ORIGINAL ARTICLE

LncRNA DACH1 protects against pulmonary fibrosis by binding to SRSF1 to suppress CTNNB1 accumulation

Jian Sun\textsuperscript{a,b,f,\dagger}, Tongzhu Jin\textsuperscript{a,b,\dagger}, Zhihui Niu\textsuperscript{a,b,\dagger}, Jiayu Guo\textsuperscript{a,b,\dagger}, Yingying Guo\textsuperscript{a,b}, Ruoxuan Yang\textsuperscript{a,b}, Qianqian Wang\textsuperscript{a,b}, Huiying Gao\textsuperscript{a,b}, Yuhan Zhang\textsuperscript{a,b}, Tianyu Li\textsuperscript{a,b}, Wenxin He\textsuperscript{c}, Zhixin Li\textsuperscript{c}, Wenchao Ma\textsuperscript{a,b}, Wei Su\textsuperscript{a,b}, Liangliang Li\textsuperscript{a,b}, Xingxing Fan\textsuperscript{d}, Hongli Shan\textsuperscript{a,b,c,\*}, Haihai Liang\textsuperscript{a,b,c,\*}

\textsuperscript{a}Department of Pharmacology (State-Provence Key Laboratories of Biomedicine-Pharmaceutics of China, Key Laboratory of Cardiovascular Research, Ministry of Education), College of Pharmacy, Harbin Medical University, Harbin 150081, China
\textsuperscript{b}Northern Translational Medicine Research and Cooperation Center, Heilongjiang Academy of Medical Sciences, Harbin Medical University, Harbin 150081, China
\textsuperscript{c}Department of Thoracic Surgery, Shanghai Pulmonary Hospital, Tongji University, Shanghai 200433, China
\textsuperscript{d}State Key Laboratory of Quality Research in Chinese Medicine/Macau Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Macau (SAR), China
\textsuperscript{e}Research Unit of Noninfectious Chronic Diseases in Frigid Zone (2019RU070), Chinese Academy of Medical Sciences, Harbin 150081, China
\textsuperscript{f}Zhuhai People’s Hospital, Guangdong Provincial Key Laboratory of Tumor Interventional Diagnosis and Treatment, Zhuhai Hospital Affiliated with Jinan University, Jinan University, Zhuhai 519000, China

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Abstract
Idiopathic pulmonary fibrosis (IPF) is a progressive disease with unknown etiology and limited therapeutic options. Activation of fibroblasts is a prominent feature of pulmonary fibrosis. Here we report that LncRNA DACH1 (dachshund homolog 1) is downregulated in the lungs of IPF patients and in an experimental mouse model of lung fibrosis. LncDACH1 knockout mice develop spontaneous pulmonary fibrosis, whereas overexpression of LncDACH1 attenuated TGF-β1-induced aberrant activation,
collagen deposition and differentiation of mouse lung fibroblasts. Similarly, forced expression of LncDACH1 not only prevented bleomycin (BLM)-induced lung fibrosis, but also reversed established lung fibrosis in a BLM model. Mechanistically, LncDACH1 binding to the serine/arginine-rich splicing factor 1 (SRSF1) protein decreases its activity and inhibits the accumulation of Ctnnb1. Enhanced expression of SRSF1 blocked the anti-fibrotic effect of LncDACH1 in lung fibroblasts. Furthermore, loss of LncDACH1 promoted proliferation, differentiation, and extracellular matrix (ECM) deposition in mouse lung fibroblasts, whereas such effects were abolished by silencing of Ctnnb1. In addition, a conserved fragment of LncDACH1 alleviated hyperproliferation, ECM deposition and differentiation of MRC-5 cells driven by TGF-β1. Collectively, LncDACH1 inhibits lung fibrosis by interacting with SRSF1 to suppress Ctnnb1 accumulation, suggesting that LncDACH1 might be a potential therapeutic target for pulmonary fibrosis.

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1. Introduction

Pulmonary fibrosis is the end-stage outcome of a large variety of lung diseases characterized by excessive fibroblast proliferation, aberrant extracellular matrix (ECM) deposition with inflammatory injury, and destruction of tissue structure. The most common disease type is idiopathic pulmonary fibrosis (IPF), a severe interstitial lung disease that leads to progressive loss of lung function, resulting in significant medical burden due to its severe clinical manifestations, poor prognosis, and high mortality. Increasing evidence suggests that repeated injury to the alveolar epithelium leads to excessive accumulation of fibroblasts and extracellular matrix proteins, which in turn leads to scarring and diminished respiratory function. Pirfenidone and nintedanib are currently approved for the treatment of IPF, but whether these two agents can improve the survival of patients with IPF and their existing adverse drug reactions remains controversial. The molecular mechanisms of IPF are not fully understood, and there is still a lack of effective treatments for IPF other than lung transplantation.

Noncoding RNAs such as microRNAs have been suggested to be involved in the development of pulmonary fibrosis. Long non-coding RNAs (lncRNAs) are defined as RNA transcripts more than 200 nucleotides with little protein-coding function. Initial studies identified altered expression levels of certain lncRNAs in cancer cells and that these lncRNAs could serve as markers and potential drug targets for cancer diagnosis. Moreover, recent studies have reported that the lncRNAs are highly associated with drug metabolism and drug resistance. Mutations and dysregulation of lncRNAs have been successively linked to various human diseases, such as cardiovascular diseases, neurological disorders, and organ fibrosis. Feng et al. reported that lncRNA Erbb4-IR produces a marked effect in renal fibrosis by targeting Smaβd7 in the TGF-β/Smad pathway. In our previous study we found that lncRNA PFAβ played a corresponding role in IPF.

LncRNAs are able to regulate gene expression at multiple levels. In simple terms, lncRNAs are able to participate in epigenetic regulation, transcriptional regulation, as well as post-transcriptional regulation. Mechanistically, lncRNAs can affect gene expression by directly binding to proteins, influencing histone modifications, interfering with mRNA splicing, sponging microRNAs, etc. Cai et al. have made outstanding contributions to the study of the role of LncRNA DACH1 (dachshund homolog 1), which could directly bind to sarcoplasmic reticulum calcium ATPase 2a (SERCA2a) and enhance its ubiquitination and degradation, leading to impairment of cardiac function. On the other hand, LncDACH1 could also regulate the PP1A/YAP1 pathway to participate in cardiac repair and regeneration after myocardial infarction. Although a large number of studies have demonstrated the essential role of lncRNAs in human diseases, the action of LncDACH1 in IPF pathogenesis and its underlying molecular mechanism needs to be further explored.

In this study, we found that LncDACH1 was decreased in lung of both IPF patients and bleomycin (BLM)-induced mice, suggested that LncDACH1 may play critical roles in pulmonary fibrosis. Consistent with this finding, LncDACH1-KO mice exhibit lung pathological changes, collagen deposition, lung function decline and remodeling. We found that LncDACH1 modulates serine/arginine-rich splicing factor 1 (SRSF1) protein, ultimately suppressing lung fibroblast activation.

2. Materials and methods

2.1. Mouse model

Male C57BL/6 mice aged 6–8 weeks with an average body weight of 18–23 g were used. A mouse model of pulmonary fibrosis was generated by a single intratracheal administration of BLM (3 mg/kg, Selleck) under anesthesia resulting in an experimental pulmonary fibrosis model 21 days later. Control mice received saline. Prevention and treatment groups were induced with adenovirus-associated virus 5 carrying lncRNA DACH1 (AAV5-LncDACH1) at a dose of 2 × 10^{10} particles by intratracheal administration. Animals were randomly assigned for each experimental group. Assessors were un-blinded to group allocation. All animal experiments were approved by the Harbin Medical University College of Pharmacy Ethical committee (A-IRB3001721, China).

2.2. Cell culture and treatment

Primary mouse lung fibroblasts (PMLFs) were extracted from the lung tissues of 3-day-old mice. After digestion and filtration, the extracted PMLFs were cultured in DMEM (Biological Industries, Israel), supplemented with 10% FBS (Biological Industries, Israel), and 1% penicillin–streptomycin–amphotericin B (Solarbio, Beijing, China) under sterile conditions. The medium was changed,
non-adherent cells were removed differentially after 6 h, and further manipulations were performed when cells were well-established.

The human fetal lung fibroblast cell line MRC-5 was obtained commercially from the Cell Bank of Chinese Academy of Sciences. Cells were cultured at 37 °C in a 5% CO2 incubator. Cells were allowed to grow and adhere to the wall. After starvation in serum-free medium for 6 h, the cells were transfected with 2 μg of plasmid followed by treatment with or without recombinant human TGF-β1 (10 ng/mL; PeproTech, USA). MRC-5 authentication was determined by short tandem repeat (STR) profiling and free from mycoplasma contamination.

Adult mouse lung fibroblasts (AMLFs) were obtained from 6–8-week-old C57/BL6 mice. Mouse lung tissues were harvested, minced into centrifuge tubes on an ultraclean table. After pancreatin digestion, the supernatant was aspirated and the reaction was terminated by adding culture medium. Pure AMLFs were filtered and placed in culture medium as described above.

2.3 Patients samples

Patient samples of IPF and non-IPF were provided by Shanghai Pulmonary Hospital. Informed consent was obtained from the patients and the study was approved by the Harbin Medical University College of Pharmacy Ethical Committee (A-IRB3001721) and conformed to the Declaration of Helsinki. Patient information is displayed in Supporting Information Table S1.

2.4 Cell transfection

PMLFs/MRC-5 were transiently transfected with LncDACH1 plasmid or small interference RNA (siRNA) of LncDACH1/Srsf1/Ctnnb1 using Lipo2000 (Invitrogen) according to the manufacturer’s instructions. SiRNA and a negative control siRNA (siNC) were synthesized by Ribobio (Guangzhou, China). SiRNA sequences are displayed in Supporting Information Table S2.

2.5 Quantitative real-time RT-PCR

Total RNA was extracted from the lung tissues, PMLFs, AMLFs and MRC-5 cells using TRIzol reagent. The concentration and purity of the RNA was determined with a NanoDrop 8000 (Thermo). cDNA reverse transcription kits (5× All-in-One RT Master Mix, TransGene Biotech, China) were used to reverse transcribe total RNA using random primers. The mRNA was quantified by SYBR Green on ABI 7500 Fast Real-time PCR system (Applied Biosystems, USA). The GAPDH gene was used for normalization. Primer efficiency was assessed by a series of dilutions, and a dissociation curve was used to assess the primer specificity. Primers sequences are shown in Supporting Information Table S3. The data were analyzed by 2−ΔΔCt method.

2.6 Western blot

Protein samples were extracted from cells and tissues using RIPA lysis buffer with protease inhibitor (Beyotime, Jiangsu, China). The proteins were separated on a 10% or 8% SDS polyacrylamide gel and transferred to a nitrocellulose membrane (Pall Life Sciences, Ann Arbor, MI, USA). Proteins were identified with antibodies against FN1 (1:500, Proteintech, Wuhan, China), Collagen I (1:500, Affinity Biosciences, OH, USA), SRSF1 (1:500, Proteintech, Wuhan, China), and β-catenin (1:500, Proteintech, Wuhan, China), β-Actin (1:1000, Proteintech, Wuhan, China) served as a loading control. The membranes were incubated with secondary antibodies (1:8000, Abcam, USA) for 1 h at room temperature. Finally, Odyssey (Odyssey CLX, Biosciences, USA) was used to detect and quantify protein expression.

2.7 Masson’s trichrome staining and immunohistochemistry

Lung tissues were fixed in 4% paraformaldehyde (Solarbio, Beijing, China) and embedded in paraffin to prepare 5-μm tissue sections. The tissue sections were deparaffinized and rehydrated in xylene and different concentrations of ethanol. After incubation with 3% hydrogen peroxide, the sections were heated to repair antigen and cooled to room temperature. The sections were blocked with 50% normal goat serum albumin for 1 h, followed by incubation with Collagen I antibody and α-SMA antibody at 4 °C overnight. The next day, sections were incubated with horseradish peroxidase-conjugated secondary antibodies and colored by 3,3′-diaminobenzidine (ZsBio, Beijing, China). IHC analysis was measured by a fluorescence microscopy (DP80, Olympus, Japan). Hematoxylin and eosin (H&E) and Masson’s trichrome staining were performed according to the instructions of the kit (Solarbio, Beijing, China), and histopathological changes were observed under a microscope.

2.8 Wound healing assay

The PMLFs or MRC-5 cells were cultured in 6-well plates overnight to determine the migratory ability of lung fibroblasts. The cell monolayer was scratched on the tip of 200 μL plastic micropipette and cultured under standard culture conditions. The cells then were transfected and observed with Nikon Tsi100 microscope (Nikon, Tokyo, Japan) at 0 and 24 h.

2.9 EdU fluorescence staining

A Cell-Light EdU Apollo 567 In Vitro Kit (RiboBio, Guangzhou, China) was used to detect cell proliferation. PMLFs or MRC-5 cells were cultured in 24-well plates. At 48 h after transfection the experiment was carried out according to the manufacturer’s protocol.

2.10 Immunofluorescence staining

Lung fibroblasts were seeded into 24-well cell culture plates. After transfection and treatment, the cells were fixed in 4% paraformaldehyde at room temperature and then solubilized and blocked. The cells were incubated with anti-α-SMA (1:200, Abcam), anti-SRSF1 (1:50, Proteintech) and anti-β-catenin (1:200, Affinity) at 4 °C overnight. On the second day, FITC-conjugated goat anti-mouse antibody (1:500, Alexa Fluor 594, Life Technology) or goat anti-rabbit antibody (1:500, Alexa Fluor 488, Life Technology) was added and incubated for 1 h in the dark condition. The nuclei were counterstained with DAPI for 5 min. Immunofluorescence was observed and analyzed under a fluorescence microscope. For immunofluorescence of tissue cryosections, retrieved lung tissues were fixed with 4% paraformaldehyde and embedded with Tissue-Tek OCT (Sakura) at −80 °C. Six μm-thick sections were cut in a Leica cryostat at −20 °C, post-fixed in acetone, and labeled with the appropriate primary and secondary antibody.

2.11 Fluorescent in situ hybridization (FISH)

Fluorescent In Situ Hybridization Kit (RiboBio, Guangzhou, China) was used to detect the location of LncDACH1. Lung
fibroblasts were fixed in 4% paraformaldehyde and solubilized with 0.4% Triton-100 for 10 min before blocking. Under dark conditions, the cells were pre-hybridized at 37 °C for 30 min, and the lncRNA FISH probe mixture was hybridized overnight at 37 °C. The cells were then washed under different conditions, and the nuclei were stained with DAPI (Roche, Basel, Switzerland) for 5 min. Fluorescence microscopy was used to observe the samples.

2.12. Micro computed tomography (Micro-CT) of mouse lung

The lung tissue density of mice was quantitatively measured by CT, and the degree of pulmonary fibrosis in each group was determined. CT plain scan was performed by the following methods: after anesthesia, the mice were placed on the scanning bed and kept in a supine position, then the chest of the mice was fixed with tape, and the living lung tissue of the mice was scanned after setting the scanning parameters. The images were automatically generated by the system workstation, and the images were post-processed by the relevant system software. The structural and morphological changes of lung tissue were observed to evaluate the different degrees of pulmonary fibrosis with comparison to the control group.

2.13. RNA pull-down

LncRNA DACH1 probe was labeled with biotin and co-incubated with streptavidin-coated magnetic beads to form complexes, followed by protein enrichment by co-incubation with cell lysates. After washing the beads, protein sample buffer was added and heated in boiling water for 10 min. The SRSF1 target band was detected after samples were electrophoretically separated (12.5% SDS-PAGE) and assayed by Western blotting.

![Figure 1](image)

**Figure 1** Loss of LncDACH1 results in pulmonary fibrosis in mice. (A) Micro-CT shows that the shadow area of the lungs in LncDACH1-KO mice was significantly greater than that of WT; n = 4. (B) Forced vital capacity (FVC), forced expiratory flow (FEF), FEV0.1, FEV0.1/FVC, inspiratory capacity (IC) as well as Flow-volume loops were reduced in LncDACH1-KO mice as detected by a flexiVent system; n = 4. (C) qRT-PCR validated that mRNA expression of Fn1, Coll1a1, Coll3a1 and Acta2 was promoted by LncDACH1 deficiency; n = 6. (D) The protein levels of FN1 and Collagen I detected by Western blot assays; n = 6. (E) Hydroxyproline content in LncDACH1-KO mice compared to WT mice; n = 6. (F, G) H&E and Masson staining reveal more exacerbated lung tissue morphology and increased fibrotic areas in LncDACH1-KO mice compared with WT mice; bar = 50 μm; n = 3. (H, I) Collagen I and α-SMA were increased in LncDACH1-KO mice as shown by immuno-histochemical analysis; scale bar = 50 μm; n = 3. (J, K) Collagen I and α-SMA were stained by immunofluorescence analysis; bar = 50 μm; n = 3. Data are presented as mean ± SEM; *P < 0.05, **P < 0.01; ns, no significance. Micro-CT, micro computed tomography.
Figure 2  Anti-fibrotic effects of LncDACH1 in primary mouse lung fibroblasts. (A) qRT-PCR analysis shows that transfected LncDACH1 was overexpressed in PMLFs; n = 6. (B) The relative mRNA levels of Fn1, Col1a1, Col3a1, and Acta2 in LncDACH1-transfected cells after TGF-β1 induction; n = 8. (C) LncDACH1 inhibited the protein expression of fibrotic markers caused by TGF-β1 in PMLFs; n = 5. (D) The fluorescence results of an EdU assay show the proliferation of PMLFs; scale bar = 50 μm; n = 5. (E) The migratory ability of PMLFs was attenuated by LncDACH1, as detected by the wound healing assay; scale bar = 200 μm; n = 5. (F) Immunofluorescence staining indicated that TGF-β1-induced α-SMA positive cells were impeded by the enhanced expression of LncDACH1 in PMLFs (scale bar = 20 μm; n = 5). Data are presented as mean ± SEM; *P < 0.05, **P < 0.01. PMLFs, primary mouse lung fibroblasts.
2.14. Determination of hydroxyproline

The collagen content in mouse lung tissues was assessed using the hydroxyproline method. A 45-mg tissue sample was precisely weighed and put into a test tube for hydrolysis according to the instructions of the hydroxyproline assay kit (Jiangcheng, Nanjing, China). After adjusting the pH, the supernatant was taken to determine the absorbance value at 550 nm.

2.15. Protein degradation assay

PMLFs were transfected with an LncDACH1 overexpression plasmid at 48 h with the addition of translational inhibitor cycloheximide (CHX, 50 μg/mL, Selleck, Shanghai, China) or proteasome inhibitor MG132 (50 μmol/L, Selleck, Shanghai, China), and then proteins were extracted after 0, 1, 2, and 4 h and SRSF1 degradation was assessed by Western blot assay.

2.16. Lung function measurements

Invasive pulmonary function testing was measured with the flexiVent system (flexiVent; SCIREQ, Montreal, QC, Canada). In brief, anesthetized mice were intubated and connected to a computer-controlled ventilator system (tidal volume of 10 mL/kg and frequency of 150 beats/min). Three acceptable measurements were recorded (coefficient of determination >0.95) for each mouse and the average was calculated.

2.17. RNA-binding protein immunoprecipitation (RIP)

A MagnaRIPTM RNA-Binding Protein Immunoprecipitation Kit (Millipore, USA) was used to perform RNA-binding protein immunoprecipitation (RIP) according to the manufacturer’s instructions. Briefly, RIP assays were performed with SRSF1 antibody or negative control immunoglobulin G (IgG; using Millipore, USA). Cells were collected and lysed in RNA lysis buffer. Then, 5 μg of SRSF1 antibody or IgG was incubated with 50 μL of magnetic bead suspension for 30 min at room temperature to prepare the beads for immunoprecipitation. The magnetic beads were resuspended with RIP immunoprecipitation buffer and incubated with the cells at 4°C overnight. The next day, the RNA/magnetic bead complex was washed and resuspended in proteinase K buffer to isolate proteins. The immunoprecipitated RNA was then purified for agarose gel electrophoresis or qRT-PCR analysis. IgG enrichment served as negative controls.

2.18. Statistics and analysis

Data are presented as the mean ± standard error of mean (SEM) of at least three independent experiments. Statistical significance was calculated using a two-tailed t-test or one-way analysis of variance (ANOVA) with a post-test Bonferroni-corrected t-test. P < 0.05 was considered a statistically significant difference. Statistical analyses were carried out using GraphPad Prism 8.0.

3. Results

3.1. LncDACH1 deficiency causes pulmonary fibrosis in mice

In previous studies, we generated LncDACH1 knockout (LncDACH1-KO) mice, which exhibited a marked reduction of LncDACH1 in the lungs (Supporting Information Fig. S1A). Unexpectedly, we observed that the lung shadow of LncDACH1-KO mice was increased compared with that of wild-type (WT) mice according to the results of micro computed tomography (Micro-CT, Fig. 1A). In addition, forced vital capacity (FVC), forced expiratory capacity (FEF), FEV0.1, FEV0.1/FVC, inspiratory capacity (IC) as well as flow-volume loops were also attenuated in LncDACH1-KO mice (Fig. 1B), indicating that LncDACH1 was closely related to the lung function of mice. We further detected that loss of LncDACH1 induced mRNA levels of Fn1, Coll1a1, Col3a1 and Acta2, and protein expression of FN1 and Collagen I, which are associated with pulmonary fibrosis (Fig. 1C and D). Hydroxyproline was the most abundant in collagen, an indicator that was elevated by knockout of LncDACH1 (Fig. 1E). Using H&E staining and Masson’s trichrome staining on lung tissue sections, we observed that knockout of LncDACH1 promoted collagen deposition and increased fibrotic area (Fig. 1F and G). Consistent with these results, immunohistochemistry and immunofluorescence confirmed the upregulated expression of Collagen I and α-SMA in the lungs of LncDACH1-KO mice (Fig. 1H–K), indicating that LncDACH1-deficient mice exhibit spontaneous pulmonary fibrosis.

To evaluate the role of LncDACH1 in pulmonary fibrosis, we examined the expression of LncDACH1 in lung scar foci. As shown in Fig. S1B and S1C, qRT-PCR analysis showed that the expression of LncDACH1 was downregulated in lung tissues of IPF patients and in BLM-treated mice.

Considering the heterogeneity of cells, we performed scRNA-seq analysis of cells isolated from IPF and normal lungs. Firstly, the GSE135893 dataset was analyzed by IPF Cell Atlas (http://ipfcellatlas.com/)17. Based on relative similarity, four cell-type groups were formed: Epithelial, Endothelial, Mesenchymal and Immune. We found the expression of DACH1, which is the LncDACH1 conserved sequence, was higher in fibroblasts rather than in AT2 cells (Supporting Information Fig. S2A and S2B). ScRNA-seq results from the Human Protein Atlas website (https://www.proteinatlas.org/) showed that DACH1 was highly expressed in endothelial cells, granulocytes cell and fibroblasts (Fig. S2C and S2D). Consistent with the results in vivo, LncDACH1 expression was considerably decreased both in the lung fibroblasts isolated from BLM-induced adult mice and in the TGF-β1-induced primary mouse lung fibroblasts (PMLFs) (Fig. S2E and S2F).

3.2. Overexpression of LncDACH1 alleviates TGF-β1-induced fibrogenesis in mouse lung fibroblasts

To further understand whether LncDACH1 acts on fibroblasts, we constructed LncDACH1 plasmids to successfully enhance LncDACH1 expression in PMLFs (Fig. 2A). The mRNA expression of Fn1 and Coll1a1 and Col3a1 and Acta2 was upregulated in PMLFs induced by TGF-β1, but these alterations were reversed after LncDACH1 overexpression (Fig. 2B). At the same time, the protein expression of FN1 and Collagen I was lower in LncDACH1-transfected PMLFs than that in TGF-β1-induced cells (Fig. 2C). Since activation of fibroblasts is an important feature of pulmonary fibrosis, we evaluated the regulatory effects of LncDACH1 on the function of PMLFs. LncDACH1 was confirmed to suppress the increased cell proliferation induced by TGF-β1 according to EdU fluorescence staining (Fig. 2D). The results of a wound-healing assay showed that LncDACH1 inhibited the migratory ability of lung fibroblasts (Fig. 2E). Immunofluorescence staining also showed that the transition of fibroblasts into α-
**Figure 3**  
*LncDACH1* regulates SRSF1 expression. (A) A FISH probe demonstrates that *LncDACH1* was mainly expressed in the nucleus in PMLFs; scale bar = 20 µm; *n* = 4. (B) A Venn diagram shows the number of *LncDACH1*-binding proteins predicted by RBPmap, RBPDB and catRAPID database. (C) The protein level of SRSF1 in lung tissue of *LncDACH1*-KO mice was significantly higher than that of WT mice; *n* = 5. (D) In PMLFs, the expression of SRSF1 was regulated by *LncDACH1*; *n* = 5. (E) Cells were treated with CHX for 0, 1, 2, 4 h, respectively, to observe the degradation of SRSF1 protein by *LncDACH1*; *n* = 3. (F) The ability of *LncDACH1* full length or *LncDACH1* (835-1251) to bind SRSF1 was confirmed by RNA pull-down; *n* = 3. (G) RNA-binding protein immunoprecipitation (RIP) confirmed the interaction between *LncDACH1* and SRSF1 in vitro; *n* = 3. (H) FISH and immunofluorescence staining in PMLFs was performed to detect expression and localization of *LncDACH1* and SRSF1; scale bar = 10 µm; *n* = 4. Data are presented as mean ± SEM; *P* < 0.05; **P** < 0.01. WT, wild-type; PMLFs, primary mouse lung fibroblasts; CHX, cycloheximide.
LncDACH1 inhibits lung fibrosis 3609

3.3. LncDACH1 binds to SRSF1 to regulate its expression and activity

The cellular localization of lncRNAs largely determines their mechanism of action. Therefore, we designed FISH probes and found that LncDACH1 was expressed in both cytoplasm and nucleus, but mainly localized in the nucleus under physiological conditions (Fig. 3A). Recently, studies have reported that lncRNAs could directly bind to proteins and then regulate protein expression and activity. We hypothesized that LncDACH1 participated in fibrogenesis by interacting with nuclear proteins. By using the RNA binding protein interaction database, including RBPmap (http://rbpmap.technion.ac.il/manual.html)16, RBPDB (http://rbpdb.ccbrc.utoronto.ca/)19 and catRAPID (http://service.tartaglialab.com/page/catrapid_group)20, the proteins binding with LncDACH1 were predicted (Fig. 3B). Among the 9 predicted proteins, SRSF1 attracted our interest because it primarily binds the LncDACH1 conserved sequence (Supporting Information Fig. S3A–S3D). As illustrated in Fig. 3C, the protein level of SRSF1 was increased in the lungs of LncDACH1-KO mice. PMLFs transfected with LncDACH1 overexpression plasmid inhibited the upregulation of SRSF1 induced by TGF-β1 (Fig. 3D). In addition, we used the translational inhibitor cycloheximide to detect the effect of LncDACH1 on SRSF1 stability in a time-dependent manner. Protein analysis showed that the overexpression of LncDACH1 augmented the degradation of SRSF1 protein (Fig. 3E). Moreover, the proteasome inhibitor MG132 did not inhibit degradation of SRSF1 (Supporting Information Fig. S4A), and there was no significant change in the amount of ubiquitin protein binding to SRSF1 in the control group and the overexpressed LncDACH1 group (Fig. S4B). These results indicate that LncDACH1 does not rely on the proteasome or ubiquitination to degrade SRSF1. We next performed an RNA pull-down assay to explore whether LncDACH1 directly bound to SRSF1. As expected, protein expression of SRSF1 was detected by Western blotting after extraction of RNA—protein complexes (Fig. 3F). An RNA binding protein immunoprecipitation (RIP) assay was carried out to evaluate the relationship between LncDACH1 and SRSF1. We observed an enrichment of LncDACH1 in the anti-SRSF1 antibody precipitates compared with the IgG precipitates (Fig. 3G). Moreover, overexpression LncDACH1 visibly attenuated the SRSF1 fluorescence in PMLFs (Fig. 3H). Taken together, our results show that LncDACH1 binds to SRSF1 to regulate its expression.

3.4. SRSF1 is necessary for the anti-fibrotic effects of LncDACH1

To investigate the role of the LncDACH1 interaction with SRSF1 in lung fibrosis, we simultaneously transfected an LncDACH1 overexpression plasmid and an adenovirus-containing SRSF1 overexpression plasmid (Adv-SRSF1) into PMLFs. The infection efficiency of Adv-SRSF1 was determined (Supporting Information Fig. S5A). At the mRNA level, enhanced expression of SRSF1 inhibited the negative regulation of LncDACH1 on Fn1, Col1a1, Col3a1, and Acta2 (Fig. 4A). SRSF1 also exhibited a promoting effect on the protein expression of FN1 and Collagen I (Fig. 4B), indicating that the inhibitory effect of LncDACH1 on fibrosis and collagen deposition was restored by the expression of SRSF1.

Likewise, PMLFs with increased SRSF1 showed stronger proliferative ability compared with LncDACH1-overexpressing cells (Fig. 4C). In the context of TGF-β1, SRSF1 impeded the inhibitory effect of LncDACH1 on cell migration and promoted the transition of fibroblasts into myofibroblasts (Fig. 4D and E). These findings suggest that SRSF1 acts as a downstream target gene of LncDACH1, and mediates the antifibrotic effect of LncDACH1.

3.5. LncDACH1 inhibits Cmbnl expression by targeting SRSF1

Previous studies have shown that SRSF1 promotes β-catenin protein accumulation by recruiting Cmbnl mRNA and enhancing its biosynthesis21. Cmbnl is a key effector of the Wnt signaling pathway and is involved in the pathogenesis of pulmonary fibrosis22. We took lung tissues from LncDACH1-KO mice for Western blot assays and found significant upregulation of the β-catenin protein level (Fig. 5A). Subsequently, we found that LncDACH1 could reverse the increased β-catenin expression induced by TGF-β1 in vitro (Fig. 5B). However, the suppression of β-catenin protein by LncDACH1 was abolished with SRSF1 overexpression (Fig. 5C). Moreover, immunofluorescent staining showed that forced expression of LncDACH1 inhibited the expression and nuclear translocation of β-catenin in TGF-β1-treated PMLFs, whereas this effect was significantly attenuated by SRSF1 overexpression (Fig. 5D).

Next, we constructed small interfering RNAs (siRNAs) to silence LncDACH1 and Cmbnl and verified their efficiency (Supporting Information Fig. S5B and S5C). As illustrated in Fig. 5E, silencing of LncDACH1 promoted the expression and nuclear translocation of β-catenin, whereas this effect was markedly inhibited by Cmbnl knockdown. qRT-PCR results showed that LncDACH1 deficiency led to the upregulation of Fn1, Col1a1, Col3a1, and Acta2, while silencing Cmbnl reversed the above changes (Fig. 5F). Knockdown of Cmbnl inhibited the increased protein expression of FN1, Collagen I, SRSF1 and β-catenin caused by LncDACH1 deficiency (Fig. 5G). In addition, LncDACH1 knockdown was sufficient to induce the proliferation and migration of PMLFs, which was abolished by knockdown of Cmbnl (Fig. 5H and I). According to the results of immunofluorescence, the expression of α-SMA was increased in LncDACH1-silenced cells, while the inhibition of Cmbnl reversed the differentiation of fibroblasts into myofibroblasts (Fig. 5J). Transfection with si-LncDACH1 promoted β-catenin expression, and si-Srsf1 co-expression attenuated expression (Supporting Information Fig. S6). Thus, our data reveal that Cmbnl is a key step in the regulation of lung fibrosis by LncDACH1-targeting of SRSF1.

3.6. Enhanced expression of LncDACH1 prevents BLM-induced pulmonary fibrosis in mice

To verify whether LncDACH1 exerts anti-fibrotic effects in vivo, we constructed an adenovirus associated virus 5 (AAV-5) overexpression plasmid containing LncDACH1, named AAV5-LncDACH1. We administered AAV5-LncDACH1 intratracheally 14 days before BLM induction to observe the preventative effect of LncDACH1 on lung fibrosis. Mice were euthanized on Day 21 after BLM injection and lung tissues were obtained for subsequent studies (Fig. 6A). LncDACH1 increased in the lungs of AAV5-LncDACH1-treated mice and decreased in the lungs of BLM-treated mice (Supporting Information Fig. S7A). According to qRT-PCR analysis, the mRNA levels of Fn1, Col1a1, Col3a1 and Acta2 were suppressed by prophylactic LncDACH1 (Fig. 6B).
Figure 4  Overexpression of SRSF1 limits LncDACH1 function. (A) qRT-PCR results indicate that the overexpression of SRSF1 normalized the inhibitory effect of LncDACH1 on the mRNAs of fibrosis-related genes; n = 6. (B) Protein expression of FN1, Collagen I and SRSF1 after simultaneous overexpression of LncDACH1 and SRSF1; n = 5. (C) EdU results (scale bar = 50 μm; n = 5) and (D) wound healing assays (scale bar = 200 μm; n = 5) show the proliferation and migration of PMLFs after Adv-SRSF1 and LncDACH1 treatment. (E) Immunofluorescence analysis shows that LncDACH1 inhibited the transition of fibroblasts into myofibroblasts by suppressing the expression of SRSF1. This effect of LncDACH1 was abolished after overexpression of SRSF1. Scale bar = 20 μm; n = 5. Data are presented as mean ± SEM; *P < 0.05; **P < 0.01. Adv-SRSF1, SRSF1 overexpression adenovirus.
Western blots detected that FN1 and Collagen I induction by BLM was impeded by AA V5-LncDACH1 (Fig. 6C). In addition, SRSF1 and β-catenin, the downstream targets of LncDACH1, were similarly downregulated (Fig. 6C). Hydroxyproline content in lung tissues of AA V5-LncDACH1-treated mice was lower compared with that in AA V5-pcDNA3.1-treated mice after treatment with BLM (Fig. 6D). After the lung tissues were fixed with paraformaldehyde and subjected to H&E and Masson staining, the mice injected with AA V5-LncDACH1 in advance showed obvious improvement in lung tissue morphology and fibrotic area, indicating that LncDACH1 inhibited fibrogenesis in vivo (Fig. 6E and F). As shown by immunohistochemistry and immunofluorescence, the increased expression of Collagen I and α-SMA induced by BLM was significantly inhibited by LncDACH1 prevention (Fig. 6G–J). These results illustrate that LncDACH1 plays a protective role in BLM-induced pulmonary fibrosis.

3.7. Forced expression of LncDACH1 attenuates the progression of lung fibrosis in an established experimental model of lung fibrosis in mice

Because of the complex triggers and long disease duration of pulmonary fibrosis, early awareness and treatment are effective ways to improve survival. In the therapeutic protocol, we injected AA V5-LncDACH1 five days after BLM induction and sacrificed the mice 21 days after BLM intratracheal administration (Fig. 7A). LncDACH1 was overexpressed in the lungs of mice treated with AA V5-LncDACH1 (Fig. S7B). The experimental
Figure 6  BLM-induced lung fibrosis is suppressed by LncDACH1. (A) Diagram of the animal experimental model of preventive LncDACH1. Mice received AAV5-LncDACH1 intratracheally 14 days before BLM induction, and lung tissues were harvested 21 days later. (B) The relative expression of fibrosis-related genes induced by BLM was suppressed by prior administration of AAV5-LncDACH1; n = 6. (C) The effect of LncDACH1 on the protein levels of FN1, Collagen I, β-catenin and SRSF1 in vivo was assessed by Western blotting; n = 4. (D) Hydroxyproline content was significantly reduced in the lungs of AAV5-LncDACH1-treated mice; n = 6. (E, F) H&E and Masson staining indicated that advance treatment with LncDACH1 inhibited BLM-induced lung morphological changes and the increase in fibrotic area; scale bar = 50 μm; n = 3. (G–J) Immunohistochemical experiments and immunofluorescent staining detected lighter staining for Collagen I and α-SMA in LncDACH1-treated BLM-mice; scale bar = 50 μm; n = 3. Data are presented as mean ± SEM; *P < 0.05; **P < 0.01. BLM, bleomycin; AAV5-LncDACH1, adenovirus-associated viruses 5 containing LncRNA DACH1.
Figure 7  Inhibitory effect of therapeutic LncDACH1 on pulmonary fibrosis. (A) Diagram of the animal experimental model of therapeutic LncDACH1. Mice were intratracheally administered AAV5-LncDACH1 on Day 5 after induction by BLM and euthanized 21 days later. To observe the ameliorative effect of LncDACH1 on lung fibrosis progression. (B) mRNA levels of the fibrosis markers, Fn1, Col1a1, Col3a1, and Acta2, after LncDACH1 treatment; n = 6. (C) After fibrosis formation, LncDACH1 inhibited the relative protein expression of FN1, Collagen I, β-catenin and SRSF1; n = 4. (D) BLM increased hydroxyproline content, which was reversed upon AAV5-LncDACH1 treatment; n = 6. (E, F) Therapeutic LncDACH1 alleviated BLM-induced fibrotic changes in lung tissue; scale bar = 50 μm; n = 3. (G–J) Immunohistochemical staining and immunoﬂuorescent staining show that AAV5-LncDACH1 regulated the expression of Collagen I and α-SMA in lung tissues; scale bar = 50 μm; n = 3. Data are presented as mean ± SEM; *P < 0.05; **P < 0.01. AAV5-LncDACH1, adenovirus-associated viruses 5 containing LncRNA DACH1.
Figure 8 Effects of the conserved sequence of LncDACH1 on human lung fibroblasts (MRC-5). (A, B) As shown in the qRT-PCR experiments, the suppression of mRNA expression of fibrosis markers by LncDACH1(835–1251) was consistent with that of full length LncDACH1; n = 6. (C) In MRC-5 cells, LncDACH1(full-length) and LncDACH1(835–1251) showed obvious inhibitory effects on TGF-β1-induced upregulation of FN1, Collagen I, β-catenin and SRSF1 at the protein levels; n = 4. The proliferation and migration of MRC-5 cells was detected by (D) EdU
results are consistent with the preventative regimen. After BLM induction of pulmonary fibrosis in mice, the mRNA and protein levels of fibrosis-related genes were suppressed by the enhanced expression of LncDACH1 (Fig. 7B and C). The protein expression of SRSF1 and β-catenin was similarly regulated by LncDACH1 treatment (Fig. 7C). Hydroxyproline content was not obviously different from normal control mice after LncDACH1 treatment, but significantly decreased in mice exposed to BLM (Fig. 7D). In response to therapeutic AAV-LncDACH1 treatment, the lung tissues of mice showed less fibrotic area, lighter Collagen I and α-SMA staining (Fig. 7E–J), indicating that LncDACH1 is able to impede the progression of lung fibrosis. What’s more, we concurrently overexpressed LncDACH1 and SRSF1 in mice. Our results reveal that LncDACH1 attenuated BLM-induced fibrosis in mice, and SRSF1 reversed the function of LncDACH1 (Supporting Information Fig. S8). These results demonstrate the antifibrotic effects of the LncDACH1/SRSF1/Cnmbl axis.

3.8. Anti-fibrotic role of the conserved sequence of LncDACH1 in MRC-5 cells

LncDACH1 exhibited potent protective effects against pulmonary fibrosis in a mouse model and in primary mouse lung fibroblasts. We wished to determine if LncDACH1 also had the same effect on human derived fibroblasts (MRC-5 cells). Since the excessively long nucleotide sequence of LncDACH1 limits its medicinal potential, we identified a sequence of LncDMCH1 (800–1200) likely to bind to SRSF1 (shown in Fig. 3B), based on a previous study that showed that the LncDMCH1(835–1251) fragment is functional and highly conserved. We detected SRSF1 and LncDACH1 (835–1251) interaction by RNA-pull down assay (Fig. 3H). qRT-PCR analysis showed that LncDACH1(835–1251) expression was suppressed in MRC-5 cells after induction by TGF-β1 (Fig. 8A). With regard to fibrogenesis, LncDMCH1(835–1251) significantly alleviated TGF-β1-induced mRNA expression of FN1, COL1A1, COL3A1 and ACTA2, while suppressing the up-regulated protein level of FN1, Collagen I, β-catenin and SRSF1, and the efficiency was consistent with that of full-length LncDACH1 (Fig. 8B and C). In addition, we found that the LncDMCH1(835–1251) inhibited migration, proliferation and differentiation of MRC-5 cells (Fig. 8D–F). Immunofluorescence assays showed that LncDMCH1(835–1251) inhibited the activity of β-catenin by reducing its nuclear translocation (Fig. 8G). In summary, we have demonstrated that the conserved sequence of LncDACH1 has an ameliorative effect on the activation of human fetal lung fibroblasts.

4. Discussion

In the present study, we found that LncDACH1 was downregulated in lung tissues of IPF patients and in BLM-treated mice, and LncDACH1-KO mice spontaneously exhibit pulmonary fibrosis. Mechanistically, we further show that LncDACH1 modulated Cnmbl expression by targeting SRSF1, ultimately suppressing lung fibroblast differentiation and ECM deposition. Moreover, we identified the anti-fibrotic effects of LncDACH1 in vivo, providing a potential preventive and therapeutic target for IPF.

A growing understanding of IPF processes suggests that the heterogeneity analyses in cells contributes to mechanistic exploration. Due to the limitation of single cell sequencing in the detection of lncRNA, we analyzed the human DACH1 (LncDACH1 conserved sequence) expression in different cell types. The results suggest that DACH1 is mainly enriched in fibroblasts and endothelial cells. Also, the successful clinical trials of pirfenidone and nintedanib, FDA-approved antifibrosis agents, highlight the importance of the epithelial cells and fibroblasts in pulmonary fibrosis.

We used mouse lung fibroblasts and human fetal lung fibroblast MRC-5 cells to investigate the underlying mechanism of LncDACH1, and found that LncDACH1 can inhibit the activation of lung fibroblasts by negatively regulating the expression and activity of SRSF1. We validated Cnmbl as a downstream target of SRSF1 in fibroblasts, and the upregulation of SRSF1 promoted β-catenin protein expression. As expected, overexpression of SRSF1 alleviated the protective effect of LncDACH1 against fibrogenesis, both in PMLFs and in MRC-5. Correspondingly, the knockdown of Cnmbl rescued the fibrogenesis triggered by LncDACH1 silencing. We carried out an in-depth study on the protective effect of LncDACH1 and found that whether used for treatment or prevention, LncDACH1 could attenuate the extent and progression of pulmonary fibrosis in BLM-induced mice. Based on the present results, we demonstrate that the LncDACH1/SRSF1/YCTNNB1 pathway plays a crucial role in pulmonary fibrosis and may be a novel drug target.

LncDACH1 initially attracted attention because its upregulation in the failing hearts of humans and mice. Many studies have confirmed the crucial role of lncRNAs. A clinical analysis by Song et al. demonstrated that LncITPF could be used as a diagnostic marker for IPF patients. Some of our previous studies of lncRNAs in pulmonary fibrosis have focused on lncRNAs as ceRNAs to regulate miRNAs, which in turn affect the expression of downstream target genes. A previous study from our group has reported that enhanced expression of lncRNA PEAR promotes lung fibrosis by targeting miR-138/YAP1-Twist axis. However, the functions of lncRNAs are considered to be more complex and diverse. Since LncDACH1 expression is mainly localized in the nucleus in fibroblasts, we predicted proteins that are likely to have the potential to bind to LncDACH1 using the catRAPID, RBPmap and RBPD database, in which we focused on the splicing factor SRSF1, and the above observations were further confirmed by RNA pull-down assay. Our work reveals for the first time that LncDACH1 competitively binds to SRSF1 in lung fibroblasts, and thereby inhibits fibroblast proliferation and differentiation, ameliorating pulmonary fibrosis.

SRSF1 is a classical member of the SR protein family, which as a regulator of alternative splicing can produce different mRNA isoforms. The necessity of the SRSF1 protein was illustrated as a regulator of alternative splicing can produce different mRNA isoforms. The necessity of the SRSF1 protein was illustrated as a regulator of alternative splicing can produce different mRNA isoforms. The necessity of the SRSF1 protein was illustrated as a regulator of alternative splicing can produce different mRNA isoforms. The necessity of the SRSF1 protein was illustrated as a regulator of alternative splicing can produce different mRNA isoforms.
in vivo, and overexpression of SRSF1 promotes the activation of lung fibroblasts. With the further understanding of SRSF1, it has been found that SRSF1 is not only involved in alternative splicing but also closely relate to mRNA transcription, nuclear output, translation, and protein sumoylation. According to research from Fu et al., SRSF1 can enhance the biosynthesis of CTNNB1, leading to the accumulation of β-catenin protein, thus leading to tumorigenesis. CTNNB1 is an essential part of the Wnt signaling pathway and also plays an important role in fibrosis. Therefore, as a downstream target of SRSF1, CTNNB1 has attracted our attention. Here, we found that the enhanced expression of SRSF1 increases the protein level of β-catenin, which further leads to the upregulation of fibrosis-relevant genes. However, the function of SRSF1 in fibrotic diseases is just beginning to be recognized.

The risk factors of IPF are very complex, and with the background of the COVID-19 pandemic, some studies have suggested that pulmonary fibrosis may be a serious consequence of SARS-CoV-2 infection. Therefore, it is more urgent to develop anti-pulmonary fibrosis drugs. Based on our results, we believe that LncDACH1/ SRSF1/CTNNB1 may become a new target for the prevention or treatment of pulmonary fibrosis. In recent years, drugs and other novel agents have been found to alleviate a variety of diseases by targeting lncRNAs. Sprangers et al. used liposomal spherical nucleic acid, which showed dose-dependent inhibition of lncRNA Malat1 and reduced cancer metastasis. Oligonucleotide-based drugs have been designed for therapeutic applications concerning lncRNAs because of the ability to precisely target RNAs. Therefore, based on our study, LncDACH1 expression was reduced in lung fibrosis models in vitro and in vivo, and overexpression of LncDACH1 alleviated collagen deposition and lung fibroblast overactivation. To further illustrate the protective role of LncDACH1 in pulmonary fibrosis, we constructed a conserved sequence plasmid of LncDACH1 containing functional domains, which was consistent with the efficiency of the full-length sequence, and similarly exhibited anti-fibrotic effects in human fetal lung fibroblasts. The discovery of functional domains largely solves the problem of excessively long nucleotide sequences of lncRNAs, which makes LncDACH1 promising as a lncRNA-targeted drug for pulmonary fibrosis.

There are some limitations to our present study. Although we demonstrated that upregulation of SRSF1 promotes the protein expression and nuclear translocation of β-catenin, we did not determine whether SRSF1 affects the degradation process of CTNNB1. Moreover, lncRNA regulates post-translational modification of proteins through ubiquitination, phosphorylation, acetylation and autophagy, thus affecting protein expression level and activity. Wang et al. found that lncRNA LINRIS promotes aerobic glycolysis in colorectal cancer by preventing the degradation of IGF2BP2 through the autophagy–lysosome pathway. Therefore, more comprehensive studies are warranted to determine how lncRNA alter protein levels. As this research was carried out without a pulmonary function test in the therapy experiment, this could be considered a limitation to this study.

5. Conclusions
Our study demonstrates that LncDACH1 negatively regulates the level of CTNNB1 by binding to SRSF1 and inhibiting SRSF1 expression, which inhibits the activation of lung fibroblasts and extracellular matrix deposition, thus participating in the process of alleviating lung fibrosis. Therefore, it is possible to utilize replacement of LncDACH1 to enhance its expression for the prevention and treatment of IPF.

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Author contributions
Haihui Liang and Hongli Shan conceived the project, designed the experiments and edited the manuscript. Jian Sun, Jiayu Guo and Tongzhu Jin planned the experiments, integrated data and wrote the manuscript. Jian Sun, Zhihui Niu, Liangliang Li, Tianyu Li, Huiying Gao and Yuhang Zhang performed cellular and molecular biological experiments and executed the data analysis. Wenxin He and Zhixin Li enrolled patients and collected human samples. Yingying Guo, Tongzhu Jin, Ruoxuan Yang, Qianqian Wang, Wenchao Ma, Wei Su, and Xingxing Fan performed animal studies and analyzed the data.

Conflicts of interest
The authors declared no conflict of interest.

Appendix A. Supporting information
Supporting data to this article can be found online at https://doi.org/10.1016/j.apsb.2022.04.006.

References
1. Liu S, Ly X, Wei X, Liu C, Li Q, Min J, et al. BTRIB3–GSK-3 interaction promotes lung fibrosis and serves as a potential therapeutic target. Acta Pharm Sin B 2021;11:3105–19.
2. George PM, Spagnolo P, Kreuter M, Altinisik G, Bonifazi M, Martinez FJ, et al. Progressive fibrosing interstitial lung disease: clinical uncertainties, consensus recommendations, and research priorities. Lancet Respir Med 2020;8:925–34.
3. Liu G, Philp A, Corte T, Travis M, Schilter H, Hansbro N, et al. Therapeutic targets in lung tissue remodelling and fibrosis. Pharmacol Ther 2021;225:107839.
4. Cameli P, Refini R, Bergantini L, d’Alessandro M, Alonzi V, Magnoni C, et al. Long-term follow-up of patients with idiopathic pulmonary fibrosis treated with pirfenidone or nintedanib: a real-life comparison study. Front Mol Biosci 2020;7:581828.
5. Li H, Zhao X, Shan H, Liang H. MicroRNAs in idiopathic pulmonary fibrosis: involvement in pathogenesis and potential use in diagnosis and therapeutics. Acta Pharm Sin B 2016;6:531–9.
6. Zhang X, Hong R, Chen W, Xu M, Wang L. The role of long non-coding RNA in major human disease. Bioorg Chem 2019;92:103214.
7. Ranmarine V, Kobelev M, Gibb E, Nouri M, Lin D, Wang Y, et al. The evolution of long noncoding RNA acceptance in prostate cancer initiation, progression, and its clinical utility in disease management. Eur Urol 2019;76:546–59.
LncRNA DACH1 inhibits lung fibrosis

8. Wang Y, Fang Z, Hong M, Yang D, Xie W. Long-noncoding RNAs (lncRNAs) in drug metabolism and disposition, implications in cancer chemoresistance. *Acta Pharmac Sin B* 2020;10:105–12.

9. Cai B, Wu W, Wang X, Sukhareva N, Hua B, Zhang L, et al. Targeting lncDACH1 promotes cardiac repair and regeneration after myocardial infarction. *Cell Death Differ* 2020;27:2158–75.

10. Deng Y, Chen D, Wang L, Gao F, Jin B, Lv H, et al. Silencing of long noncoding RNA Nespa aggravates microglial cell death and neuro-inflammation in ischemic stroke. *Stroke* 2019;50:1850–8.

11. Jiang X, Zhang F. Long noncoding RNA: a new contributor and potential therapeutic target in fibrosis. *Epigenomics* 2017;9:1233–41.

12. Feng M, Tang P, Huang X, Sun S, You Y, Xiao J, et al. TGF-β mediates renal fibrosis via the Smad3–Erbb4–IR long noncoding RNA axis. *Mol Ther* 2018;26:148–61.

13. Zhao X, Sun J, Chen Y, Su W, Shan H, Li Y, et al. lncRNA PFAR promotes lung fibroblast activation and fibrosis by targeting miR-138 to regulate the YAP1–Twist axis. *Mol Ther* 2018;26:2206–17.

14. Dykes I, Emanuelli C. Transcriptional and post-transcriptional gene regulation by long non-coding RNA. *Dev Reprod Biol* 2017;15:177–86.

15. Cai B, Zhang Y, Zhao Y, Wang J, Li T, Zhang Y, et al. Long noncoding RNA-DACH1 (Dachshund homolog 1) regulates cardiac function by inhibiting SERCA2a (sarcoplasmic reticulum calcium ATPase 2a). *Hypertension* 2019;74:833–42.

16. Habermann A, Gutierrez A, Bui L, Yahn S, Winters N, Calvi C, et al. Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *Sci Adv* 2020;6:eaaba1972.

17. Neumark N, Cosme C, Rose K, Kaminski N. The idiopathic pulmonary fibrosis cell Atlas. *Am J Physiol Lung Cell Mol Physiol* 2020;319:L887–93.

18. Paz I, Kosti I, Ares M, Cline M, Mandel-Gutfreund Y. RBPmap: a web server for mapping binding sites of RNA-binding proteins. *Nucleic Acids Res* 2014;42:W361–7.

19. Cook K, Kazan H, Zuberi K, Morris Q, Hughes T. RBPDDB: a database of RNA-binding specificities. *Nucleic Acids Res* 2011;39:D301–8.

20. Agostini F, Zanoni A, Klus P, Marchese D, Cirillo D, Tartaglia G. catRAPID omics: a web server for large-scale prediction of protein–RNA interactions. *Bioinformatics* 2013;29:2928–30.

21. Fu Y, Huang B, Shi Z, Han J, Wang Y, Huangfu J, et al. SRSF1 and SRSF9 RNA binding proteins promote Wnt signalling-mediated tumorigenesis by enhancing beta-catenin biosynthesis. *EMBO Mol Med* 2013;5:737–50.

22. Cao P, Aoki Y, Badri L, Walker N, Manning C, Lagstein A, et al. Autocrine lysosphosphatic acid signaling activates β-catenin and promotes lung allograft fibrosis. *J Clin Invest* 2017;127:1517–30.

23. Noble P, Albera C, Bradford W, Costabel U, Glassberg M, Kardatzke D, et al. Pirfenidone in patients with idiopathic pulmonary fibrosis (CAPACITY): two randomised trials. *Lancet* 2011;377:1760–9.

24. Richeldi L, du Bois R, Raghu G, Azuma A, Brown K, Costabel U, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med* 2014;370:2071–82.

25. Song X, Xu P, Meng C, Song C, Blackwell T, Li R, et al. IncITPF promotes pulmonary fibrosis by targeting hnRNP-L depending on its host gene ITGB1. *Mol Ther* 2019;27:380–93.

26. Das S, Krainer A. Emerging functions of SRSF1, splicing factor and oncoprotein, in RNA metabolism and cancer. *Mol Cancer Res* 2014;12:1195–204.

27. Möröy T, Heyd F. The impact of alternative splicing in vivo: mouse models show the way. RNA 2007;13:1155–71.

28. Paz S, Ritchie A, Mauer C, Caputi M. The RNA binding protein SRSF1 is a master switch of gene expression and regulation in the immune system. *Cytokine Growth Factor Rev* 2021;57:19–26.

29. Sheng J, Zhao Q, Zhao J, Zhang W, Sun Y, Qin P, et al. SRSF1 modulates PTPMT1 alternative splicing to regulate lung cancer cell radiosensitivity. *EBioMedicine* 2018;38:113–26.

30. Pozzi B, Mammì P, Bragado L, Giono L, Strebrow A. When SUMO met splicing. *RNA Biol* 2018;15:689–95.

31. Miao J, Liu J, Niu J, Zhang Y, Shen W, Luo C, et al. Wnt/β-catenin/RAS signaling mediates age-related renal fibrosis and is associated with mitochondrial dysfunction. *Aging Cell* 2019;18:e13004.

32. Wu Y, Xu L, Cao G, Min L, Dong T. Effect and mechanism of Qingfei Paidu decoction in the management of pulmonary fibrosis and COVID-19. *Am J Chin Med* 2022;50:33–51.

33. Sprangers A, Hao L, Banga R, Mirkin C. Liposomal spherical nucleic acids for regulating long noncoding mas in the nucleus. *Small* 2017;13:1602753.

34. Chen Y, Li Z, Chen X, Zhang S. Long non-coding RNAs: from disease code to drug role. *Acta Pharm Sin B* 2021;11:340–54.

35. Wang Y, Lu J, Wu Q, Jin Y, Wang D, Chen Y, et al. LncRNA LINRIS stabilizes IGF2BP2 and promotes the aerobic glycolysis in colorectal cancer. *Mol Cancer* 2019;18:174.