, USA, 1:1,000); anti-LAMIN B1, anti-FIBRONECTIN and anti-COL1A1 (Proteintech, Wuhan, China, 1:1,000); anti- α -SMA, anti-phospho- β -CATENIN (Ser33/37/Thr41), and anti- β -CATENIN (Cell Signaling Technology, MA, USA, 1: 1000); and anti-GAPDH and anti- β -ACTIN (Abcam, MA, USA, 1:3000). GAPDH bands were used as the loading control for cytoplasmic or total proteins, and LAMIN B1 bands were used as the loading control for nuclear proteins. Detection was performed using a chemiluminescent substrate system (Bio-Rad Laboratories, CA, USA). The gray values were analyzed with ImageJ software.

Cell proliferation assay

PMLFs were cultured in 96-well plates at a density of 2×10^3 cells/well. Cell proliferation was then measured using the EdU proliferation assay (Ribobio, Guangzhou, China) as previously reported²¹. Briefly, 18 h after being seeded in the plates, cells were labeled with EdU for 2 h at 37°C, treated with 100 μ L of Apollo reaction cocktail and stained with 100 μ L of Hoechst 33342. Finally, the cells were observed under a fluorescence microscope (Olympus, Shinjuku, Japan).

Cell migration assay

Cell migration assays were performed using Transwell inserts with a membrane with a pore size of 8.0 mm (Corning, MA, USA) according to previously reported methods³⁰. The cells (2.5×10^4) were resuspended in 2% serum-containing medium and seeded into the upper chambers. The lower chambers

were filled with complete culture medium containing 10% FBS to function as a chemoattractant. After incubation at 37°C for 24 h, the cells migrated through the membrane filter and were stained with 0.1% crystal violet (Sigma-Aldrich, St. Louis, MO, USA). The experiment was performed in triplicate.

Coimmunoprecipitation assay

Immunoprecipitation was performed according to a previous protocol¹⁴. Anti-ACP5 and anti- β -Catenin antibodies were used to form immune complexes with the ACP5 and β -Catenin proteins in lysates that were immunoprecipitated with magnetic beads (Cell Signaling Technology, Danvers, MA, USA). Finally, equivalent protein samples were subjected to Western blot analysis for ACP5 and β -Catenin.

Immunohistochemistry

Immunohistochemistry (IHC) was performed on 3 μ m adjacent lung sections according to the protocol in a previous report³¹. The primary antibodies used were anti-Pcna (Cell Signaling Technology, MA, USA, 1:100) and anti-Fsp1 (Proteintech, Wuhan, China, 1:100). The primary antibodies were incubated with the sections overnight at 4 °C, followed by incubation with a secondary antibody for 1 h at room temperature. Finally, the sections were stained with DAB reagent for 2 min at room temperature, and the nuclei were then counterstained with hematoxylin. Stained cells were observed under an optical photographic light microscope at $\times 400$ magnification.

Nanoparticles

SiRNA-loaded liposomes were prepared as reported³². A lipid solution in which the lipidoid, cholesterol, DSPC and mPEG-DMG at a molar ratio of 50:38.5:10:1.5 were dissolved in a solution of 90% ethanol and 10 mM sodium citrate was prepared. Then, siRNA was dissolved in 10 mM citrate buffer, and the lipid components were mixed with the dissolved siRNA by vortexing such that the final weight ratio of lipidoid:siRNA was 5:1. The next step was ultrafiltration centrifugation to exclude free siRNA. Finally, the siRNA-liposomes were diluted in PBS. The hydrodynamic diameter, polydispersity, zeta potential and stability of the liposomes were measured by dynamic light scattering (DLS) (Malvern Zetasizer Nano-ZS, UK). A RiboGreen assay was employed to calculate the siRNA entrapment efficiency. After staining with 2% phosphotungstic acid, the liposomes were characterized by transmission electron microscopy (TEM, Jeol, Japan).

Statistical analyses

All statistical analyses were performed using GraphPad Prism (San Diego, CA, USA) or SPSS 25.0 (IBM, Armonk, NY, USA). Correlations between ACP5 expression and the clinical pathological features of IPF patients and control subjects were analyzed by the χ^2 test or Fisher's exact test. Other data are expressed as the mean $\pm$ SEM, and an independent Student's t-test was administered to analyze the statistical significance of differences between two groups. $p < 0.05$ was used to indicate statistical significance. All data were tested for normalization before analysis.

Results

IPF is characterized by altered ACP5 expression

We first sought to examine the levels of ACP5 in the sera of IPF patients and control subjects. Interestingly, ACP5 levels in the serum derived from control subjects were low, and higher levels of ACP5 were detected in the serum samples from IPF patients (Figure 1A). Then, we assessed the correlation between the levels of ACP5 and disease severity, which were estimated by the lung's gas diffusing capacity³³ (as measured by the diffusing capacity of the lungs for carbon monoxide, DLCO). Notably, the levels of ACP5 were higher in severe IPF patients whose DLCO was less than 40% of the predicted value than in patients with less severe IPF (Figure 1B). To further address the role of ACP5 in pulmonary fibrosis, we then detected the expression of ACP5 in the lungs of IPF patients and mice with the onset of BLM-induced pulmonary fibrosis. Interestingly, the lung homogenates of IPF patients exhibited 6-fold higher ACP5 expression than those of control subjects, and markedly higher expression levels of COL1A1 and α -SMA, markers of fibrosis, were also noted in IPF patients (Figure 1C). Similarly, significantly higher Acp5 expression was detected in the lungs of mice following BLM injection than in those of saline-treated mice (Figure 1D). Consistent results were also obtained by RT-PCR analysis of Acp5 expression (Figure 1E). Next, we sought to examine cells showing altered ACP5 expression in the lung sections from IPF patients and control subjects. Coimmunostaining showed that ACP5 was almost undetectable in the lung sections from control subjects, while IPF patient-derived lung sections were characterized by a large amount of lung fibroblast aggregation, manifesting as high levels of ACP5 revealed by costaining for ACP5 with fibroblast specific protein 1 (FSP1), a fibroblast marker (Figure 1F). We further detected lung sections from mice after BLM injection and found that the progression of pulmonary fibrosis was highly correlated with the severity of fibroblast accumulation and Acp5 overexpression in the lungs (Figure 1G).

Acp5 is upregulated in a canonical TGF- β signaling-dependent manner

To further confirm the above results, we cultured primary lung fibroblasts from WT mice and control individuals and then stimulated them with TGF- β 1. Indeed, low levels of ACP5 were observed in fibroblasts before stimulation. Upon TGF- β 1 stimulation, a substantial increase in ACP5 levels in primary mouse pulmonary fibroblasts (PMLFs) (Figure 2A) and primary human pulmonary fibroblasts (PHLFs) (Figure 2B) was noted. Given that TGF- β /Smad3 signaling plays a pivotal role in fibroblast differentiation induced by TGF- β 1³⁴, we conducted coimmunostaining for Acp5 and p-Smad3 in PMLFs after 1 h of TGF- β 1 treatment. Interestingly, Acp5 colocalized with p-smad3 in cultured fibroblasts (Figure 2C). These findings suggested to us that Acp5 may be a downstream target of canonical TGF- β signaling. To assess this hypothesis, we treated PMLFs with TGF- β 1 and SB431542, a specific inhibitor of TGF- β type I receptor (TGF β RI). As expected, the expression of Fibronectin, Col1a1 and α -SMA was significantly enhanced following TGF- β 1 treatment (Figure 2D). However, inhibition of TGF β RI activation by SB431542 absolutely abolished the upregulation of these fibrotic proteins. Surprisingly, the overexpression of Acp5 induced by TGF- β 1 was reversed after SB431542 treatment (Figure 2D). Interestingly, we observed similar results when PMLFs were stimulated with TGF- β 1 and SIS3-HCl (a specific inhibitor of smad3

phosphorylation) (Figure 2E). Collectively, these data support the notion that Acp5 expression in fibroblasts is controlled by canonical TGF- β signaling, of which TGF β R1/Smad3 are essential mediators.

ACP5 is essential for fibroblast differentiation, proliferation and migration

To assess the functional role of ACP5 in lung fibroblasts, PMLFs were generated from WT and *Acp5*^{-/-} mice and then subjected to TGF- β 1 stimulation. Compared with fibroblast differentiation in WT PMLFs, the loss of *Acp5* significantly attenuated the differentiation of fibroblasts into myofibroblasts, as evidenced by the decreased expression of myofibroblast markers (fibronectin, Col1a1 and α -SMA) following TGF- β 1 treatment (Figure 3A). Consistently, RT-PCR analysis of the expression of these genes demonstrated similar results (Figure 3B). To further confirm this result, we then performed ACP5 gain- and loss-of-function assays in PHLFs following TGF- β 1 treatment. As expected, the expression of ACP5 was efficiently silenced or enhanced following ACP5 siRNA or ACP5 plasmid transfection (Supplementary figure 2A and B). Notably, both Western blot and RT-PCR analyses demonstrated that fibroblast differentiation to myofibroblast was abrogated in *ACP5* siRNA-transfected PHLFs after TGF- β 1 induction (Figure 3B), while a significant increase in myofibroblast markers was observed in ACP5-overexpressing PHLFs (Supplementary figure 3). Additionally, we estimated the impact of ACP5 on the proliferation of PMLFs and PHLFs by EdU staining and CFSE staining, respectively. Fewer EdU-positive cells were noted among *Acp5*^{-/-} PMLFs than among WT PMLFs (Figure 3E). Consistently, the silencing of *ACP5* expression remarkably reduced the proliferation of fibroblasts induced by TGF- β 1 (Figure 3F). Similar data were also detected in PMLFs and PHLFs by CFSE staining (Supplementary figure 4A and B). Furthermore, we next examined the migration of fibroblasts by Transwell assay. Notably, the loss of ACP5 significantly suppressed the migration of PMLFs and PHLFs across the Transwell membrane (Figure 3G, H).

ACP5 interacts with β -catenin and regulates the degradation of β -catenin

β -Catenin signaling is known to be critical in the process of pulmonary fibrosis³⁵. Therefore, we investigated the impact of Acp5 on β -catenin signaling in TGF- β 1-stimulated fibroblasts. As expected, high levels of β -catenin were detected in WT PMLFs after 24 h of TGF- β 1 treatment. However, the protein but not mRNA levels of β -catenin were much lower in *Acp5*^{-/-} PMLFs, indicating that the decrease in β -catenin levels was not due to decreased gene transcription (Figure 4A and Supplementary figure 5A). Consistently, the knockdown of *ACP5* expression in PHLFs also revealed lower levels of β -catenin following TGF- β 1 induction compared to those in the group transfected with scrambled siRNA. To confirm this result, the expression of *ACP5* was enhanced in *Acp5*^{-/-} PMLFs and PHLFs by plasmid transfection. In contrast, a remarkable increase in β -catenin was observed in both PMLFs and PHLFs after *ACP5* plasmid transduction followed by TGF- β 1 treatment (Figure 4C and D). Nevertheless, in this experiment, the mRNA levels of β -catenin were also not affected by ACP5 overexpression.

Given that β -catenin translocation into the nucleus is critical for its function³⁶, we next estimated the localization of Acp5 and β -catenin in PHLFs. Immunofluorescence assays revealed that both Acp5 and β -catenin were mainly localized to the cytoplasm before treatment, while β -catenin translocation into the

nucleus upon TGF- β 1 stimulation was noted, and high expression of Acp5 was noted in the cytoplasm. However, much lower levels of β -catenin were detected in *Acp5*^{-/-} PMLFs than in WT PMLFs (Figure 4E). To further quantify the subcellular transfer of β -catenin, we used a Western blot assay. Indeed, significantly higher levels of β -catenin were detected in cytoplasmic and nuclear proteins from WT PMLFs than in those from *Acp5*^{-/-} PMLFs (Figure 4F).

As compelling evidence suggests that Acp5 is a phosphatase, we hypothesized that Acp5 dephosphorylates some sites on phosphorylated β -catenin, such as the sites Ser33, Ser37 and Thr41, which are involved in the process of β -catenin degradation³⁷. Interestingly, Acp5 could interact with β -catenin in PMLFs (Figure 5A and B) and PHLFs (Figure 5C and D), as shown by co-IP assay. Furthermore, we discovered higher levels of β -catenin phosphorylated at Ser33, Ser37 and Thr41 in *Acp5*^{-/-} PMLFs (Figure 5E) and *ACP5*-silenced PHLFs (Figure 5F) than in WT PMLFs and scrambled RNA-transfected PHLFs, respectively. In contrast, the opposite was observed in *ACP5*-overexpressing fibroblasts (Figure 5G and H), indicating that Acp5 appears to dephosphorylate the Ser33, Ser37 and Thr41 sites in p- β -catenin, by which it inhibits the process of degradation. To further confirm this result, we constructed 4 plasmids containing mutated β -catenin (MU1, MU2, MU3 and MU4) (Figure 5I) and transfected the mutant β -catenin plasmids and *Acp5* plasmid into PMLFs, followed by TGF- β 1 stimulation. As expected, low levels of p- β -catenin (33/37/41) and high levels of β -catenin were noted in PMLFs transfected with the MU4- β -catenin plasmid. Furthermore, these changes were also observed in PMLFs transfected with the other MU plasmids except for MU2 (Figure 5J), indicating that Acp5 dephosphorylated the Ser33 and Thr41 sites on β -catenin and inhibited the degradation of β -catenin.

Global deletion of *Acp5* protected mice from BLM-induced lung injury and fibrosis

To assess the requirement of *Acp5* in the development of lung fibrosis, *Acp5*^{-/-} and WT mice were subjected to BLM treatment (intratracheal injection), and lung injury and fibrosis were assessed after 21 days of induction. Hematoxylin and eosin (H&E), Masson's trichrome and Sirius red staining of the lung sections from mice in the BLM group demonstrated a remarkable increase in lung parenchymal fibrotic lesions compared to those in the saline group. Notably, compared with *Acp5*^{-/-} mice, BLM-treated WT mice were more susceptible to bleomycin toxicity, as evidenced by higher Ashcroft scores (Figure 6A), increased levels of hydroxyproline (Figure 6B) and lower survival rates (Figure 6C). To further quantitatively determine whether the loss of *Acp5* could inhibit fibrotic marker expression and ECM production in the lungs of BLM-treated mice, we evaluated the protein and mRNA expression levels of fibrotic genes by Western blot and RT-PCR, respectively. As illustrated in Figure 6D and E, the protein and mRNA levels of fibronectin, collagen, and α -SMA after BLM treatment were significantly lower in the *Acp5*^{-/-} mice than in the WT mice. Additionally, *Acp5* deficiency remarkably attenuated the transition of fibroblasts to myofibroblasts, as evidenced by decreased fluorescence intensity for Fsp1⁺/ α -SMA⁺ cells in the fibrotic lesion (Figure 6F). Consistent with the effect of ACP5 on fibroblast proliferation *in vitro*, staining for PCNA and Fsp1 in the adjacent lung sections showed the decreased ability of fibroblasts in *Acp5*^{-/-} mice to proliferate (Supplementary figure 6). Furthermore, the levels of β -catenin and p- β -catenin

were also detected in WT and *Acp5*^{-/-} mice following BLM induction. Consistently, Western blot analysis demonstrated that the levels of β -catenin were increased and the levels of p- β -catenin were decreased in the lung homogenates derived from the BLM-induced mice compared to uninduced mice (Figure 6G), and loss of *Acp5* largely reversed changes in the levels of β -catenin and p- β -catenin induced by BLM (Figure 6G). Collectively, our data demonstrate that the loss of *Acp5* protected mice against BLM-induced lung injury and fibrosis.

***In vivo* treatment with nanoparticles carrying *Acp5* siRNA evoked an antifibrotic response in BLM-induced pulmonary fibrosis**

Finally, we sought to transform the above discoveries into a therapeutic approach to remedy pulmonary fibrosis. First, we verified the knockdown efficiency of three siRNAs in PMLFs (Figure 7A and B) and chose the most efficient siRNA (#3) to generate lipid-based nanoparticles loaded with *Acp5* siRNA (Figure 7C). The prepared liposomes demonstrated a >90% entrapment efficiency for loading siRNA with a zeta potential of 4.1 mV (Supplementary figure 7A). Additionally, as observed in the transmission electron microscopy (TEM) images (Supplementary figure 7B), the prepared liposomes showed an average diameter of ~100 nm and a uniform sphere morphology. Furthermore, those liposomes illustrated a normal hydrodynamic diameter distribution (Supplementary figure 7C) and were continuously stable over 24 hours (Supplementary figure 7D). To demonstrate the distribution of liposomes in the lung following BLM induction, DiR-labeled liposomes were used. Interestingly, most of the fibroblasts overlapped the liposomes, which were mainly located in the fibrotic area, revealing the highly efficient absorption of fibroblasts (Figure 7D). Then, the temporal expression of *Acp5* was assessed after delivery of the liposomes. Notably, a significant decline in *Acp5* was noted, and the lowest *Acp5* expression was detected at day 3 after the intratracheal delivery of *Acp5* siRNA-loaded liposomes; however, *Acp5* expression was restored to normal levels at day 5 (Figure 7E).

Ultimately, the WT mice were administered scrambled siRNA- or *Acp5* siRNA-loaded liposomes on day 14 and day 18, respectively, by intratracheal instillation (100 nmol/kg). Indeed, the therapeutic effects of siRNA-loaded liposomes were validated in BLM-induced mice, as manifested by the results of histopathological analysis (Figure 7F), fibrotic scores (Figure 7F) and decreased levels of hydroxyproline in the lung (Figure 7G). Consistently, mice administered *Acp5* siRNA-loaded liposomes exhibited marked attenuation of fibrotic markers (fibronectin, Col1a1, α -SMA) at the protein level (Figure 7H) and mRNA level (Figure 7I). Collectively, our experiments reveal the clinical potential of intratracheal *Acp5* siRNA-loaded liposome administration in the treatment of pulmonary fibrosis.

Discussion

As outcomes in pulmonary fibrosis, especially IPF, remain poor despite the emergence of some new antifibrotic agents, an improved understanding of factors that influence gene expression and fibroblast differentiation is required. Although ACP5 has extensive pathophysiological functions depending on the cell type and disease^{38,39}, to the best of our knowledge, our study provides for the first direct experimental

proof that ACP5 plays a crucial role in the pathogenesis of fibrosis. We detected a marked increase in the levels of ACP5 within human IPF samples compared to samples from control subjects. Remarkably, higher levels of ACP5 were noted in the sera of IPF patients with lower DLCO values. Furthermore, *Acp5* deficiency protected mice from BLM-induced lung injury and fibrosis, with decreases in the differentiation of fibroblasts to myofibroblasts and proliferation. Mechanistic experiments revealed that *Acp5* was upregulated by TGF- β 1 in a TGF β R1/*smad3*-dependent manner, after which *Acp5*, a phosphatase, specifically bound p- β -catenin and dephosphorylated the sites Ser33 and Thr41, by which it inhibited the degradation of β -catenin and enhanced β -catenin signaling in the nucleus to promote the differentiation and proliferation of fibroblasts (Figure 8). Notably, the treatment of fibrotic mice with *Acp5* siRNA-loaded liposomes reversed pulmonary fibrosis in the mice. Taken together, our results not only provide novel insights into the understanding of IPF pathogenesis but also strongly suggest that strategies aimed at silencing ACP5 could be viable approaches for the treatment of pulmonary fibrosis in clinical settings.

Previous studies, including ours, have demonstrated that ACP5 is upregulated in the progression of carcinoma and regulates tumor cell proliferation, migration and apoptosis¹⁴⁻¹⁷. As pathways and risk factors are often shared by cancer and IPF, we detected the levels of ACP5 in sera and lung samples from IPF patients. Interestingly, high levels of ACP5 were noted in sera and lung sections from IPF patients. The most exciting discovery in this report is that the serum concentration of ACP5 was associated with decreased lung function in IPF patients. Specifically, higher levels of ACP5 were observed in patients with severe IPF (DLCO<40% predicted), indicating that ACP5 may be a surrogate biochemical marker for the severity of IPF. Similar to previous data⁴⁰, serum ACP5 is a useful marker of bone resorption and bears clinical applicability in the diagnosis and management of metabolic and pathologic bone diseases, such as bone metastasis.

We next focused on assessing cells that show altered ACP5 expression in lung sections from IPF patients. Interestingly, we illustrated that ACP5 was predominantly localized within FSP1⁺ fibroblasts in IPF; similarly, *Acp5* was noted to be overexpressed in fibroblasts derived from mice with BLM-induced fibrosis. Additionally, the expression of *Acp5* following TGF- β 1 treatment was detected in PMLFs and PHLFs. Indeed, *Acp5* was upregulated in myofibroblasts induced with TGF- β 1. Furthermore, we showed that *Acp5* was expressed in myofibroblasts in a TGF β R1- and *Smad3*-dependent manner, as manifested by the detection of decreased levels of *Acp5* in myofibroblasts treated with SB431542 or SIS3-HCL followed by TGF- β 1 induction. Myofibroblasts are critical in the process of pulmonary fibrosis by their secretion of ECM proteins, leading to tissue stiffness and respiratory failure⁴¹. Recent study has demonstrated that myofibroblasts in fibrotic lungs are mainly derived from resident lung fibroblasts⁹. This discovery prompted us to assess the impact of *Acp5* on fibroblasts following TGF- β 1 treatment. Indeed, *Acp5* deficiency and silencing significantly abolished the differentiation, proliferation and migration of PMLFs and PHLFs, respectively. As a result, mice deficient in *Acp5* exhibited a significant reduction in myofibroblast differentiation from fibroblasts and fibroblast proliferation in the lung following BLM induction.

The next important issue addressed was how Acp5 expression promotes the differentiation, proliferation and migration of fibroblasts. Previous studies have illustrated that aberrant β -catenin activation is involved in the pathogenesis of IPF^{42,43}. In addition, β -catenin signaling is required for TGF- β -induced fibroblast differentiation, proliferation and migration^{44,45}. We therefore examined the effects of Acp5 on β -catenin signaling in fibroblasts. Indeed, the loss or knockdown of Acp5 robustly decreased the levels of β -catenin in the cytoplasm and nucleus. Consistently, more β -catenin was observed in Acp5 plasmid-transfected fibroblasts than in control fibroblasts. It is likely that the impact of Acp5 on β -catenin is not regulated at the transcriptional level, as β -catenin mRNA was not altered in Acp5-knockdown or Acp5-overexpression fibroblasts. A previous study showed that cytoplasmic β -catenin in the destruction complex is phosphorylated at residues Ser33, Ser37 and Thr41 in the absence of Wnt ligands; phosphorylated β -catenin is recognized by the E3 ubiquitin ligase β -transducin repeat-containing protein (β -TrCP) and subsequently degraded by the ubiquitin-dependent proteasome pathway⁴⁶. Indeed, high levels of p- β -catenin were observed in Acp5-deficient or Acp5-silenced fibroblasts. In particular, further data showed that Acp5, a phosphatase, bound p- β -catenin and then dephosphorylated the Ser33 and Thr41 sites, subsequently inhibiting the degradation of β -catenin.

Given that until now, no effective therapies to halt the progression of IPF have been available, we assessed the efficacy of targeting Acp5 in mice with fibrosis induced by BLM. Previous studies have established liposomes as drug carriers for inhalation owing to their safety and ability to provide controlled drug release in the lung³². Similarly, in our study, Acp5 siRNA-loaded liposomes were efficiently taken up by fibroblasts in lung lesions after intratracheal injection. Notably, liposomes carrying Acp5 siRNA significantly attenuated lung fibrosis during the “fibrotic” phase of the model, which is more applicable to the clinical management of IPF patients and reflective of IPF.

Our study has some limitations. First, although we demonstrated that Acp5 specifically bound p- β -catenin and dephosphorylated the sites Ser33 and Thr41, until now, no commercial antibodies for p- β -catenin (Ser33), p- β -catenin (Thr41) or p- β -catenin (Ser33 and Thr41), except for commercial antibodies for p- β -catenin (Ser33, Ser37 and Thr41), have been available. Second, the use of a specific Acp5-knockout mouse model would make our data more compelling. Third, in our study, we illustrated that Acp5 was upregulated by TGF- β 1 in a TGF β R1/Smad3-dependent manner, but the detailed mechanism needs to be further explored.

In conclusion, this report demonstrates that ACP5 plays a crucial role in the progression of pulmonary fibrosis. High serum levels of ACP5 were significantly associated with the severity of IPF in patients. In addition, mice with *Acp5* deficiency were protected from BLM-induced lung injury and fibrosis. Mechanistic experiments showed that Acp5 selectively dephosphorylates p- β -catenin at Ser33 and Thr41 in the cytoplasm, reducing the degradation of β -catenin, by which the levels of β -catenin in the nucleus are enhanced, promoting fibroblast differentiation, proliferation and migration. Together, our data indicate that targeting Acp5 may represent a promising therapeutic approach for the treatment of pulmonary fibrosis in clinical settings.

Declarations

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Duality of interest

There are no potential conflicts of interest relevant to this article to report.

Author Contributions:

YW and WNX designed, edited and led out the experiments of this study. YNH, JY, QW and QZ conducted the experiments, data analysis, and critical discussions of the results. YHD, JL, GRW, LZ and YJX provided material support and study supervision. All authors contributed to the writing and editing of the manuscript and approved the final draft of the manuscript.

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Table

Table 1. Characteristics of the Patients at Baseline[#]

	Serum samples			Lung tissue samples		
Variable	IPF patients (n = 20)	Control subjects (n = 13)	p-Value	IPF patients (n = 20)	Control subjects (n = 13)	p-Value
Age, years (mean $\pm$ SD)	64.30 $\pm$ 12.84	59.00 $\pm$ 13.74	0.268	56.67 $\pm$ 4.096	58.33 $\pm$ 3.844	0.7815
Gender						
Male	12 (60%)	6 (46.15%)	0.493	2 (66.7%)	2 (66.7%)	
Female	8 (40%)	7 (53.85%)		1 (33.3%)	1 (33.3%)	
Pulmonary Function						
FVC, % predicted	70.06 $\pm$ 13.26	NA		60.33 $\pm$ 16.42	NA	
DLCO, % predicted	48.25 $\pm$ 18.18	NA		38.25 $\pm$ 14.89	NA	

IPF, Idiopathic pulmonary fibrosis; FVC, Forced vital capacity; DLCO, diffusing capacity of the lung for carbon monoxide.

#Continuous variables listed as mean (SD) and differences assessed by two-sample t test; Categorical variables listed by count (percentage) and differences assessed by Fisher's exact test.

Supplementary Figure Legends

Supplemental Figure 1. Genotyping results of WT and *Acp5*^{-/-} allele. The wildtype allele is none and the *Acp5*^{-/-} allele is 490 bp (up). The wildtype allele is 484 bp and the *Acp5*^{-/-} allele is none (below)

Supplemental Figure 2. A-B: Western blot analysis of the levels of β -catenin in ACP5 siRNA or Scrambled siRNA treated PHLFs (A) and ACP5 plasmid or Vector treated PHLFs (B). The data are represented as the mean $\pm$ SEM of three independent experiments. ***, $p < 0.001$.

Supplemental Figure 3. Western blot analysis of FIBRONECTIN, COL1A1 and α -SMA expression in ACP5 plasmid or Vector treated PHLFs following TGF- β 1 induction. The data are represented as the mean $\pm$ SEM of three independent experiments. *, $p < 0.05$; ***, $p < 0.001$.

Supplemental Figure 4. CFSE staining analysis of proliferation in WT and *Acp5*^{-/-} PMLFs (A), ACP5 siRNA or Scrambled siRNA treated PHLFs (B)

Supplemental Figure 5. A-B: RT-PCR analysis of the levels of β -catenin in WT and *Acp5*^{-/-} PMLFs (**A**) and *ACP5* plasmid or Vector treated PHLFs (**B**). The data are represented as the mean $\pm$ SEM of three independent experiments. N.S, no significant difference between two groups

Supplemental Figure 6. Representative IHC staining of adjacent lung tissue sections for Fsp1 and Pcn α . Nine mice were included in each study group. The data are represented as the mean $\pm$ SEM. ***, $p < 0.001$.

Supplemental Figure 7. A: The prepared nanoparticles demonstrated >90% entrapment efficiency for loading siRNA with a Zeta-potential of 4.1 mv. **B:** A representative image taken by transmission electron microscope (TEM). **C-D:** A normal distribution of hydrodynamic diameter of those nanoparticles with continuous stability within 24 hours.

Figures

Figure 1

Analysis of ACP5 levels in IPF patients and mice with BLM induction. A: ELISA analysis of ACP5 levels in the serum of patients with IPF (n = 20) and healthy subjects (n = 13). B: Analysis of the correlation between ACP5 levels with DLCO% predicted in IPF patients. C: Western blot analysis of ACP5, COL1A1 and α-SMA expression in the lungs of control subjects (n = 3) and IPF patients (n = 3). D-E: Western blot (D) and RT-PCR (E) analysis of Acp5 expression in the lung homogenate of Saline (n = 5) and BLM-

induced (n = 5) mouse model. F: Representative results for co-immunostaining of ACP5 and FSP1 (a fibroblast marker) in the lung sections from patients with IPF (n = 3) and healthy subjects (n = 3). G: Results for co-immunostaining of Acp5 and Fsp1 in BLM-induced lung sections. The nuclei were stained blue by DAPI, and the images were taken under original magnification $\times 400$. The data are represented as the mean $\pm$ SEM. *, p < 0.05; **, p < 0.01; ***, p < 0.001.

Figure 2

Acp5 is upregulated in fibroblasts in a TGFβRI/Smad3-dependent manner. A-B: Western blot analysis of ACP5 expression in primary mice lung fibroblasts (PMLFs, A) and primary human lung fibroblasts (PHLFs, B) following TGF-β1 induction. C: Representative results for co-immunostaining of Acp5 and p-Smad3 in PMLFs following 1h of TGF-β1 induction. The nuclei were stained blue by DAPI, and the images were taken under original magnification ×400. D-E: Western blot analysis of fibronectin, Col1a1, α-SMA and Acp5 expression in PMLFs following SB431542 (D) and SIS3-HCL (E) treatment. The data are represented as the mean ± SEM of three independent experiments. ***, p < 0.001.

Figure 3

The impact of Acp5 on the differentiation, proliferation and migration of fibroblasts. A-B: Western blot (A) and RT-PCR (B) analysis of fibronectin, Col1a1 and α -SMA expression in PMLFs from WT or Acp5^{-/-} mice following TGF- β 1 treatment. C-D: Western blot (C) and RT-PCR (D) analysis of FIBRONECTIN, COL1A1 and α -SMA expression in ACP5 siRNA or Scrambled siRNA treated PHLFs following TGF- β 1 induction. E-F: Representative results for EdU staining in WT or Acp5^{-/-} PMLFs (E) and ACP5 siRNA or Scrambled siRNA treated PHLFs (F). G-H: Representative results for Transwell assay in WT or Acp5^{-/-} mice derived PMLFs (G) and ACP5 siRNA or Scrambled siRNA treated PHLFs (H). The data are represented as the mean $\pm$ SEM of three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 4

Altered Acp5 expression affects the levels of β-catenin. A-D: Western blot analysis of the levels of β-catenin in WT or *Acp5*^{-/-} PMLFs (A), ACP5 siRNA or Scrambled siRNA treated PHLFs (B), Acp5 plasmid or Vector treated *Acp5*^{-/-} PMLFs (C) and ACP5 plasmid or Vector treated PHLFs (D). E: Representative results for co-immunostaining of Acp5 and β-catenin in PMLFs from WT and *Acp5*^{-/-} PMLFs following TGF-β1 induction. The nuclei were stained blue by DAPI, and the images were taken under original

magnification $\times 400$. F Western blot analysis of the levels of β -catenin in cytoplasm and nuclear. The data are represented as the mean $\pm$ SEM of three independent experiments. *, $p < 0.05$; **, $p < 0.01$.

Figure 5

Acp5 dephosphorylates β -catenin at Ser33 and Thr41. A-D: Co-immunoprecipitation of Acp5 and β -catenin in PMLFs (A-B) and in PHMLs (C-D). E-H: Western blot analysis of the levels of p- β -catenin (S33, S37 and T41) in WT and Acp5^{-/-} PMLFs (E), ACP5 siRNA and Scrambled siRNA treated PHLFs (F), Acp5

plasmid and Vector treated *Acp5*^{-/-} PMLFs (G) and *Acp5* plasmid and Vector treated PHLFs (H). I: the schematic results showing the mutant plasmids (MU1-3) of these three phosphorylated sites. Each of mutant plasmids (MU1-3) of these three phosphorylated sites maintained one normal site and two mutant sites (red boxes), and all phosphorylated sites were deleted in MU4. J: Western blot analysis of the levels of β -catenin and p- β -catenin (S33, S37 and T41) in PMLFs following plasmids transduced. The data are represented as the mean $\pm$ SEM of three independent experiments. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 6

Comparison of the severity of lung fibrosis between WT and Acp5^{-/-} mice after BLM induction. A: Histological analysis of the severity of lung fibrosis in mice after BLM induction. Left panel: representative images for H&E (top), Masson staining (middle) and Sirius red (bottom). Right panel: A bar graph showed the quantitative mean score of the severity of fibrosis. Images were captured at $\times 200$ magnification. B: Quantification of hydroxyproline contents in WT and Acp5^{-/-} mice. C: the survival ratio in WT and Acp5^{-/-} mice after BLM induction. D-E: Western blot (D) and RT-PCR (E) analysis of fibronectin, Col1a1 and α -SMA expression. F: Co-immunostaining of Fsp1 and α -SMA in the lung sections. The nuclei were stained blue by DAPI, and the images were taken under original magnification $\times 400$. G: Western blot analysis of the levels of β -catenin and p- β -catenin (S33, S37 and T41) in WT and Acp5^{-/-} mice after BLM challenge. Nine mice were included in each study group. The data are represented as the mean $\pm$ SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 7

Administration of Acp5 siRNA-loaded liposomes protected mice from BLM-induced lung injury and fibrosis. A-B: Western blot (A) and RT-PCR (B) analysis of the interfering efficiency of Acp5 siRNAs in PMLFs. C: Schematic diagram for preparation of liposomes carrying Acp5 siRNA. D: Representative images of immunofluorescence for the biodistribution of liposomes in lungs from BLM-induced mice. The nuclei were stained blue by DAPI, and the images were taken under original magnification $\times 400$. E:

Temporal Acp5 expression changes in lungs from transfected mice. F: Histological analysis of the severity of lung fibrosis in mice after BLM induction. Left panel: representative images for H&E (left), Masson staining (middle) and Sirius red (right). Right panel: A bar graph showed the quantitative mean score of the severity of fibrosis. Images were captured at $\times 200$ magnification. B: Quantification of hydroxyproline contents in Scrambled or Acp5 siRNA-loaded liposomes treated mice after BLM injection. H-I: Western blot (H) and RT-PCR (I) analysis of fibronectin, Col1a1, α -SMA and Acp5 expression. Nine mice were included in each study group. The data are represented as the mean $\pm$ SEM. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Figure 8

Schematic illustration of the mechanisms of Acp5 in fibroblasts. Acp5 is upregulated by TGF-β1 in a TGFβR1/smad3 depend manner, and then Acp5 specially binds to p-β-catenin and dephosphorylate the sites of Ser33 and Thr41, by which it resists the degradation of β-catenin and enhanced β-catenin signaling in the nuclear to promote the differentiation, proliferation and migration of fibroblasts

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