

Research on the role and mechanism of SLC7A5 in idiopathic pulmonary fibrosis

MENGHAN WANG^{1*}, YAO YU^{1*}, ZHAOYU LIU^{1*}, DANDAN XU², YUPENG LI¹,
XING ZOU³, NING SHAN¹, XINING LIU¹, CE SHI³ and HONG CHEN¹

¹Department of Pulmonary and Critical Care Medicine, The Second Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150086, P.R. China; ²Department of Respiratory Medicine, Heilongjiang Provincial Hospital, Harbin, Heilongjiang 150001, P.R. China; ³NHC Key Laboratory of Cell Transplantation, The First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang 150001, P.R. China

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Abstract. Idiopathic pulmonary fibrosis (IPF) is a progressive, chronic and fatal interstitial lung disease. The etiology of IPF remains to be fully elucidated and its prognosis remains poor with a short survival period. *Solute carrier family 7 member 5 (SLC7A5)* is an amino acid transporter that is responsible for the transportation of large neutral amino acids. The expression of *SLC7A5* was found to be significantly upregulated in patients with IPF, as well as in the lung tissues of mice with bleomycin (BLM)-induced pulmonary fibrosis and in human lung fibroblasts induced by transforming growth factor- β 1 (TGF- β 1). Both *SLC7A5* knockdown and the use of the selective *SLC7A5* inhibitor JPH203 affected the function of TGF- β 1-induced myofibroblasts by inhibiting the PI3K/AKT/mTOR pathway. *In vivo*, JPH203 improved BLM-induced pulmonary fibrosis in mice by inhibiting the activation of the PI3K/AKT/mTOR

signaling pathway. Therefore, *SLC7A5* may represent a new therapeutic target for IPF. Future research should focus on therapeutic strategies regulating this target for translation into potential effective treatments for IPF in clinical practice.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a progressive, chronic and fatal interstitial lung disease, yet the cause of this disease remains ambiguous (1). The pathological features of IPF include fibroblast proliferation, myofibroblast differentiation, collagen synthesis, increased extracellular matrix (ECM) production and interstitial thickening, which lead to gas-exchange impairment and ventilation restriction (2). The primary clinical features of IPF include decreased lung function, aggravated pulmonary fibrosis shown by high-resolution computed tomography, deterioration of symptoms and patient quality of life, and premature patient mortality (3). However, the preferred anti-fibrotic drugs for IPF treatment, nintedanib and pirfenidone, have been shown to only slow the decline of lung function in a subset of patients and cannot prolong patient survival (4–6). Therefore, it is necessary to conduct in-depth research on the pathogenesis of IPF in order to identify potential therapeutic targets and improve the prognosis of this disease, thereby extending the survival period of patients with IPF.

Myofibroblasts represent the typical pathological fibroblast phenotype in the lungs of patients with IPF and several mediators, including transforming growth factor- β (TGF- β)1, have been shown to promote the differentiation of fibroblasts into myofibroblasts (7). Myofibroblasts secrete a large amount of ECM, including components such as collagen and fibrin, which: i) Thickens the alveolar walls; ii) leads to the formation of fibrous scars, the destruction of alveolar structures and functional loss in the alveoli; and iii) ultimately results in pulmonary fibrosis (8). The anti-fibrotic drugs pirfenidone and nintedanib have been shown to exert anti-fibrotic effects in IPF by inhibiting the transformation of pulmonary fibroblasts into pulmonary myofibroblasts (9).

The *solute carrier family 7 member 5 (SLC7A5)* gene encodes L-type amino acid transporter 1, which is one of the members of the system L amino acid transporter protein

Correspondence to: Professor Ce Shi, NHC Key Laboratory of Cell Transplantation, The First Affiliated Hospital of Harbin Medical University, 23 Youzheng Street, Nangang, Harbin, Heilongjiang 150001, P.R. China
E-mail: shice@hrbmu.edu.cn

Professor Hong Chen, Department of Pulmonary and Critical Care Medicine, The Second Affiliated Hospital of Harbin Medical University, 246 Xuefu Road, Nangang, Harbin, Heilongjiang 150086, P.R. China
E-mail: chenhong744563@aliyun.com

*Contributed equally

Abbreviations: BCAA, branched-chain amino acid; BLM, bleomycin; DLCO, diffusing capacity for carbon monoxide; ECM, extracellular matrix; FVC, forced vital capacity; GSEA, Gene Set Enrichment Analysis; H&E, hematoxylin and eosin; IPF, idiopathic pulmonary fibrosis; PPI, protein-protein interaction; TGF- β 1, transforming growth factor- β 1

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family (10). *SLC7A5* is responsible for transporting various important amino acids, such as histidine, isoleucine and leucine, as well as a number of hormones and drugs, across cell membranes (11). The abnormal expression of *SLC7A5* has been shown to associate with a variety of diseases, such as cancer, insulin resistance, immune disorders and a number of neurological diseases (12). On the other hand, JPH203 is a novel selective inhibitor targeting *SLC7A5*. Due to its therapeutic efficacy in tumor models, as demonstrated in a preclinical experiment, JPH203 represents a promising anti-tumor drug (13). However, there are no clear reports on the roles of *SLC7A5* and JPH203 in the treatment of pulmonary-fibrotic diseases.

The present study demonstrated that *SLC7A5* was upregulated in patients with IPF and animal and cell models of lung fibrosis. By inhibiting the expression or function of *SLC7A5*, the transformation of lung fibroblasts into lung myofibroblasts can be inhibited by blocking the activation of the PI3K/AKT/mTOR signaling pathway. Finally, the present study demonstrated that JPH203 exerted anti-fibrotic effects in lung-fibrosis model mice. Based on these findings, the present study elucidated the potential impact of *SLC7A5* on IPF, providing a theoretical basis for identifying new targets for the prevention and treatment of IPF.

Materials and methods

Analysis of differentially expressed genes. The Gene Expression Omnibus (GEO) database was searched for datasets on patients with IPF, and differential gene analysis was subsequently conducted on datasets GSE49072, GSE40839, GSE135065 and GSE150910 (14-17) using the 'limma' package (version 3.66.0) in R (4.5.2). The screening criterion for differentially expressed genes included $|\log_2\text{fold-change}| > 1$ and an adjusted P-value < 0.05 . Venn diagrams were generated using the R package ggvenn (version 0.1.19).

Survival analysis. The GEO database was searched for datasets containing survival information regarding patients with IPF. Survival analysis was conducted using datasets GSE70866 and GSE27957 (18,19). The Kaplan-Meier method was used to analyze the overall survival period of patients with IPF, defining death as the endpoint event. Patients in each dataset were divided into high- and low-expression groups based on the optimal cutoff value of *SLC7A5* mRNA expression, which was determined separately for each dataset: 6.78 for GSE70866 and 7.03 for GSE27957, and log-rank tests were performed to compare the survival of patients between groups. The survival curves were visualized using the R package 'survminer' (version 0.4.9) and a risk table was provided (Fig. 1).

Transcriptome sequencing analysis. RNA was extracted from HFL1 cells using the total RNA extraction kit [Seven Innovation (Beijing) Biotechnology Co., Ltd.; cat. no. SM132] and sent to Novogene for sequencing. The quality and integrity of the processed RNA samples were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Sequencing libraries were prepared using the Fast RNA-seq Lib Prep Kit V2 (cat. no. RK20306; ABclonal Biotech Co., Ltd.) according to the manufacturer's instructions. The library

concentration was determined by quantitative PCR (qPCR), and the final loading concentration of the library was 1.5 ng/ μ l. Sequencing was performed on the NovaSeq X Plus platform (Illumina, Inc.) with paired-end 150 bp (PE150) sequencing reads. The 'limma' package (version 3.66.0) in R (4.5.2) was used to conduct differential analysis on the expression data from transcriptome sequencing.

Gene Ontology (GO) functional enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were both conducted using the 'clusterProfiler' package in R. Gene Set Enrichment Analysis (GSEA) was performed using the GSEA_4.3.2 software. The hallmark gene sets, C2 curated gene set collection and KEGG subset from the Molecular Signatures Database (MSigDB; <https://www.gsea-msigdb.org/gsea/msigdb/collections.jsp#C2>) was used for GSEA. GSEA was performed on the transcriptomic data of datasets GSE101286 (20) and GSE135065. Furthermore, the differentially expressed genes identified via transcriptome sequencing were inputted into the Search Tool for the Retrieval of Interacting Genes/Proteins database platform (v12.0; cn.string-db.org) in order to conduct protein-protein interaction (PPI) analyses. $P < 0.05$ and false discovery rate < 0.25 were considered to indicate statistically significant differences.

Animal breeding and in vivo experiments. In the present study, healthy wild-type male C57BL/6J mice aged 8-10 weeks were selected as the experimental animals. Mice were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Charles River Laboratories, Inc.) and each mouse weighed ~ 25 g. The mice used in the present study were raised in a specific pathogen-free environment. The environmental conditions were maintained at a constant indoor temperature of 20-26°C with a relative humidity of 40-70%, 12-h light/dark cycle, and noise controlled < 60 dB to provide a quiet environment. The mice were given *ad libitum* access to sufficient drinking water and food, which had been sterilized. The mouse cages and bedding materials were sterilized by autoclaving (high-pressure steam) and regularly replaced each week. All animal experiments involved in the present study were approved by the Experimental Animal Protection and Ethics Committee of Harbin Medical University (Harbin, China; approval no. YJSDW2023-024).

A pulmonary fibrosis animal model was established in C57BL/6J mice via intratracheal instillation of bleomycin (BLM; cat. no. s1214; Selleck Chemicals). Mice were fasted and dehydrated for 1-2 h before anesthesia. Subsequently, mice were anesthetized by inhalation of isoflurane (induction concentration, 4%; maintenance concentration, 2%) and 3.5 mg/kg of BLM was injected into the trachea of model mice to establish a model of IPF; mice in the normal saline (NS) control group were injected with the same volume of NS.

JPH203 (cat. no. TQ0081; TargetMol Chemicals Inc.) was used to treat mice with pulmonary fibrosis induced by BLM. From days 1 to 13 following the establishment of the BLM model, mice were administered JPH203 by intraperitoneal injection daily, with mice divided into additional groups based on drug doses (12.5 and 25 mg/kg/d).

Immediately after the BLM infusion was completed, the anesthesia recovery status of mice was observed, which included monitoring the breathing, heart rate and body temperature of

mice. After modeling, mice in the NS, BLM group and BLM + JPH203 groups were housed separately. The health status of mice was observed daily for 14 consecutive days without interruption after BLM induction, which involved monitoring for changes in body weight, appearance of the mice, clinical symptoms, behavioral responses and social behaviors. On the 14th day following BLM infusion, the mice were euthanized and lung, heart, liver, spleen and kidney tissues were collected. Mice were euthanized before reaching the experimental endpoint if the following humane endpoints were displayed: i) A decrease in body weight of 20-25%; ii) cachexia or wasting symptoms; iii) breathing difficulties, specifically regarding saliva flowing from the mouth and nose, cyanosis, arching of the back or lying down; iv) a complete loss of appetite for 24 h or a poor appetite (<50% of normal appetite) for 3 days; v) inability to eat or drink without anesthesia; or vi) mice were only able stand with great difficulty for 24 h. These humane endpoints were similar to those reported in previous studies (21,22). Euthanasia was performed by inducing deep anesthesia via inhalation of 4% isoflurane followed by rapid dislocation of the head and neck. After euthanasia, death was confirmed via the following observations: i) No respiratory movement in the chest or nostrils; ii) a lack of heartbeat observed through palpation of the femoral artery; iii) using a flashlight to illuminate the pupils, no contraction response was observed; and iv) no pain response or limb withdrawal after pressing the toes of euthanized mice.

A total of 42 mice were used in the present study (NS group, n=14; BLM group, n=14; BLM + 12.5 mg/kg group, n=7; BLM + 25 mg/kg group, n=7). A total of 2 mice were euthanized prematurely, and 3 mice died prior to reaching humane endpoint criteria; the deaths of these mice were observed between two daily welfare checks, predominantly occurring between days 1 to 7 following model establishment, which is referred to as the acute lung injury stage (23,24). Before these 3 mice died, they did not exhibit any of the typical symptoms of respiratory distress, such as saliva flowing from the mouth and nose, cyanosis, arching of the back or lying down, nor did these mice meet the other predetermined humane endpoint criteria of the present study. The cause of the death in these mice was speculated to be BLM-related lung injury and respiratory deficiency (21,25,26). The mortality rate of BLM-induced model mice in the present study was within the range of 10-80% as reported in previous literature (27-29). The remaining 37 mice all reached the 14-day set endpoint.

Cell culture. The human embryonic lung fibroblast cell lines HFL1 (iCell-h017) and MRC-5 (iCell-h146) were both purchased from iCell Bioscience Inc. The authenticity of these cell lines was confirmed through short tandem repeat analysis. MRC-5 cells were cultured in DMEM (cat. no. C3103-0500; Shanghai VivaCell Biosciences, Ltd.), whereas HFL1 cells were cultured in F12K medium (cat. no. iCell-0007; iCell Bioscience Inc.). Cells were cultured in an incubator at a constant temperature of 37°C with 5% CO₂. Before experiments using these cells, all cells were tested for mycoplasma and those showing negative results for mycoplasma contamination were used for subsequent formal experiments.

Recombinant TGF-β1 protein (cat. no. TMPY-02638; TargetMol Chemicals Inc.) was added to the culture medium of both cell lines at a concentration of 10 ng/ml. After incubation

with recombinant TGF-β1 for 48-72 h, the cells were collected for subsequent experiments. For the JPH203-treatment group in each cell line, 10 ng/ml of recombinant TGF-β1 protein and the corresponding concentration of JPH203 (cat. no. TQ0081; TargetMol Chemicals Inc.) were added simultaneously to the cell culture media. For control cells, an equal volume of dimethyl sulfoxide (DMSO; cat. no. KGL2329-100; Jiangsu KeyGen Biotech Co., Ltd.) was added to the culture media, with the total concentration of DMSO not exceeding 1%.

Cell transfection with siRNA. HFL1 and MRC-5 were transfected with siRNAs using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions, the final concentration of siRNAs is 30 nM. Cells were maintained at 37°C in a 5% CO₂ incubator. The transfection medium was removed after 8 h and replaced with fresh culture medium. For verification of siRNA knockdown efficiency, cells were harvested at 24 h post-transfection for RNA extraction and at 48 h post-transfection for protein extraction. For wound healing assays, cells were harvested at 48 h post-transfection for both RNA and protein analyses. For all other experiments, cells were harvested at 96 h post-transfection for RNA and protein extraction. The siRNA sequences used were synthesized by Seven Innovation (Beijing) Biotechnology Co., Ltd. The specific sequences of siRNAs were as follows: siRNA sequence targeting *SLC7A5* (si-*SLC7A5*), forward GGGUGAUGUGUCCAAUCUA, reverse UAGAUGGACACAUCACCC; and negative control siRNA (si-NC), forward UUCUCCGAACGUGUCACGU, reverse ACGUGACACGUUCGGAGAA.

Transfection of overexpression plasmids. The coding sequence for human *SLC7A5* was cloned into the pCDNA3.4 vector to create a pCDNA3.4-*SLC7A5* overexpression construct. The unmodified pCDNA3.4 plasmid was used throughout the present study as an empty vector control; both the blank vector and *SLC7A5* overexpression plasmid were synthesized by Wuhan MiaoLing Biotech Science Co., Ltd. HFL1 cells were transiently transfected with the overexpression plasmid using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. A total of 4 μg plasmid DNA and 10 μl Lipofectamine® 2000 were diluted separately in Opti-MEM® (cat. no. 31985062; Thermo Fisher Scientific, Inc.), gently mixed and incubated at room temperature for 5 min to form a complex. Then, this complex was added to the cells. Cells were maintained at 37°C in a 5% CO₂ incubator. The transfection medium was removed after 8 h and replaced with fresh culture medium. Total RNA and proteins were extracted from transfected HFL1 cells 48-72 h after transfection and subjected to RT-qPCR and western blotting assays respectively to verify the overexpression of the target protein. For cells in the JPH203 co-treatment group, cells were incubated with JPH203 (20 μM) 24 h after transfection.

Wound healing assay. A tightly packed monolayer of HFL1 cells was scratched in a straight line (when cells were completely attached), and siRNAs or JPH203 were added as appropriate. After the scratch, the cells were cultured in 1% serum (341506; Sigma-Aldrich; Merck KGaA) medium.

Images were captured at the same position under an optical microscope (TH4-200; Olympus Corporation) after incubation at 37°C for 0, 24, and 48 h. The scratch area was calculated using the ImageJ 1.54d image analysis software (National Institutes of Health).

EdU cell proliferation assay. Cells were treated with the EdU-555 Cell Proliferation Assay Kit (Beyotime Biotechnology) according to the manufacturer's instructions. Cells were observed and images were subsequently captured under a fluorescence microscope (TH4-200; Olympus Corporation).

Cell Counting Kit-8 (CCK-8). Cell viability in HFL1 cells was detected using a Cell Counting Kit [Seven Innovation (Beijing) Biotechnology Co., Ltd.] according to the manufacturer's instructions. A blank well containing culture medium and CCK-8 solution without cells was used for background correction. The optical density value of each well was measured using an enzyme detector (SpectraMax M5; Molecular Devices, LLC) at a wavelength of 450 nm. The following formula was used to calculate cell viability: Cell viability (%) = $[A(\text{with drug}) - A(\text{blank})] / [A(\text{no drug}) - A(\text{blank})] \times 100$ (A = optical density).

Apoptosis assay. HFL1 cells were divided into three groups: DMSO, TGF- β 1 + DMSO and TGF- β 1 + 20 μ M JPH203. After processing the cells, the same number of cells (1×10^4) were collected from each treatment group. According to the manufacturer's instructions (cat. no. C1062; Beyotime Biotechnology), annexin V-fluorescein isothiocyanate and propidium iodide were used to stain the cells. The apoptotic rate of the cells was detected using a flow cytometer (BD FACSCelesta; BD Biosciences), and the results were analyzed using FlowJo_v10.8.1 software (BD Biosciences).

Protein extraction and western blotting. PMSF solution (cat. no. ST506; Beyotime Biotechnology) and phosphatase inhibitors (cat. no. HY-K0021; MedChemExpress) were added to RIPA lysis buffer (cat. no. P0013B; Beyotime Biotechnology) to prepare the cell lysis solution. A total of 1×10^6 cells were lysed in approximately 100 μ l of cell lysis solution, and cells were harvested by gently scraping with a cell scraper. For mouse tissues, 20–30 mg of lung tissue was added to 250 μ l lysis solution and subsequently ground on ice using an electric grinder. Cells or tissues were lysed on ice for 30 min and subsequently centrifuged at 14,000 $\times$ g for 10 min at 4°C. Protein concentration was measured using a BCA kit (cat. no. P0010; Beyotime Biotechnology). The standard curve formula for protein concentration measurements was as follows: $y = 0.397x + 0.191$; R^2 , 0.998. Proteins in each group were adjusted to reach the same volume and mass (15 μ l; 20 μ g) using the cell lysis solution and loading buffer (cat. no. LT101S; Shanghai Epizyme Biopharmaceutical Technology Co., Ltd.; Ipsen Pharma) and were subsequently heated at 100°C for 10 min.

PAGE gel (7.5%) was prepared according to the instructions of the PAGE Gel Rapid Preparation Kit (cat. no. PG111; Shanghai Epizyme Biopharmaceutical Technology Co., Ltd.; Ipsen Pharma). Denatured protein samples were added to the wells of the gel and pre-stained protein markers (cat. no. WJ103;

Shanghai Epizyme Biopharmaceutical Technology Co., Ltd.; Ipsen Pharma) were added at both ends of the protein sample. Electrophoresis was performed using fresh electrophoretic solution (cat. no. P0561; Beyotime Biotechnology) at a constant voltage of 80 V until the samples entered the stacking gel (~30 min), and then at 120 V until the bromophenol blue dye front reached the bottom of the separating gel (approximately 1–2 h). Proteins were transferred onto PVDF membranes (cat. no. 03010040001; Roche Diagnostics (Shanghai) Co., Ltd.) at 300 mA for 90 min. The membranes were blocked with 5% non-fat milk [SW128; Seven Innovation (Beijing) Biotechnology Co., Ltd.] in TBS with 0.05% Tween [TBST; SW142; Seven Innovation (Beijing) Biotechnology Co., Ltd.] for 1 h at room temperature. The membrane was washed with TBST and then incubated with primary antibodies at 4°C overnight. The membranes were subsequently incubated with the appropriate secondary antibodies. For mouse primary antibodies, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (1:5,000; cat. no. RS0001; Immunoway) was used. For rabbit primary antibodies, HRP-conjugated goat anti-rabbit IgG (1:5,000; cat. no. RS0002; Immunoway) was used. Incubation was performed at room temperature for 1 h with gentle shaking. After washing with TBST, the membranes were exposed to the MeilunBio® fg super sensitive ECL luminescence reagent (cat. no. MA0186, MeilunBio) for protein band visualization. For target proteins with similar or identical molecular weights, the same membrane was used for sequential detection by stripping and reprobing. After imaging the first target protein, the membrane was incubated with a stripping buffer (cat. no. CW0056M; Jiangsu CoWin Biotech Co., Ltd.) at room temperature for 15 min to remove the primary and secondary antibodies, washed thoroughly with TBST, blocked with 5% non-fat milk in TBST for 1 h at room temperature, and then re-probed with the antibody against the second target protein. Band intensity was detected using the Tanon 5200 (Tanon Science and Technology Co., Ltd.) and the gray values of the bands were analyzed using the ImageJ 1.54d software (National Institutes of Health). Primary antibodies used in the present study included: GAPDH (1:5,000; cat. no. 10494-1-AP; Proteintech Group, Inc.), β -actin (1:5,000; cat. no. 66009-1-Ig; Proteintech Group, Inc.), human SLC7A5 (1:1,000; cat. no. ab305251; Abcam), mouse SLC7A5 (1:1,000; cat. no. DF8065; Affinity Biosciences), fibronectin 1 (FN1; 1:2,000; cat. no. 66042-1-Ig; Proteintech Group, Inc.), collagen type 1 α 1 (COL1A1; 1:1,000; cat. no. 14695-1-AP; Proteintech Group, Inc.), α -smooth muscle actin; (α -SMA; 1:1,000; cat. no. ab5694; Abcam), Bcl-2 (1:1,000; cat. no. F0125; Selleck Chemicals), Bax (1:1,000; cat. no. F0037; Selleck Chemicals), phosphorylated-(p)-PI3K (1:1,000; cat. no. AP0427; ABclonal Biotech Co., Ltd.), PI3K (1:1,000; cat. no. ab191606; Abcam), p-AKT (1:1,000; cat. no. 4060; CST Biological Reagents Co., Ltd.), AKT (1:1,000; cat. no. 4691; CST Biological Reagents Co., Ltd.), p-mTOR (1:1,000; cat. no. 5536; CST Biological Reagents Co., Ltd.) and mTOR (1:1,000; cat. no. 2983; CST Biological Reagents Co., Ltd.).

RT-qPCR. Total RNA was extracted from HFL1 cells, MRC-5 cells and mouse lung tissues using the total RNA extraction kit [Seven Innovation (Beijing) Biotechnology Co., Ltd.; cat. no. SM132]. cDNA was synthesized according

Table I. Primer sequences.

Gene	Forward sequence, 5'-3'	Reverse sequence, 5'-3'
Human SLC7A5	GGGGACCATTATCGGCTCG	CCGCCCCGATTTGGAGATGG
Human FN1	GCGAAGGGAAGCAAACCTGG	ATTTGCTGAGCCTGCCTCTT
Human COL1A1	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAAC
Human α -SMA	AAAGCAAGTCCTCCAGCGTT	TTCACAGGATTCTGGGAGCG
Human GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
Mouse SLC7A5	GGTCTCTGTTTCACGTCCTCAAG	GAACACCAGTGATGGCACAGGT
Mouse FN1	ATGTGGACCCCTCCTGATAGT	GCCCAGTGATTTTCAGCAAAGG
Mouse COL1A1	TAAGGGTCCCAATGGTGAGA	GGGTCCCTCGACTCCTACAT
Mouse α -SMA	TGTGACAGAGGCACCACTGAA	CAGTTGTACGTCCAGAGGCATAG
Mouse GAPDH	TGGCCTTCCGTGTTCTCTAC	GAGTTGCTGTTGAAGTCGCA

SLC7A5, solute carrier family 7 member 5; FN1, fibronectin 1; COL1A1, collagen type 1 α 1; α -SMA, α -smooth muscle actin.

to the instructions of the Uni All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (TransGen Biotech Co., Ltd.; cat. no. AU341-02-V2). RT-qPCR was performed using the PerfectStart® Green qPCR SuperMix (TransGen Biotech Co., Ltd.). The thermocycling conditions were as follows: initial denaturation at 94°C for 30 sec, followed by 40 cycles of denaturation at 94°C for 5 sec and annealing/extension at 60°C for 30 sec. The qPCR was performed on the SLAN-96S using the SLAN 8.2.2.7z software (Shanghai Hongshi Medical Technology Co., Ltd.). Gene expression was calculated using the following formulae: $\Delta Cq = Cq(\text{target gene}) - Cq(\text{reference gene})$; $\Delta\Delta Cq = \Delta Cq(\text{study sample}) - \Delta Cq(\text{control sample})$; total fold-change = $2^{-\Delta\Delta Cq}$ (30). The sequences of the primers used are detailed in Table I.

Immunofluorescence. HFL1 cells were subjected to immunofluorescence analysis in the following groups: si-NC group, si-NC + TGF- β 1 group, si-SLC7A5 + TGF- β 1 group, DMSO group, DMSO + TGF- β 1 group, and DMSO + 20 μ M group. The tissue was embedded in paraffin and the section thickness was 3 μ m. Cells and tissue sections were fixed with 4% paraformaldehyde at room temperature for 15 min. After fixing the cells and tissue sections, 0.1% Triton permeabilization solution was added. After washing, the samples were blocked with 5% goat serum at room temperature for 1 h. The cells were then incubated with primary antibodies overnight at 4°C. The samples were incubated with CoraLite488-conjugated goat anti-rabbit IgG (H+L) (1:250, cat. no. SA00013-2; Proteintech Group, Inc.) and CoraLite594-conjugated goat anti-mouse IgG (H+L) (1:250, cat. no. SA00013-3; Proteintech Group, Inc.) for rabbit and mouse primary antibodies, respectively, at room temperature for 1 h in the dark. Nuclei were counterstained with DAPI at room temperature for 10 min. After counterstaining with DAPI, images of samples were captured under a fluorescence microscope (TH4-200; Olympus Corporation). The primary antibodies used in the present study included: COL1A1 (1:200; cat. no. 14695-1-AP; Proteintech Group, Inc.) for analysis of HFL1 cells, SLC7A5 (1:100; cat. no. DF8065; Affinity Biosciences) and α -SMA (1:100; cat. no. F2514; Selleck Chemicals) for analysis of mouse lung tissues.

Immunohistochemistry. The tissue was embedded in paraffin and sectioned at 3 μ m. After tissues were dewaxed and hydrated, an endogenous peroxidase inhibitor (cat. no. PV6001; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.) was added and the sections were rinsed with PBS. Subsequently, sections were placed in immunohistochemical antigen retrieval buffer (EDTA method; cat. no. MVS0099; Fuzhou Maixin Biotech Co., Ltd.) and boiled for 10-20 min. After sections were cooled to room temperature, 5% BSA (Wuhan Servicebio Technology Co., Ltd.) was used for blocking for 30 min at room temperature. After blocking, sections were incubated with the primary SLC7A5 antibody (1:50; cat. no. DF8065; Affinity Biosciences) at 4°C overnight. The sections were then washed with PBS and incubated with the secondary antibody (cat. no. PV6001; Beijing Zhongshan Jinqiao Biotechnology Co., Ltd.), which is conjugated to horseradish peroxidase (HRP) via a polymer backbone, for 30 min at room temperature. Color development was performed according to the instructions of the DAB color development kit (cat. no. DAB0031; Fuzhou Maixin Biotech Co., Ltd.) and sections were counterstained with hematoxylin for 30 sec at room temperature. After dehydration and clearing, the sections were sealed, before sections were observed and imaged under a light microscope (VS200; Olympus Corporation). Images were processed using the Olympus OlyVIA 4.1 software. The percentage of the positive area was calculated using ImageJ 1.54d software (National Institutes of Health).

Detection of branched-chain amino acid (BCAA) content within cells. The BCAA detection kit (Beyotime Biotechnology) was used to detect BCAA content within HFL1 cell samples and was used in accordance with the manufacturer's instructions. The optical density value of each well was measured using an enzyme detector (SpectraMax M5; Molecular Devices, LLC) at a wavelength of 450 nm. A standard curve was established based on the standard sample: $y = 0.0014x + 0.1765$; R^2 , 0.9989. Subsequently, the concentration of BCAA in each sample was calculated based on the standard curve.

Hematoxylin and eosin (H&E) and Masson's trichrome staining. The stained tissues included the lungs, heart, liver, spleen and kidneys. Fresh tissues were fixed in 4%

paraformaldehyde at room temperature for 24 h. The tissue was embedded in paraffin and sectioned at 3 μ m. Samples were stained using the improved H&E staining kit [Seven Innovation (Beijing) Biotechnology Co., Ltd.; cat. no. SI107] at room temperature for 5 min in hematoxylin and 2 min in eosin. Masson's trichrome staining was performed at room temperature according to the manufacturer's protocol (Beijing Solarbio Science & Technology Co., Ltd.; cat. no. G1346), with the following key steps: Hematoxylin staining for 2 min, Ponceau-acid fuchsin staining for 5 min, phosphomolybdic acid treatment for 5 min and aniline blue staining for 5 min. Samples were observed under a light microscope and images were captured (VS200; Olympus Corporation). Images were processed using the Olympus OlyVIA 4.1 software.

Statistical analysis. All experiments were repeated at least three times. GraphPad Prism 10 software (Dotmatics) was used for the visualization and statistical analysis of the experimental results in the present study. For comparisons between two groups, unpaired t-tests were used for analysis. Pearson's correlation analysis was used to evaluate the linear correlation between two continuous variables. Two-way ANOVA was used to analyze the influence of two independent variables on the dependent variable, followed by Bonferroni's post-hoc test. One-way ANOVA was used to analyze the influence of one independent variable on the dependent variable, for which Bonferroni's post-hoc test was selected. Data are shown as mean $\pm$ SD. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Expression level of SLC7A5 is correlated with IPF. In order to explore the relationship between the expression level of SLC7A5 and IPF, differential gene analysis was conducted on the GSE49072, GSE40839, GSE135065 and GSE150910 datasets, which were retrieved from the GEO database and contained data regarding the clinical samples of patients with IPF. Differential genes identified in the four datasets were integrated and visualized in a Venn diagram (Fig. 1A). The results showed that three genes, SLC7A5, versican (VCAN) and methylenetetrahydrofolate dehydrogenase 2 (MTHFD2), were significantly differentially expressed simultaneously in all four datasets, and the expression levels of these genes were increased in IPF. Notably, VCAN and MTHFD2 have been reported to be associated with pulmonary fibrosis in several studies (31-34). However, the relationship between the high expression of SLC7A5 and the occurrence and development of IPF remains to be fully elucidated. Therefore, a deeper exploration of the correlation between SLC7A5 and patients with IPF was conducted in the present study. Compared with the healthy control group, SLC7A5 was significantly upregulated in alveolar macrophages (Fig. 1B), lung fibroblasts (Fig. 1C and D) and lung tissues (Fig. 1E) in patients with IPF, suggesting that the upregulation of SLC7A5 expression may have been associated with IPF. To explore the correlation between the expression level of SLC7A5 and related proteins in pulmonary fibrosis, transcriptomic data from the GSE40839 dataset was analyzed. Pearson's correlation analysis showed that the expression level of SLC7A5 was significantly positively

correlated with that of α -SMA, COL1A1, connective tissue growth factor (CTGF) and serpin E1 (SERPINE1) (Fig. 1F-I), further indicating the correlation between SLC7A5 expression and pulmonary fibrosis.

The results of pulmonary function tests in patients with IPF frequently show a series of characteristic changes, primarily including restrictive ventilation disorder, which manifests as a decline of indicators such as forced vital capacity (FVC) and total lung capacity, as well as reduced diffusion function, which manifests as a decline in diffusing capacity for carbon monoxide (DLCO) (35). Pearson's correlation analysis was conducted on the lung function test data of patients with IPF from two platforms (GPL6480 and GPL14550) in the GSE47460 dataset. The results of this analysis showed that in the GPL6480 platform, the expression level of SLC7A5 was negatively correlated with DLCO %pred (percent of predicted value) and FVC %pred in patients with IPF (Fig. 1J and K). In the GPL14550 platform, the expression level of SLC7A5 also exhibited a negative correlation with the DLCO %pred and FVC %pred values of patients with IPF (Fig. 1L and M). This indicated that the upregulation of SLC7A5 was correlated with the decline of pulmonary function in patients with IPF.

To explore whether the expression level of SLC7A5 affected the survival of patients with IPF, the present study conducted survival analyses using the GSE70866 and GSE27957 datasets. The results showed that in the GSE70866 dataset, high expression levels of SLC7A5 in the bronchoalveolar lavage fluid of patients with IPF was associated with a significantly higher mortality rate than patients with low SLC7A5 expression (Fig. 1N). In the GSE27957 dataset, the high expression of SLC7A5 in the peripheral blood mononuclear cells of patients with IPF was also associated with a significantly increased mortality rate compared with patients with low SLC7A5 expression (Fig. 1O). These results demonstrated that high SLC7A5 expression associated with a poor prognosis in patients with IPF. Based on the results of these bioinformatics analyses, it can be concluded that SLC7A5 was highly expressed in patients with IPF, and that the expression level of SLC7A5 was closely related to IPF development.

SLC7A5 is highly expressed in the lung tissue of mice with BLM-induced pulmonary fibrosis. To further investigate the effect of SLC7A5 on IPF, a mouse model of pulmonary fibrosis was established via intratracheal BLM administration. Compared with the NS control group, the lung tissues of mice in the BLM group showed notable morphological changes, including thickening of the alveolar walls, destruction of the alveolar septa and increased collagen content (Fig. 2A and B). The results of western blotting showed that compared with NS group mice, the expression levels of the pulmonary fibrosis markers FN1, COL1A1 and α -SMA, as well as those of the SLC7A5 protein, were significantly upregulated in the lung tissues of the BLM group mice (Fig. 2C). RT-qPCR also achieved consistent results (Fig. 2D). The results of immunohistochemical staining showed that SLC7A5 staining, as shown by the brown staining of lung tissues in the figure, was darker and covered a significantly higher area proportion in BLM group mice compared with control mice (Fig. 2E). To investigate the localization of SLC7A5 in the lung tissues of mice, the present study performed immunofluorescence co-staining for the lung

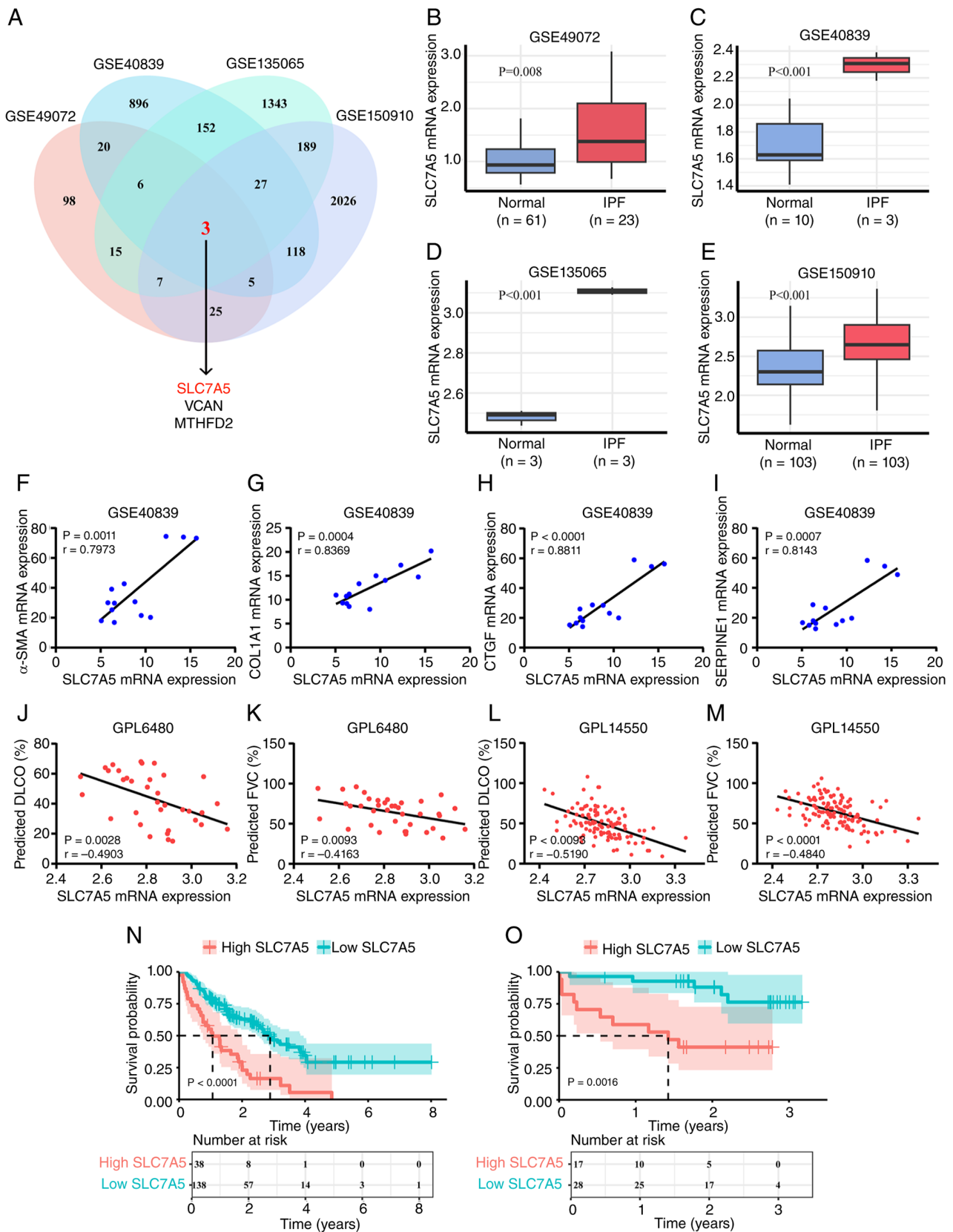


Figure 1. Correlation between *SLC7A5* expression and IPF. (A) Venn diagram showing differentially expressed genes across four GEO datasets. The expression of *SLC7A5* in control and IPF samples in the (B) GSE49072, (C) GSE40839, (D) GSE135065 and (E) GSE150910 datasets (unpaired t-test). (F) The correlation between the expression of the *SLC7A5* and α -SMA (Pearson's correlation analysis). (G) The correlation between the expression of *SLC7A5* and *COL1A1* (Pearson's correlation analysis). (H) The correlation between the expression of *SLC7A5* and *CTGF* (Pearson's correlation analysis). (I) The correlation between the expression of *SLC7A5* and *SERPINE1* (Pearson's correlation analysis). The correlation between (J) *SLC7A5* expression and DLCO and (K) *SLC7A5* expression and FVC in the GPL6480 platform of the GSE47460 dataset (Pearson's correlation analysis). The correlation between (L) *SLC7A5* expression and DLCO and (M) *SLC7A5* expression and FVC in the GPL14550 platform of the GSE47460 dataset (Pearson's correlation analysis). (N) Survival analysis of patients grouped by *SLC7A5* expression in the (N) GSE70866 and (O) GSE27957 dataset (log-rank test). DLCO, diffusing capacity for carbon monoxide; FVC, forced vital capacity; GEO, Gene Expression Omnibus; IPF, idiopathic pulmonary fibrosis.

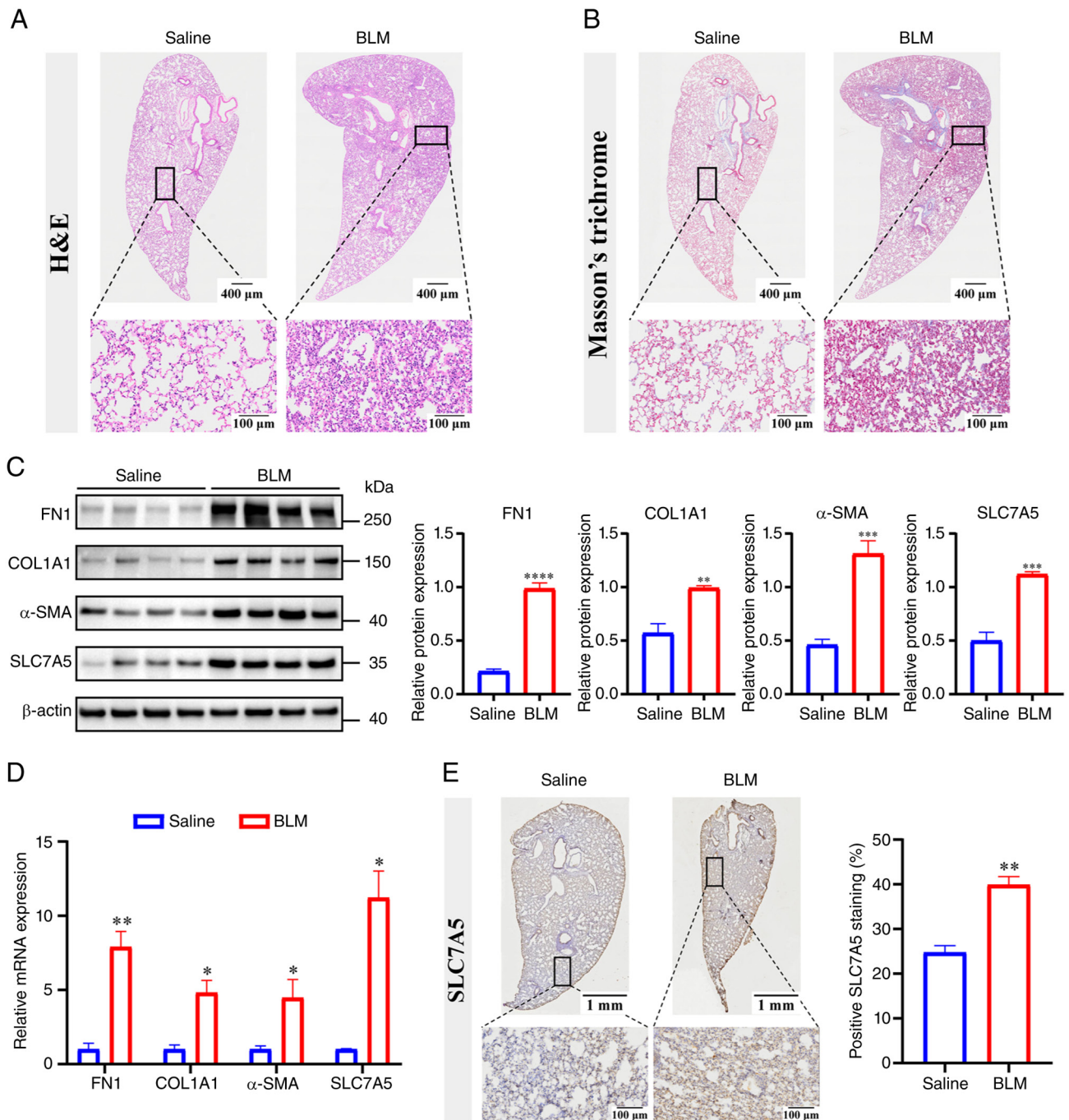


Figure 2. *SLC7A5* expression in the lung tissues of mice in the BLM group. (A) Hematoxylin and eosin and (B) Masson's trichrome staining of lung tissue sections from BLM-treated mice (n=4). (C) The expression of *SLC7A5*, *FN1*, *COL1A1* and α -SMA in the lung issue of BLM-treated mice was detected by western blotting and semi-quantified (n=4; data are shown as mean $\pm$ SD; **P<0.01, ***P<0.001 and ****P<0.0001 vs. saline group; unpaired t-test). (D) Detection of the expression of *SLC7A5*, *FN1*, *COL1A1* and α -SMA in the lung tissues of BLM mice by reverse transcription-quantitative PCR (n=4; data are shown as mean $\pm$ SD; *P<0.05 and **P<0.01; unpaired t-test). (E) Immunohistochemical staining of *SLC7A5* in control and model mice (n=4), and statistical analysis of the percentage of *SLC7A5*-positive staining (n=4; data are shown as mean $\pm$ SD; **P<0.01 vs. saline group; unpaired t-test). BLM, bleomycin; H&E, hematoxylin and eosin; FN1, fibronectin 1; COL1A1, collagen type 1 α 1; α -SMA, α -smooth muscle actin.

myofibroblast marker α -SMA together with *SLC7A5*. The results showed that in the BLM group, the expression levels of both α -SMA and *SLC7A5* were markedly upregulated, and the *SLC7A5* protein was predominantly aggregated in proximity to the α -SMA positive regions (Fig. S1). This indicated that *SLC7A5* was predominantly localized on lung myofibroblasts. The aforementioned results indicated that the *SLC7A5* was upregulated in the lung tissues of the BLM group mice, and

this upregulation was demonstrated *in vivo*, supporting the differential expression of *SLC7A5* in the pulmonary fibrosis model.

SLC7A5 is highly expressed in pulmonary myofibroblasts and is associated with the mTOR pathway. In order to verify the expression of *SLC7A5* in the *in vitro* experiments using human lung fibrosis cell models, the TGF- β 1 recombinant protein was

used to induce HFL1 and MRC-5 cell lines to transform into myofibroblasts. Western blotting and RT-qPCR results showed that in HFL1 cells, after treatment with the TGF- β 1 recombinant protein, the protein and mRNA expressions of *FNI*, *COLIA1* and α -*SMA* were significantly upregulated compared with untreated cells, indicating that fibroblasts transformed into myofibroblasts. The expression of *SLC7A5* was also significantly upregulated (Fig. 3A and B). The aforementioned results were also demonstrated in the MRC-5 cell line (Fig. 3C and D), indicating that *SLC7A5* was highly expressed in the TGF- β 1-induced lung fibrosis cell model.

To explore the potential mechanisms by which *SLC7A5* played a role in pulmonary fibrosis, transcriptome sequencing was performed on the mRNA of TGF- β 1-induced HFL1 cells. As shown in Fig. 3E and F, different expression patterns of differentially expressed genes were observed between the two treatment groups. Compared with the control group, a total of 2,459 genes were differentially expressed in the TGF- β 1 group. Some fibrosis markers showed increased expression levels in the TGF- β 1 group, such as *SERPINE1*, *FNI*, α -*SMA*, *IL-11*, *collagen type IV alpha 1 (COL4A1)*, *TGF- β 1* and *matrix metalloproteinase 1 (MMP1)* (Fig. 3G). Furthermore, the expression of *SLC7A5* in cells in the TGF- β 1 group was significantly upregulated (Fig. 3H). Further exploration of the relevant signaling pathways involved in the TGF- β 1-mediated induction of pulmonary fibroblasts involved GO functional enrichment analysis and KEGG pathway enrichment analysis. These analyses were conducted on the genes showing differential expression between the two treatment groups. Several IPF-related pathways were significantly enriched, including the Cell adhesion, Extracellular matrix, and TGF- β signaling pathway (Fig. 3I and J). GSEA comparing the gene expression profiles between the two groups revealed three mTOR-related signaling pathways that were enriched in different gene sets (HALLMARK, C2 and KEGG), and all of these pathways were significantly upregulated in the TGF- β 1 group (Fig. 3K). Notably, *SLC7A5*, as one of the core genes contributing notably to the enrichment score, was enriched in all three identified mTOR signaling pathways. To verify the activation of mTOR signaling in TGF- β 1-induced pulmonary fibroblasts, western blotting was used to detect p-mTOR and mTOR expression. The results showed that the p-mTOR/mTOR ratio was significantly increased in the TGF- β 1 group compared with the control group, suggesting the activation of this pathway (Fig. 3L). These results indicated that *SLC7A5* may have regulated the mTOR signaling pathway in order to affect the formation of pulmonary myofibroblasts and the occurrence and development of IPF.

Knockdown of *SLC7A5* inhibits the formation of pulmonary myofibroblasts. To investigate whether *SLC7A5* had any effect on the occurrence and development of IPF, *SLC7A5* was knocked down using siRNA in HFL1 cells. Initially, the knockdown efficiency of si-*SLC7A5* was measured via RT-qPCR and western blotting. The results showed that, compared with the si-NC group, the mRNA and protein expression levels of *SLC7A5* in the si-*SLC7A5* group were significantly decreased (Fig. 4A and B). To investigate whether silencing the expression of *SLC7A5* would affect the cell function of pulmonary myofibroblasts, the present study conducted

wound healing assays and EdU proliferation tests on transfected HFL1 cells. The results showed that, compared with the si-NC group, the cell migration and proliferation ability of si-NC-transfected cells were significantly increased after the addition of TGF- β 1. Furthermore, compared with the si-NC + TGF- β 1 group, the cell migration and proliferation ability of the si-*SLC7A5* + TGF- β 1 group were significantly decreased (Fig. 4C-E). Western blotting analysis revealed that, compared with the si-NC + TGF- β 1 group, the protein expression of *FNI*, *COLIA1* and α -*SMA* in the si-*SLC7A5* + TGF- β 1 group was significantly reduced (Fig. 4F and G). These results were also supported at the mRNA level (Fig. 4H). Immunofluorescence staining of HFL1 cells indicated that the expression of *COLIA1* in the si-*SLC7A5* + TGF- β 1 group was decreased compared with that in the si-NC + TGF- β 1 group (Fig. 4I). These results suggested that silencing *SLC7A5* expression affected the cellular functions of pulmonary myofibroblasts.

In order to verify the reliability of siRNA-mediated knockdown and the universality of subsequent changes in pulmonary fibrosis markers in different human lung fibroblast cell lines, the siRNA sequences in the present study were also used to knock down *SLC7A5* in MRC-5 cells. At both the mRNA and protein levels, significant knockdown of *SLC7A5* expression was observed (Fig. S2A and B). Similarly, western blotting and RT-qPCR results showed that after knocking down *SLC7A5*, the expression levels of *FNI*, *COLIA1* and α -*SMA*, which were also induced by TGF- β 1 treatment in MRC-5 cells, were also significantly reduced (Fig. S2C and D). These results suggested that the upregulation of *SLC7A5* may be the key factor causing the formation and development of pulmonary fibrosis.

Selective inhibitor JPH203 of *SLC7A5* inhibits the formation of pulmonary myofibroblasts. JPH203 is a selective inhibitor of *SLC7A5* that predominantly exerts effects in cancers, such as gastric, colon and bile duct cancer, by inhibiting the uptake of leucine (36). However, to the best of our knowledge, there is currently no literature reporting whether this inhibitor can be used to treat pulmonary fibrosis. Primarily, CCK-8 assays were performed on HFL1 cells treated with various concentrations of JPH203. The results of these assays indicated that, compared with the control group, JPH203 significantly inhibited the cell viability of pulmonary myofibroblasts and exerted both concentration-dependent and time-dependent effects (Fig. 5A). This inhibitory effect was partially reversed via overexpression of *SLC7A5* at 24 and 48 h (Fig. S3A-D). Based on the results of the CCK-8 assay, the working concentrations of JPH203 for subsequent experiments were set at 20 and 30 μ M, as these concentrations elicited moderate inhibition without excessive cytotoxicity, while higher concentrations (40 μ M) led to a marked reduction that could confound the interpretation of pathway-specific effects. Subsequently, BCAA content, including leucine, isoleucine and valine levels, was detected in HFL1 cells following treatment with JPH203. As shown in Fig. 5B, after incubating HFL1 cells with JPH203 for 48 h, the content of BCAA in the TGF- β 1 + 20 μ M and TGF- β 1 + 30 μ M groups were significantly reduced compared with the TGF- β 1 + DMSO group. These results indicated that in lung fibroblasts, JPH203 specifically exerted its pharmacological effects by inhibiting the translocation of BCAA from the extracellular space to the intracellular space by targeting *SLC7A5*.

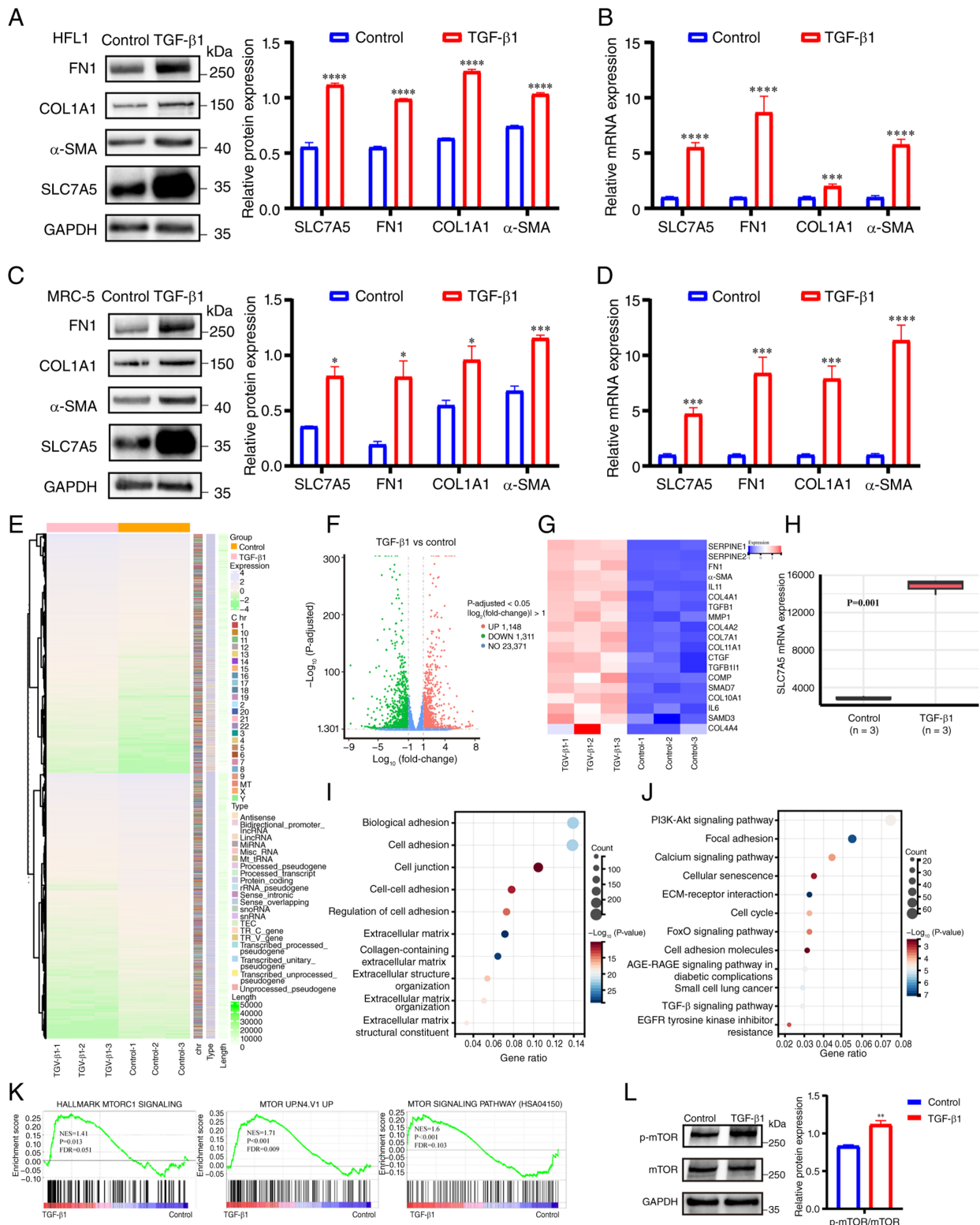


Figure 3. Transcriptome sequencing analysis revealed that *SLC7A5* was upregulated in TGF- β 1-induced lung fibroblasts and was associated with the mTOR signaling pathway. (A) The expression of *SLC7A5* and fibrosis markers in TGF- β 1-induced HFL1 cells was detected by western blotting and semi-quantified (n=3; data are shown as mean $\pm$ SD; ****P<0.0001 vs. control; unpaired t-test). (B) Relative mRNA expression of *SLC7A5* and fibrosis markers in TGF- β 1-induced HFL1 cells was detected by RT-qPCR (n=3; data are shown as mean $\pm$ SD; ***P<0.001 and ****P<0.0001 vs. control; unpaired t-test). (C) The expression of *SLC7A5* and fibrosis markers in TGF- β 1-induced MRC-5 cells was detected by western blotting and semi-quantified (n=3; data are shown as mean $\pm$ SD; *P<0.05 and ***P<0.001 vs. control; unpaired t-test). (D) Relative mRNA expression of *SLC7A5* and fibrosis markers in TGF- β 1-induced MRC-5 cells was detected by RT-qPCR (n=3; data are shown as mean $\pm$ SD; **P<0.01 and ****P<0.0001 vs. control; unpaired t-test). (E) Heatmap of differentially expressed genes obtained via transcriptome sequencing of control and TGF- β 1-induced HFL1 cells. (F) Volcano plot of differentially expressed genes between the TGF- β 1 group and the control group. (G) Heatmap of the expression of upregulated fibrosis markers in the treatment two groups. (H) The expression levels of *SLC7A5* in the TGF- β 1 and control groups (n=3; unpaired t-test). (I) Gene Ontology functional analysis comparing the two treatment groups. (J) Kyoto Encyclopedia of Genes and Genomes functional analysis comparing the two treatment groups. (K) Gene Set Enrichment Analysis comparing the two treatment groups. (L) Verification of the activation of the mTOR pathway in the TGF- β 1 group was performed via western blotting, and results were semi-quantified (n=3; data are shown as mean $\pm$ SD; **P<0.01; unpaired t-test). TGF- β 1, transforming growth factor- β 1.

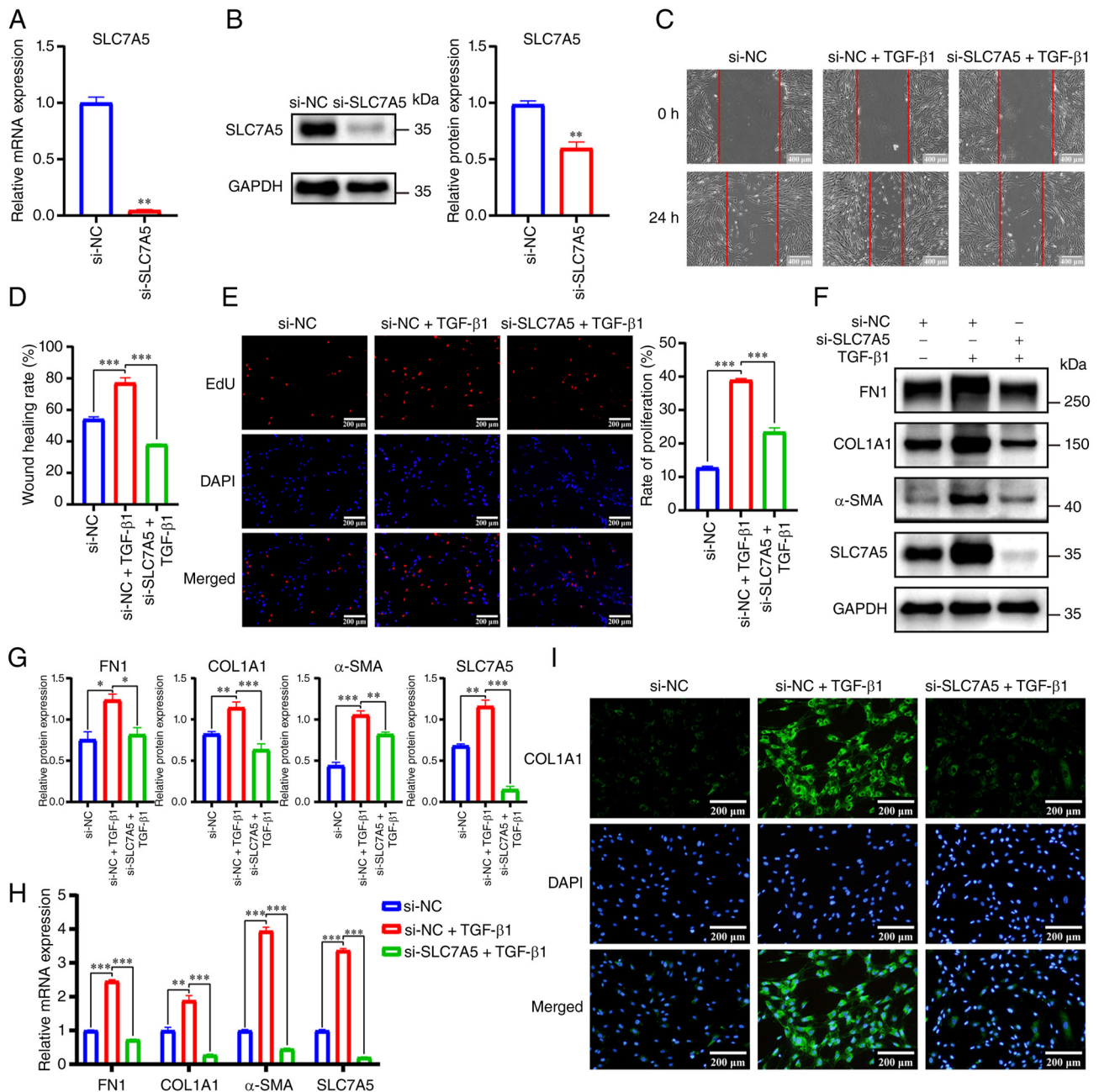


Figure 4. Knockdown of *SLC7A5* inhibits the formation of pulmonary myofibroblasts. (A) The knockdown efficiency of siRNA was verified by RT-qPCR (n=3; data are shown as mean ± SD; **P<0.01 vs. si-NC group; unpaired t-test). (B) The knockdown efficiency of siRNA was verified by western blotting (n=3; data are shown as mean ± SD; **P<0.01 vs. si-NC; unpaired t-test). (C) Wound healing assays were performed to detect the migration of lung myofibroblasts. Scale bar, 200 μm (n=3). (D) Statistical analysis of wound healing rate among the three treatment groups (n=3; data are shown as mean ± SD; ***P<0.001; one-way ANOVA). (E) The proliferation of lung myofibroblasts was detected through EdU staining (scale bar, 100 μm) and subsequently quantified (n=3; data are shown as mean ± SD; ***P<0.001; one-way ANOVA). (F) Western blotting and (G) semi-quantification of *FN1*, *COL1A1*, *α-SMA* and *SLC7A5* expression in TGF-β1-induced HFL1 cells after *SLC7A5* knockdown (n=3; data are shown as mean ± SD; *P<0.05, **P<0.01 and ***P<0.001; one-way ANOVA). (H) Detection of relative *FN1*, *COL1A1*, *α-SMA* and *SLC7A5* expression in TGF-β1-induced HFL1 cells after *SLC7A5* knockdown using RT-qPCR (n=3; data are shown as mean ± SD; **P<0.01 and ***P<0.001; one-way ANOVA). (I) The expression of *COL1A1* across the three treatment groups was observed via immunofluorescence staining. Scale bar, 100 μm (n=3). FN1, fibronectin 1; COL1A1, collagen type 1 α1; α-SMA, α-smooth muscle actin; TGF-β1, transforming growth factor-β1.

Based on the aforementioned results, 20 μM was selected as the working concentration of JPH203 for subsequent experiments. This concentration was chosen because both 20 and 30 μM yielded similar levels of target inhibition in the preliminary assays, and 20 μM was sufficient to produce a significant effect while minimizing potential off-target cytotoxicity associated with higher doses. In HFL1 cells, wound healing and EdU staining assays were used to determine the

effects of JPH203 on the migration and proliferation abilities of human lung myofibroblasts. The results demonstrated that, compared with the DMSO group, the migration and proliferation of cells in the TGF-β1 + DMSO group were significantly enhanced. Furthermore, JPH203 inhibited the enhancement of cell migration and proliferation caused by TGF-β1 treatment (Fig. 5C and D). A study has shown that TGF-β1, as a cytokine responsible for promoting fibrosis, can enhance the

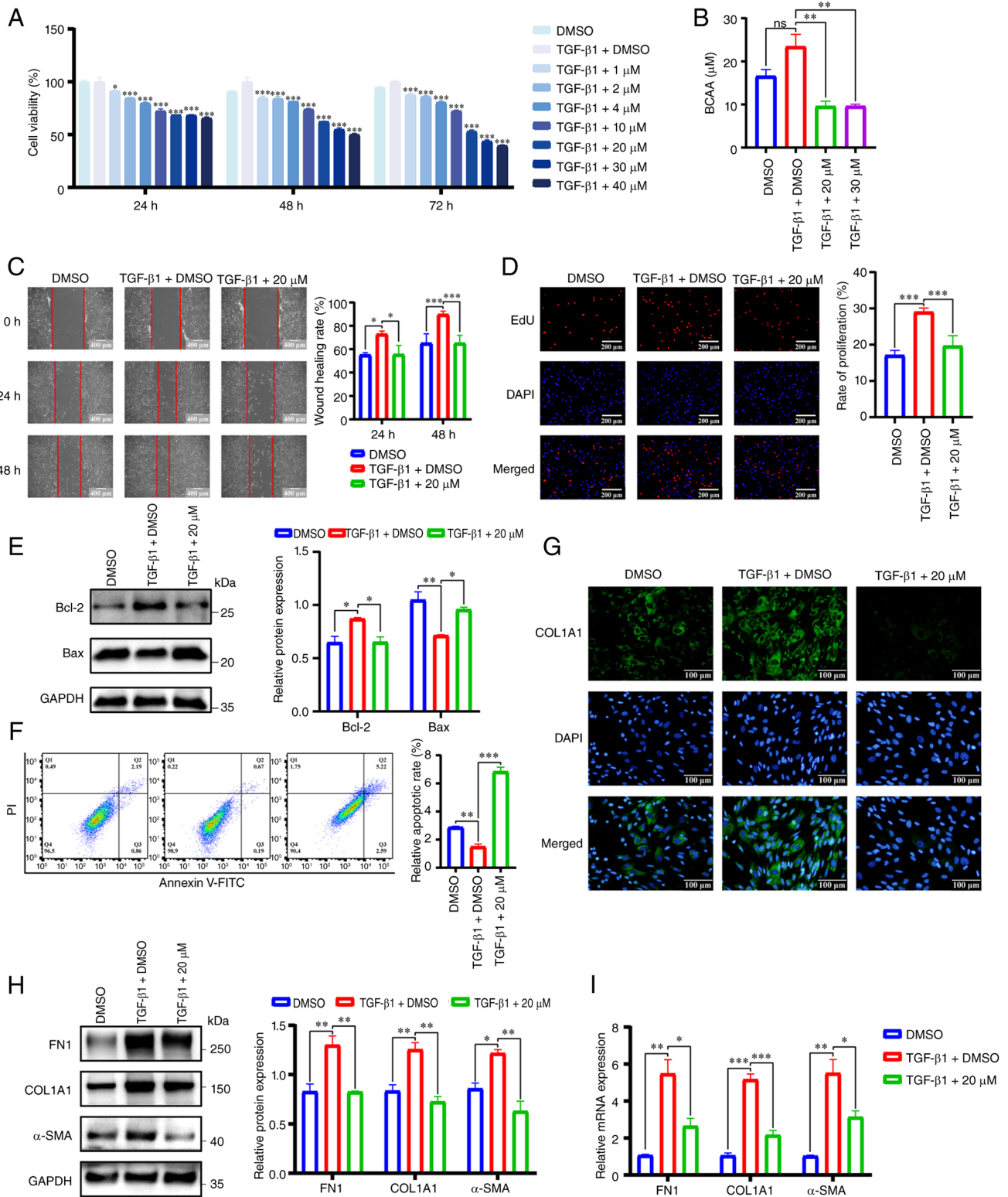


Figure 5. Selective *SLC7A5* inhibitor JPH203 inhibits the formation of pulmonary myofibroblasts. (A) The effect of JPH203 treatment on the cell viability of lung myofibroblasts at 24, 48 and 72 h was detected via Cell Counting Kit-8 assay ($n=3$; data are shown as mean $\pm$ SD; $^{*}P<0.05$ and $^{***}P<0.001$ vs. TGF-β1 + DMSO group; two-way ANOVA). (B) BCAA content in lung myofibroblasts after treatment with JPH203 ($n=3$; data are shown as mean $\pm$ SD; $^{*}P<0.01$; one-way ANOVA). (C) The migration of lung myofibroblasts after treatment with JPH203 was detected via wound healing assay (scale bar, 200 μm; $n=3$; data are shown as mean $\pm$ SD; $^{*}P<0.05$ and $^{***}P<0.001$; two-way ANOVA). (D) The proliferation of lung fibroblasts was detected through EdU staining (scale bar, 100 μm; $n=3$; data are shown as mean $\pm$ SD; $^{***}P<0.001$; one-way ANOVA). (E) The expression levels of *Bcl-2* and *Bax* in HFL1 cells treated with JPH203 were detected by western blotting and semi-quantified ($n=3$; data are shown as mean $\pm$ SD; $^{*}P<0.05$ and $^{**}P<0.01$; one-way ANOVA). (F) The proportion of apoptotic HFL1 cells after JPH203 treatment was detected using flow cytometry ($n=3$; data are shown as mean $\pm$ SD; $^{**}P<0.01$ and $^{***}P<0.001$; one-way ANOVA). (G) The expression of *COL1A1* in each treatment group was detected via immunofluorescence staining (scale bar, 50 μm; $n=3$). (H) Western blotting and semi-quantification of *FN1*, *COL1A1* and α -SMA expression in TGF-β1-induced HFL1 cells treated with JPH203 ($n=3$; data are shown as mean $\pm$ SD; $^{*}P<0.05$ and $^{**}P<0.01$; one-way ANOVA). (I) Detection of relative *FN1*, *COL1A1* and α -SMA expression in TGF-β1-induced HFL1 cells after JPH203 co-treatment using reverse transcription-quantitative PCR ($n=3$; data are shown as mean $\pm$ SD; $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$; one-way ANOVA). DMSO, dimethyl sulfoxide; FN1, fibronectin 1; COL1A1, collagen type 1 $\alpha 1$; α -SMA, α -smooth muscle actin.

resistance of lung fibroblasts to apoptosis (37), thereby inhibiting the apoptosis of lung myofibroblasts in the present study. The results of western blotting (Fig. 5E) demonstrated that the expression of *Bcl-2* in the TGF- β 1 + DMSO group was significantly elevated compared with the DMSO group, whereas the expression of *Bax* significantly decreased. Furthermore, compared with the TGF- β 1 + DMSO group, the expression of *Bcl-2* in the TGF- β 1 + 20 μ M group significantly decreased, yet the expression of *Bax* significantly increased.

In addition, the apoptotic rate of HFL1 cells treated with JPH203 was detected using flow cytometry. Compared with the DMSO group, the number of apoptotic cells in the TGF- β 1 + DMSO group significantly decreased and the proportions of early and late apoptosis were reduced. However, compared with the TGF- β 1 + DMSO group, the apoptotic rate of cells in the TGF- β 1 + 20 μ M group significantly increased, reversing the observed resistance to apoptosis induced by TGF- β 1 treatment (Fig. 5F); this indicated that JPH203 inhibited the resistance of lung myofibroblasts to cell apoptosis. In order to investigate whether JPH203 affected the expression of lung fibrosis markers, immunofluorescence staining was performed; the results of this staining showed that JPH203 co-treatment markedly inhibited the upregulation of *COL1A1* caused by TGF- β 1 treatment (Fig. 5G). Furthermore, western blotting was used to detect the protein expression of *FNI*, *COL1A1* and α -*SMA* in HFL1 lung fibroblasts following JPH203 treatment. The results showed that the protein expression levels of *FNI*, *COL1A1* and α -*SMA* in the TGF- β 1 + 20 μ M group were significantly decreased compared with the TGF- β 1 + DMSO group (Fig. 5H). These results were also supported at the mRNA level (Fig. 5I). The aforementioned results indicated that the *SLC7A5* inhibitor JPH203 inhibited the cellular functions of pulmonary myofibroblasts, and may therefore have affected the formation and development process of pulmonary fibrosis.

Modulation of SLC7A5 can inhibit the activation of the PI3K/AKT/mTOR pathway. In order to explore the mechanism by which *SLC7A5* mediated the occurrence of IPF, transcriptome sequencing analysis was conducted on the mRNA of HFL1 cells treated with si-*SLC7A5* + TGF- β 1, with the si-NC + TGF- β 1 group representing the control group. The volcano plot (Fig. S4A) and heatmap (Fig. S4B) generated from these data showed that, compared with the control group, 1,527 genes were differentially expressed in the si-*SLC7A5* + TGF- β 1 group, indicating that there were different expression patterns of genes between the two treatment groups.

Subsequently, a GO functional enrichment analysis was performed on the differentially expressed genes identified between the two treatment groups (Fig. 6A). The results demonstrated that the pathways inhibited in the si-*SLC7A5* + TGF- β 1 group included three PI3K signaling pathways. The KEGG functional enrichment analysis also demonstrated the significant downregulation of the 'PI3K-Akt signaling pathway', which plays an important role in regulating the occurrence and development of IPF (Fig. 6B). The differentially expressed genes downregulated in the si-*SLC7A5* + TGF- β 1 group were then subjected to PPI analysis; it was found that these key nodes were also enriched in the 'PI3K-Akt signaling pathway' in the KEGG dataset (Fig. 6C). Finally, GSEA was performed

between the gene expression profiles of the two groups. In the C2 gene set database, two PI3K/AKT-related signaling pathways were found to be enriched, and both pathways were significantly downregulated in the si-*SLC7A5* + TGF- β 1 group (Fig. 6D). By analyzing transcriptomic data from the GEO datasets GSE101286 and GSE135065, the PI3K/AKT-related pathway was shown to be significantly upregulated in patients with IPF (Fig. 6E). This suggested that the knockdown of *SLC7A5* inhibited certain genes in the PI3K/AKT pathway to affect the activation of the PI3K/AKT pathway.

According to the aforementioned results, *SLC7A5* may have participated in the formation of myofibroblasts by activating the mTOR signaling pathway; notably, mTOR is a downstream effector of the PI3K/AKT signaling pathway (38). Therefore, it can be inferred that inhibiting the function of the *SLC7A5* gene may have affected the occurrence of pulmonary fibrosis by inhibiting the PI3K/AKT/mTOR signaling pathway. The results of western blotting showed that, compared with the si-NC group, the p-PI3K, p-AKT and p-mTOR expression levels in the si-NC + TGF- β 1 group were significantly upregulated. Furthermore, compared with the si-NC + TGF- β 1 group, the p-PI3K, p-AKT and p-mTOR expression levels in the si-*SLC7A5* + TGF- β 1 group were significantly downregulated (Fig. 7A). On the other hand, western blotting showed that, the p-PI3K, p-AKT and p-mTOR expression levels in the TGF- β 1 + DMSO group were significantly upregulated compared with the DMSO group. Furthermore, by treating TGF- β 1-induced HFL1 cells with the JPH203 inhibitor (20 μ M), the p-PI3K, p-AKT and p-mTOR expression levels in the TGF- β 1 + JPH203 group were downregulated compared with the TGF- β 1 + DMSO group (Fig. 7B). This indicated that downregulating the expression of *SLC7A5* by siRNA-mediated knockdown or inhibiting the function of *SLC7A5* via JPH203 treatment suppressed the activation of the PI3K/AKT/mTOR signaling pathway, thereby inhibiting the formation of pulmonary myofibroblasts and possibly suppressing the occurrence and development of IPF.

JPH203 reduces the severity of pulmonary fibrosis in BLM mice by inhibiting the activation of the PI3K/AKT/mTOR pathway. In order to verify the therapeutic effect of JPH203 on pulmonary fibrosis *in vivo* and to determine whether *SLC7A5* could represent a potential therapeutic target for IPF, mice with BLM-induced pulmonary fibrosis were treated with JPH203 by intraperitoneal injection for 13 consecutive days, as shown in Fig. 8A. Intraperitoneal injection was used as the administration method of JPH203 *in vivo*, consistent with previous studies [e.g., JPH203 was administered intraperitoneally in a thyroid carcinoma mouse model (39)]. The present study selected 12.5 and 25 mg/kg as the experimental doses for JPH203 treatment. These two doses have demonstrated notable antitumor effects in multiple studies (40,41) and were tolerated well by mice in the present study.

The lung tissue sections of experimental mice were stained with H&E and Masson's trichrome, as shown in Fig. 8B. Compared with the BLM group, the pathological changes in the lungs of the BLM + 12.5 mg/kg group were notably alleviated, and the degree of pulmonary fibrosis in the BLM + 25 mg/kg group was further improved. Based on the tissue-staining results, the BLM + 25 mg/kg group was selected as the

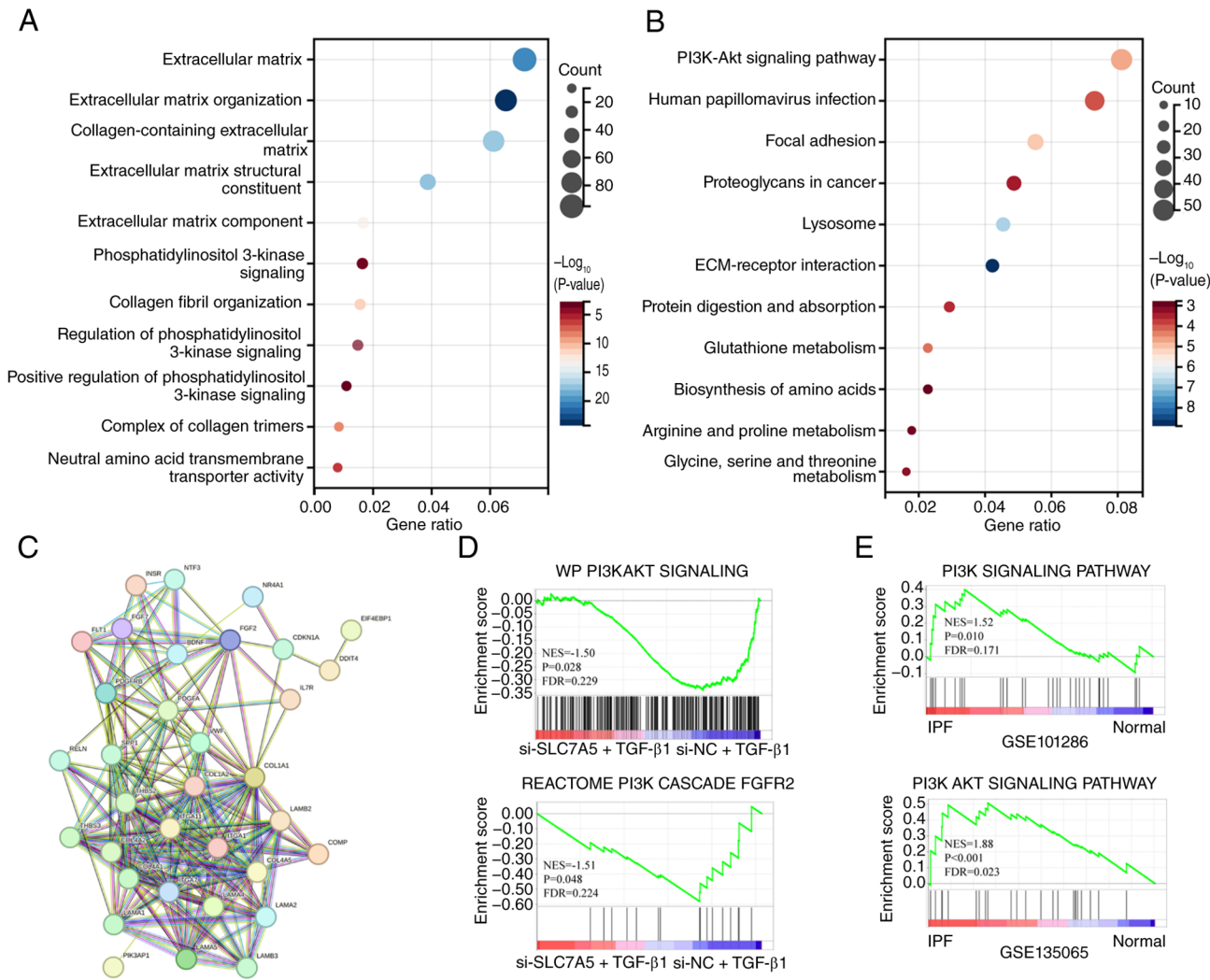


Figure 6. Functional pathway enrichment analysis of transcriptome sequencing data. (A) Gene Ontology functional analysis of transcriptome sequencing data between the si-*SLC7A5* + TGF- β 1 and si-NC + TGF- β 1 groups. (B) Kyoto Encyclopedia of Genes and Genomes functional analysis between the si-*SLC7A5* + TGF- β 1 and si-NC + TGF- β 1 groups. (C) Protein-protein interaction analysis between the si-*SLC7A5* + TGF- β 1 and si-NC + TGF- β 1 groups. (D) Gene Set Enrichment Analysis between the two transfection groups of the present study. (E) Gene Set Enrichment Analysis in GSE101286 and GSE135065 datasets. *SLC7A5*, solute carrier family 7 member 5; TGF- β 1, transforming growth factor- β 1.

treatment group for the subsequent experiments. The results of western blotting showed that, compared with the BLM group, the expression levels of *FNI*, *COL1A1* and α -*SMA* in the lung tissue of mice in the BLM + 25 mg/kg group were significantly downregulated (Fig. 8C). RT-qPCR also achieved consistent results (Fig. S5). Furthermore, the present study used western blotting to verify that the PI3K/AKT/mTOR pathway was activated in the lung tissues of mice with BLM-induced pulmonary fibrosis, whereas the activity of this pathway was significantly inhibited in the BLM + 25 mg/kg group (Fig. 8D). In addition, H&E staining pathological analysis of heart, liver, spleen and kidney tissue sections from BLM-induced mice was also conducted. The results showed that, compared with the BLM group, the experimental groups (BLM + 12.5 mg/kg and BLM + 25 mg/kg groups) demonstrated more intact tissue structures in the heart, liver, spleen and kidneys, without obvious inflammatory infiltration, cell necrosis, fibrosis or other signs of pathological damage (Fig. S6). The aforementioned results indicated that JPH203 improved BLM-induced pulmonary fibrosis in mice by inhibiting the activation of the

PI3K/AKT/mTOR pathway. Furthermore, at the therapeutic dose used in the present study, JPH203 demonstrated notable safety (Fig. S6), which provided important implications for the clinical application of JPH203.

Discussion

IPF is a fatal progressive interstitial lung disease. Current anti-fibrotic drugs have demonstrated limited efficacy, so there is a notable requirement for new therapeutic targets in IPF (1). The present study utilized bioinformatics analysis to discover that *SLC7A5* was significantly upregulated in patients with IPF; the present study also demonstrated that the expression level of *SLC7A5* was positively correlated with fibrosis markers and negatively correlated with lung function, indicating that high *SLC7A5* expression associated with poor patient prognoses. Experimental verification also showed that *SLC7A5* was upregulated in the mouse lung fibrosis model induced by BLM and in lung fibroblasts stimulated by TGF- β 1. *SLC7A5* knockdown or use of the selective *SLC7A5* inhibitor

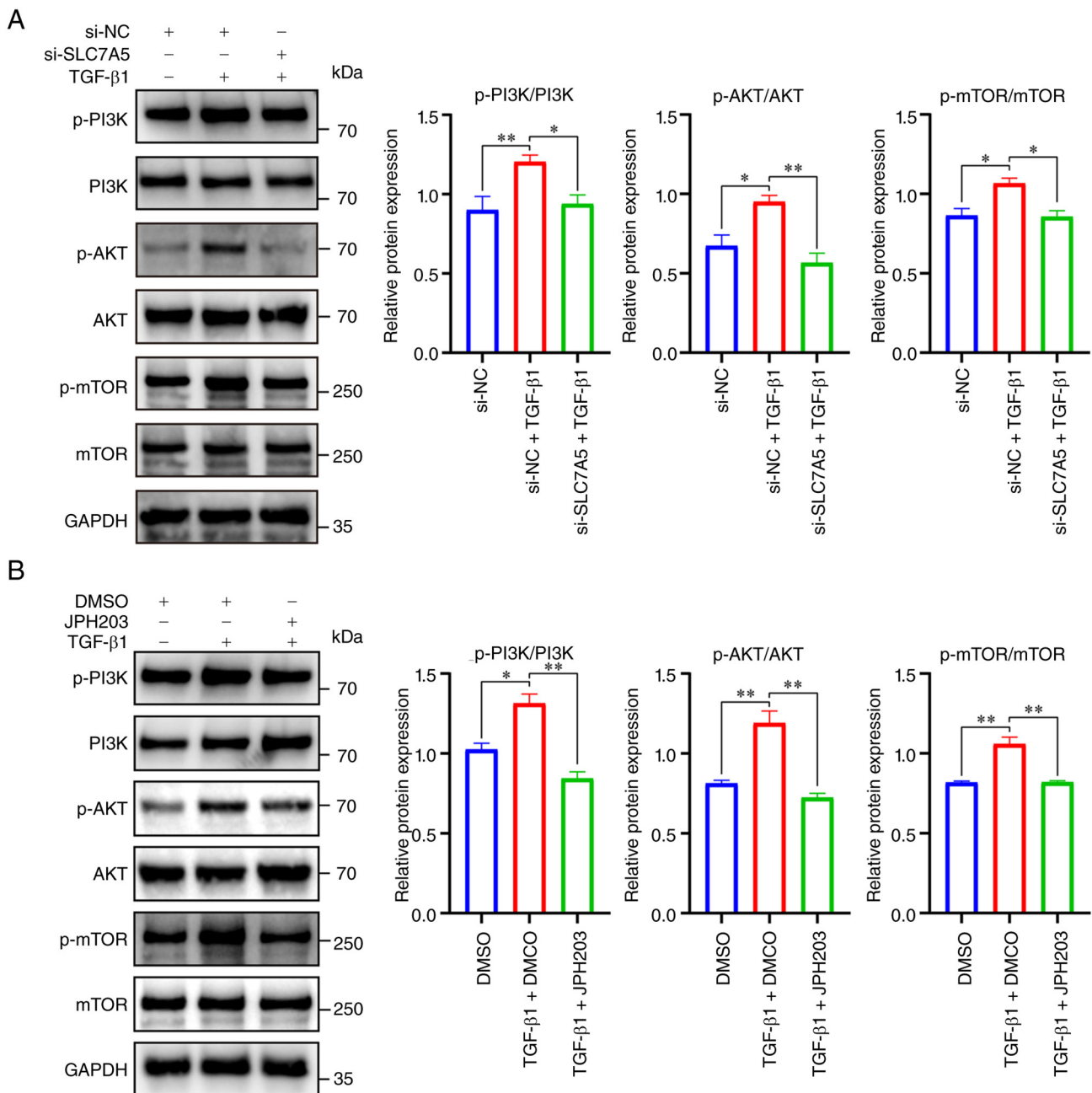


Figure 7. Regulation of *SLC7A5* on the activity of the PI3K/AKT/mTOR signaling pathway. (A) The relative expression of PI3K, p-PI3K, AKT, p-AKT, mTOR and p-mTOR in the si-*SLC7A5* + TGF- β 1 group was detected by western blotting (n=3; data are shown as mean $\pm$ SD; *P<0.05 and **P<0.01; one-way ANOVA). (B) The relative expression of PI3K, p-PI3K, AKT, p-AKT, mTOR and p-mTOR in the TGF- β 1 + JPH203 group (20 μ M) was detected by western blotting (n=3; data are shown as mean $\pm$ SD; *P<0.05 and **P<0.01; one-way ANOVA). *SLC7A5*, solute carrier family 7 member 5; TGF- β 1, transforming growth factor- β 1; p-, phosphorylated.

JPH203 inhibited BCAA transport, reduced the activity of pulmonary myofibroblasts and improved lung fibrosis in mice. Further experiments demonstrated that blocking or silencing *SLC7A5* inhibited the PI3K/AKT/mTOR pathway, prevented the formation of pulmonary myofibroblasts and suggested that this pathway may have been involved in the occurrence and development of IPF, providing a theoretical basis for the search for new therapeutic targets in IPF.

SLC7A5 is a non-sodium-dependent high-affinity amino acid transporter (42). Previous studies have shown that *SLC7A5* is highly expressed in the cardiomyocytes of diabetic mice (43) and during the TGF- β 1-induced mesenchymal-epithelial transition

process (44). These results are consistent with the findings of the present study, which found that *SLC7A5* was significantly upregulated in patients with IPF. A continuous decline of lung function represents the core pathophysiological feature of IPF; patients with IPF usually exhibit a decrease in DLCO (45). Consistent with this, the present study found that the expression level of *SLC7A5* in lung tissues was significantly negatively correlated with DLCO and FVC in patients with IPF. Furthermore, high *SLC7A5* expression was closely related to the poor prognosis of patients with IPF. As such, the expression level of *SLC7A5* was closely related to the clinical indicators of patients with IPF and may have been involved in the occurrence and development of IPF.

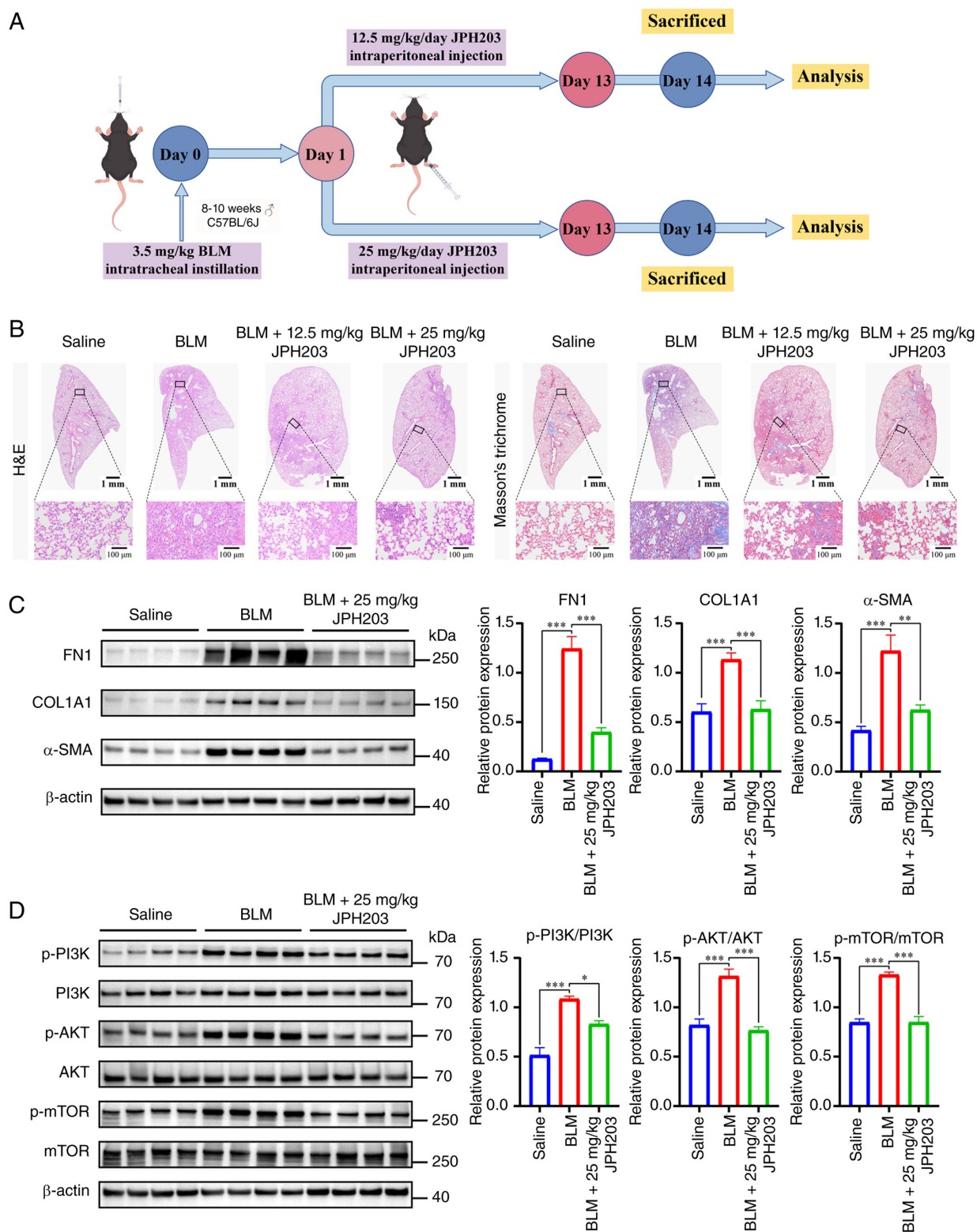


Figure 8. JPH203 improves the degree of pulmonary fibrosis induced by BLM in mice by inhibiting the activation of the PI3K/AKT/mTOR pathway. (A) Timeline of the *in vivo* mouse experiments. (B) H&E and Masson's trichrome staining of the lung tissues of JPH203-treated mice with pulmonary fibrosis (n=4). (C) The expression levels of fibrosis markers in the lungs of JPH203-treated mice with pulmonary fibrosis were detected by western blotting (n=4; data are shown as mean $\pm$ SD; **P<0.01 and ***P<0.001; one-way ANOVA). (D) The expression of PI3K, p-PI3K, AKT, p-AKT, mTOR and p-mTOR in the lungs of JPH203-treated mice with pulmonary fibrosis was detected by western blotting (n=4; data are shown as mean $\pm$ SD; *P<0.05 and ***P<0.001; one-way ANOVA). BLM, bleomycin; H&E, hematoxylin and eosin; p-, phosphorylated.

The pathogenesis of IPF is related to genetic factors, epigenetic factors and oxidative stress, and involves multiple cellular signaling pathways (46,47). Under the influence of

genetic and environmental factors, alveolar epithelial cells will develop metabolic disorders and exhibit abnormal proliferation and repair. These cells also interact with mesenchymal

cells through various signaling pathways that promote the transformation of fibroblasts into myofibroblasts, thereby leading to IPF (48). In the present study, RNA interference technology was used to knockdown the *SLC7A5* gene in pulmonary fibroblasts; it was found that in TGF- β 1-induced *SLC7A5* knockdown cells, the proliferation and migration of pulmonary fibroblasts were significantly inhibited and the myofibroblast transformation was reversed, as shown in Fig. 4. These results suggested that *SLC7A5* may have been involved in the development of pulmonary fibrosis. A notable characteristic of IPF is that fibroblasts are transformed into myofibroblasts under the influence of TGF- β 1 (49). As described in the present study, the expression levels of *FNI*, *COLIA1* and α -*SMA* in TGF- β 1-induced pulmonary fibroblasts were upregulated, suggesting that TGF- β 1 induced the differentiation process of pulmonary fibroblasts. Silencing *SLC7A5* expression significantly inhibited the upregulation of *FNI*, *COLIA1* and α -*SMA* induced by TGF- β 1 and inhibited the phenotypic transformation of fibroblasts.

SLC7A5 plays an important role in regulating the mTOR signaling pathway. As an important amino acid transporter with a high affinity for leucine and high transport capacity, *SLC7A5* is primarily responsible for the uptake of extracellular leucine (50). Leucine is not only necessary for protein synthesis but also a core signaling molecule for the activation of mTORC1 (51). Although cell membranes have various amino acid transporters, a study has shown that *SLC7A5*, due to its efficient uptake characteristics, dominates the cellular acquisition of leucine (50). In the present study, the importance of the *SLC7A5*/mTOR axis remains particularly prominent (52). By upregulating *SLC7A5*, cancer cells can take in large amounts of leucine and continuously activate the mTORC1 pathway, driving increased cell proliferation (53). When *SLC7A5* is dysfunctional or knocked out genetically, the concentrations of leucine and BCAA in the cells markedly decrease, thereby impairing the activity of the mTORC1 signaling pathway (54). The association between *SLC7A5* and mTOR is insufficiently studied in fibrotic diseases and, to the best of our knowledge, there is no report for this association in pulmonary fibrosis. The present study used transcriptome sequencing to determine that *SLC7A5* was enriched in mTOR-related pathways and support the activation of the mTOR pathway in IPF (as shown in Fig. 3K), suggesting that, in the process of pulmonary fibroblasts transforming into pulmonary myofibroblasts, *SLC7A5* may have affected this process through the mTOR pathway. Furthermore, the present study observed, that after treatment with JPH203, the lung fibroblast content of BCAA, which contained leucine, was significantly reduced. This reduction was accompanied by a decrease in the phosphorylation level of mTOR. Therefore, we hypothesize that inhibiting *SLC7A5* inhibited the mTOR pathway by suppressing the transport of amino acids, thereby inhibiting pulmonary fibrosis.

The PI3K/AKT/mTOR pathway has been shown to regulate cell proliferation, differentiation and survival, and is closely related to pulmonary fibrosis (55). A study reported by Li *et al* (56) demonstrated that duvelisib enhances autophagy and inhibits the activation of pulmonary fibroblasts by blocking the PI3K/AKT/mTOR pathway, thereby alleviating pulmonary fibrosis. Studies have also shown that the PI3K/AKT pathway is involved in the occurrence and development of myocardial,

hepatic and renal interstitial fibrosis (57,58). For example, a study reported by Wei *et al* (59) showed that the activity of the PI3K/AKT/mTOR pathway is closely linked to the growth and activation of hepatic stellate cells and can promote the progression of fibrosis in the liver. A study has also shown that the PI3K/AKT/mTOR pathway may directly participate in the occurrence and development of fibrosis via an interaction that is yet to be fully elucidated (60). A previous study has shown that the overexpression of secreted phosphoprotein 1 (*SPPI*) enhances activation of the PI3K/AKT/mTOR pathway and thereby the proliferation of fibroblasts in IPF; these effects were eliminated by treatment with PI3K inhibitors (61). The transcriptome sequencing data from the present study showed that in the TGF- β 1-induced model of IPF, activation of the PI3K/AKT pathway was inhibited. It should be noted that, although the PI3K/AKT pathway is typically regarded as an upstream pathway responsible for activating mTOR, few studies have shown that the activity of the PI3K/AKT may also be affected by *SLC7A5*. The experimental results of the cell experiments and animal models in the present study demonstrated that JPH203, by inhibiting the activity of *SLC7A5*, reduced the intracellular levels of essential amino acids, thereby blocking the activation of the PI3K/AKT/mTOR signaling pathway and exerting potential therapeutic effects in improving pulmonary fibrosis.

There are currently only two clinical anti-fibrotic treatments available for patients with IPF: Pirfenidone and nintedanib (4,62). However, treatments using these drugs have demonstrated a number of adverse reactions, which forces patients to reduce the dosage and in turn affects treatment outcomes (5,63). By shifting the goal of anti-fibrotic treatment for IPF from preventing disease progression to improving quality of life and reducing drug toxicity, it is expected that the therapies for IPF can reduce the occurrence of adverse reactions. JPH203 inhibits the function of *SLC7A5*, thereby preventing cells from absorbing essential amino acids and leading to the depletion of amino acid reserves within the cells, which subsequently inhibits cell proliferation (64). The first clinical trial (UMIN000016546) using the selective *SLC7A5* inhibitor JPH203 showed that this drug exhibits good tolerance in patients with advanced solid tumors (13). A study has shown that the expression of *SLC7A5* is markedly increased in peritoneal fibrosis, whereas JPH203 can markedly reduce the fibrotic phenotype in affected cells both *in vivo* and *in vitro* (44). However, to the best of our knowledge, there are no studies reporting the effect of JPH203 in pulmonary fibrosis.

The present study used JPH203 to treat TGF- β 1-induced pulmonary fibroblasts and found that JPH203 inhibited the proliferation, migration and anti-apoptotic ability of pulmonary myofibroblasts. JPH203 treatment also suppressed the expression levels of *FNI*, *COLIA1* and α -*SMA*, indicating that JPH203 blocked the transformation of pulmonary fibroblasts by inhibiting the transport ability of *SLC7A5* for BCAA. In a phase I clinical trial (UMIN000016546) of the selective inhibitor JPH203, patients with advanced solid tumors were treated with JPH203 daily via intravenous injection. Disease control was achieved at dose levels of 12 and 25 mg/m², with a disease control rate of 60% for cholangiocarcinoma (13). In the present study, JPH203 was able to improve BLM-induced pulmonary

fibrosis in mice and this improvement was more notable in the 25 mg/kg group. The tissue structures of heart, liver, spleen and kidney tissues of pulmonary-fibrosis model mice treated with 12.5 and 25 mg/kg doses JPH203 were intact, without obvious inflammatory infiltration, cell necrosis or pathological damage. From these results, the present study observed that JPH203 exhibited good tolerance and efficacy levels *in vivo* (as shown in Figs. 8 and S6). As such, the aforementioned results also indicated that JPH203 may represent a new therapeutic agent for IPF treatment, and a dose of 25 mg/kg JPH203 can be used for investigation in future studies.

The predominant findings of the present study involve the discovery of high *SLC7A5* expression in patients with IPF using a large sample dataset and the discovery that *SLC7A5* upregulation was associated with the decline in lung function and poor prognosis of patients with IPF. Inhibiting the function of *SLC7A5* or silencing its expression through inhibitors or siRNA sequences inhibited the function of pulmonary myofibroblasts via suppression of the PI3K/AKT/mTOR signaling pathway. Furthermore, the present study used the selective *SLC7A5* inhibitor JPH203 to treat BLM-induced pulmonary fibrosis mice and supported the anti-IPF efficacy of JPH203 *in vivo*.

However, the present study also exhibited certain limitations. Primarily, although the use of BLM to induce pulmonary fibrosis in mice is currently recognized as an effective method for establishing an animal model of IPF (65,66), pulmonary fibrosis in the BLM-induced pulmonary fibrosis mouse model is temporary and self-limiting, whereas IPF is a chronic, progressive and irreversible disease. This difference exemplifies the limitations of the IPF model (67). In addition, a limitation of the present study is that post-mortem analysis was not performed for the three mice that died before the scheduled endpoint; therefore, the exact cause of death could not be conclusively determined. Furthermore, the present study used public datasets on patients with IPF but did not include our own IPF patient cohort from inpatient records. In subsequent research, patients with IPF should be included to allow for in-depth explorations of *SLC7A5* expression in clinical samples, as well as to elucidate the association between *SLC7A5* and clinical indicators, such as disease severity. Finally, the present study did not explore whether *SLC7A5* activated the PI3K/AKT/mTOR signaling pathway by affecting the transport of specific amino acids. Future studies should explore the related pathways and downstream target genes of *SLC7A5*, as well as the association between downstream amino acids transported by *SLC7A5* and IPF development.

In conclusion, *SLC7A5* was significantly upregulated in samples from patients with IPF, and the expression of *SLC7A5* was negatively correlated with the pulmonary function of patients with IPF. The upregulation of *SLC7A5* was also associated with the poor prognosis of patients with IPF. *SLC7A5* was significantly upregulated in the lung tissues of mice with BLM-induced pulmonary fibrosis and in lung fibroblasts induced by TGF- β 1. *SLC7A5* knockdown or selective inhibition using JPH203 prevented the formation of pulmonary myofibroblasts by inhibiting the activation of the PI3K/AKT/mTOR pathway. *In vivo*, JPH203 improved the degree of fibrosis in the lungs of mice with BLM-induced pulmonary fibrosis. Therefore, *SLC7A5* may represent a new therapeutic target for IPF, and future research should focus on regulating this target and translating *SLC7A5*-targeted treatments into effective therapies for IPF in clinical practice.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author. The transcriptome sequencing data generated in the present study may be found in the Science Data Bank repository at the following URLs: <https://doi.org/10.57760/sciencedb.29996> (accession number: CSTR: <https://cstr.cn/31253.11.sciencedb.29996>, Fig. 3) and <https://doi.org/10.57760/sciencedb.30161> (accession number: CSTR: <https://cstr.cn/31253.11.sciencedb.30161>, Fig. 6).

Authors' contributions

CS and HC designed the study and supervised the project. MW, YY and ZL wrote the manuscript, conducted experiments, performed literature searches and analyzed the data. DX, YL, XZ, NS and XL contributed towards data processing and interpretation, statistical analysis, and editing the manuscript. HC and MW confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All animal experiments involved in the present study were approved by the Experimental Animal Protection and Ethics Committee of Harbin Medical University (approval no. YJSDW2023-024).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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