Circ_PWWP2A promotes lung fibroblast proliferation and fibrosis via the miR-27b-3p/GATA3 axis, thereby aggravating idiopathic pulmonary fibrosis

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Objective: This paper was to investigate the effect of circ_PWWP2A-mediated miR-27b-3p/GATA3 axis on idiopathic pulmonary fibrosis (IPF). Methods: circ_PWWP2A expression in lung fibroblasts MLg2908 induced by different concentrations of TGF-β was detected. The relationship between circ_PWWP2A or GATA3 and miR-27b-3p was analyzed by RNA immunoprecipitation and dual-luciferin reporter assay. The proliferation of MLg2908 cells was determined by MTI. GATA3, α-SMA, Collagen-I, and Collagen-III in cells were detected by RT-qPCR and Western blot. The rat model of IPF induced by bleomycin (BLM) was constructed and treated with circ_PWWP2A siRNA injection. HE and Masson staining were of utility to evaluate the pathological conditions of rat lung tissue, and circ_PWWP2A, miR-27b-3p, and GATA3 levels in lung tissues were detected by RT-qPCR. Immunohistochemistry was used to detect the staining of α-SMA, collagen I, and collagen III in the lung tissues of rats. Results: circ_PWWP2A in MLg2908 cells induced by TGF-β decreased in a concentration-dependent manner. MLg2908 cells transfected with circ_PWWP2A siRNA were induced by 5 ng/ml TGF-β, decreasing circ_PWWP2A and GATA3 levels, increasing miR-27b-3p expression, and suppressing cell proliferation. The targeting relationship between circ_PWWP2A and miR-27b-3p, as well as miR-27b-3p and GATA3, was confirmed. Depleting miR-27b-3p reduced the inhibitory effect of circ_PWWP2A down-regulation on the proliferation of TGF-β-treated MLg2908 cells, accompanied by increased expression of α-SMA, Collagen I, and Collagen III, and increased expression of GATA3. The in vivo results showed that BLM-induced fibrosis in rat lung tissue was obvious, accompanied by increased expression of circ_PWWP2A and GATA3, decreased expression of miR-27b-3p, and deepened staining of α-SMA, collagen I, and collagen III, but circ_PWWP2A siRNA could improve these phenomena. Conclusion: Silencing circ_PWWP2A can inhibit the proliferation of lung fibroblasts induced by TGF-β through the miR-27b-3p/GATA3 axis, and reduce BLM-induced pulmonary fibrosis in rats, which may be a potential therapeutic target for IPF.

Keywords: Lung fibroblasts, circ_PWWP2A, miR-27b-3p, GATA3, Idiopathic pulmonary fibrosis

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Abbreviations: circRNA, Circular RNA; IPF, Idiopathic pulmonary fibrosis

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a diffuse parenchymal lung disease, which is a group of interstitial lung diseases (Martinez et al, 2017; Xaubet et al, 2017). IPF is a representative interstitial lung disease (Sgalla et al, 2016) that is pathologically featured by diffuse alveolitis, pulmonary fibrosis, and progressive scarring. The clinical manifestations are mainly no obvious cause, and the initial symptoms are exertional dyspnea and chronic cough without sputum (Konigsberg et al, 2021; Wolters et al, 2014).

At present, there are mainly anti-inflammatory drugs (such as prednisone) for inflammation, and immunosuppressants (such as cyclophosphamide) for immune response. However, the application of these two types of drugs can affect the inflammatory response of IPF, but not improve the progress of immune response and immune response much, or increase the survival rate of patients (Hewlett et al, 2018; Somogyi et al, 2019). These include nintedanib (Ofev®) and pirfenidone (Esbriet®). These medications are called anti-fibrotic agents, Nintedanib inhibits inflammation and fibrosis by blocking a variety of tyrosine kinase receptors, including platelet-derived growth factor, vascular endothelial growth factor, and fibroblast growth factor (Hilberg et al, 2008). The main antifibrotic effect of pirfenidone is to down-regulate transforming growth factor-β (Nakayama et al, 2008). Such as Tananchai et al data confirm that multicenter phase III randomized controlled trial of pirfenidone and nintedanib (ASCEND and INPULSiS Q7) showed a similar reduction in lung function decline compared with placebo (King et al, 2014).The long-term effects of antifibrotic therapy on mortality are unknown. Real-world and registry-based studies have been published describing the long-term use of antifibrotic drugs in clinical practice (Nathan et al, 2017). These studies showed that about 40 percent of patients had drug-related adverse effects, including diarrhea, gastrointestinal distress, and elevated transaminases. However, only 5% of patients receiving antifibrotic therapy permanently discontinue treatment(Cerri et al, 2019). Such studies also add to the growing number of reports on the long-term efficacy of antifibrotic drugs. Therefore, IPF has a poor prognosis and a high mortality rate (Wakwaya & Brown, 2019). IPF generally affects men after the age of 60 years and a median survival time of 2-4 years, even worse than many cancers (Vancheri et al, 2010; Sgalla et al, 2016). It was reported that the survival rate is as low as 2–5 years after diagnosis of the IPF(Richeldi et
Therefore, how to prevent and treat IPF more effectively is a difficult problem faced by clinicians.

Circular RNA (circRNA) closed circular RNA molecules formed by covalent bonds (Kristensen et al., 2019). Studies have proved that circRNAs exerts a regulatory role in eukaryotic life activities and disease developments, such as cardiovascular diseases (Altesha et al., 2019), tumors (Lei et al., 2020; Li et al., 2020), kidney-related diseases (Jin et al., 2020), and lung-related diseases (M. et al., 2020), including IPF (Li et al., 2018). For example, hsa_circ_0058493 knockdown inhibits fibrosis by affecting the epithelial-mesenchymal transition (EMT) process in IPF (Cheng et al., 2022). Has_circ_PWPP2A, also known as hsa_circ_0074837 (Zhang et al., 2018), was found to be associated with promoting liver fibrosis (Liu et al., 2019). A reliable function of circRNAs is to directly or indirectly bind target miRNAs to inhibit miRNA function through a process commonly referred to as miRNA sponge adsorption (Huang et al., 2020). Through ENCORI comprehensive database, we found that circ_PWPP2A has a potential binding site with miR-27b-3p. miR-27b-3p has been discovered to be correlated with renal fibrosis (Conserva et al., 2019). Microarray analysis revealed that downregulation of miR-27b-3p is associated with fibrosis (Kim et al., 2017). Furthermore, miR-27b-3p expression alters significantly during lung exacerbation in cystic fibrosis patients (Stachowiak et al., 2020).

Therefore, this article targeted to reveal the mechanism related to circ_PWPP2A and the regulatory network of circ_PWPP2A/miR-27b-3p/GATA3 in IPF, with the purpose to develop molecule-based therapy.

**MATERIALS AND METHODS**

**Cell culture and treatment**

The murine lung fibroblast cell line MLg2908 (ATCC, CCL-206, USA) was maintained in EMEM (30-2003, ATCC, USA) containing 10% FBS and treated with different concentrations of recombinant human TGF-β (10 ng/ml; PeproTech, USA; 0, 5, 10 ng/ml) for 24 h (Y. J. Li et al., 2006). circ_PWPP2A sRNA#1, circ_PWPP2A sRNA#2, and NC sRNA were transfected into MLg2908 cells according to Lipofectamine 3000 reagent (L3000001, Thermo Fisher Scientific), and the more effective sRNA was selected for later experiments. MLg2908 were treated with TGF-β at an optimal concentration of 5 ng/ml. Before induction, rats were treated with 2ml Opti-MEM medium (GIBCO, USA) containing plasmids (1 μg) and Lipofectamine 3000 (2.5 μl). The medium was changed after 6 h, and the RNA extraction was performed at 48 h to verify the transfection efficiency.

**RNA immunoprecipitation (RIP)**

Protein-A/G-coated magnetic beads were resuspended in 100 μl NT-2 buffer and let stand with 5 μg AGO2-labeled antibody(Millipore, Billerica, MA, USA). Cells were centrifuged, and 100 μl of the supernatant was added to 900 μl of magnetic beads resuspended in NET-2 buffer. Appropriate antisense probes were designed according to the unique trans-cleavage site of circRNA, and the corresponding sense sequences were used as control negative probes. Briefly, cells were lysed in RIP lysis buffer, then supernatants were transferred to nuclease-free tubes, magnetic beads conjugated to Ago2 or IgG antibodies were added and incubated for 6 hours at 4°C. The immunoprecipitates bound to the beads were eluted with elution buffer, and the purified RNA fragments were analyzed by RT-qPCR.

**MTT experiment**

Cells were taken after trypsinization and centrifuged to prepare a cell suspension at 5×10^6 cells/mL. Seeded into 96-well plates to 45 Wells, 200 μl of cell suspension was cultured for 24 h and centrifuged at 1000 r/min (supernatant was removed). Afterward, 200 μl of serum-free culture medium and 20 μl of 5 mg/ml MTT were added to each well for an additional 4 h, centrifuged at 1000 rpm/min (supernatant was removed), and reacted with 200 μl DMSO to record OD_490 value on a microplate reader.

**Luciferase activity assay**

Bioinformatics software predicted the binding sites of circ_PWPP2A and miR-27b-3p, as well as miR-27b-3p and GATA3, and a miR-27b-3p recombinant firefly luciferase reporter plasmid containing the circ_PWPP2A/GATA3 binding sequence was designed and synthesized. circ_PWPP2A WT/circ_PWPP2A MUT/GATA3 WT/GATA3 MUT was co-transfected with mimic NC or miR-27b-3p mimics into MLg2908, respectively. After 48 h, the luciferase activity of the cells was detected according to the instructions of the Dual-Luciferase reporter system kit (Promega).

**Immunoblot analysis**

Proteins were extracted by lysing cells with RIPA buffer (Sigma, USA) containing protease inhibitors and subsequently quantified by a BCA protein concentration assay kit.(Beyotime Biotechnology, Nanjing, China). Total protein was loaded onto PVDF membrane (ThermoFisher Scientific, USA) after 10% SDS-PAGE gel electrophoresis, then blocked with 5% nonfat milk powder, and with primary antibodies at 4°C overnight, including z-SMA at 1 μg/ml (ab5694, Abcam), collagen I (1:5000, ab260043, Abcam), collagen III (1:5000, ab7778, Abcam), GATA3 (1:1000, ab199428, Abcam) and GAPDH (1:2500, ab9548, Abcam). The next day, secondary antibodies were added and incubated for 40 min, the membrane interacted with HRP-conjugated goat anti-rabbit IgG secondary antibody (#431460, Thermo Fisher Scientific). Immunoblots were visualized in BioRad FL1500 Intelligent Imaging System (ThermoFisher, USA) and GAPDH was used as an internal control.

**Establishment and treatment of IPF rat model**

Twenty-four SD male rats, weighing about 200 g, were treated accordingly (6 rats in each group). The rats were anesthetized by intraperitoneal 10% chloral hydrate and fixed in a supine position to expose the trachea. After anesthesia, 1 mg BLM was added to 0.5 ml PBS for intratracheal administration (Otsuka et al., 2017), while the Sham group was only given 0.5 ml PBS. Forty-four days after modeling (day 15), 1 μl/g siRNA was intravenously injected into rats at 0.75 mg/kg/day, three times every other day. Twenty-four hours after the last injection, rats were euthanized by pentobarbital sodium, and lungs were harvested for HE staining and Masson stain-
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Fibrosis was scored according to the Ashcroft assay.

**Immunohistochemistry**

Fresh paraffin tissues (4 μm) were placed in an oven at 65°C for 2 h, deparaffinized to water, and micro-waved in EDTA buffer. Sections were placed in 3% hydrogen peroxide solution for 10 min, blocked with 5% BSA for 20 min, added about 50 μl of diluted primary antibody overnightincluding α-SMA at 1 μg/ml (ab5694, Abcam), collagen I (1:5000, ab260043, Abcam), collagen III (1:5000, ab7778, Abcam), and GAPDH (1:2500, ab9548, Abcam), and 50-100 μl secondary antibody for 50 min. After the addition of DAB solution, the sections were counterstained with hematoxylin, differentiated with 1% hydrochloric acid alcohol, immersed in ammonia, dehydrated with gradient alcohol, cleared with xylene, and mounted with neutral gum.

**RT-qPCR**

Cell and tissue RNA was extracted by the Trizol method, and the concentration and quality of the RNA solution were determined on a DU730 instrument. RNA was reverse transcribed into cDNA using SuperScript IV Reverse Transcriptase (#18090010, Invitrogen) and treated with qPCR according to the SuperScript III Platinum SYBR Green One-Step qRT-PCR Kit (#11736059, Invitrogen). The primer sequences are shown in Table 1.

By the 2−∆∆Ct method.

**Statistical analysis**

SPSS 19.0 software was of utility for statistical analysis and Graphpad 6.0 was for drawing graphs. One-way analysis of variance, along with Tukey’s HSD test was suitable for assessing data collected from multiple groups. Differences were considered statistically significant at \( P < 0.05 \).

**RESULTS**

circ_PWWP2A can affect the proliferation of TGF-β-treated MLg2908

To investigate the function of circ_PWWP2A in MLg2908 cells, we first knocked it down by transforming the cells using two lentiviral vectors encoding independent shRNAs targeting circ_PWWP2A.

![Figure 1. circ_PWWP2A can inhibit the proliferation of mouse lung fibroblasts treated with TGF-β](image)

(A) circ_PWWP2A expression after MLg2908 cells were treated with different concentrations of TGF-β (0, 5, 10 ng/ml), *vs. 0 ng/ml TGF-β, \( P < 0.05 \); **vs. 5 ng/ml TGF-β, \( P < 0.05 \); (B) circ_PWWP2A expression in MLg2908 cells co-treated with circ_PWWP2A siRNA and 5 ng/ml TGF-β; (C) Proliferation of MLg2908 cells after co-treatment with cirr_PWWP2A siRNA and 5 ng/ml TGF-β; vs. Control group, \( P < 0.05 \); vs. TGF-β group and NC siRNA + TGF-β group, \( P < 0.05 \); vs. circ_PWWP2A siRNA + TGF-β group, \( P < 0.05 \); (D) qRT-PCR analysis of circ_PWWP2A levels in MLg2908 cells infected with a lentiviral vector encoding circ_PWWP2A siRNA#1 or circ_PWWP2A siRNA#2.
RT-qPCR analysis confirmed that circ_PWWP2A levels were suppressed to a greater degree by expression of circ_PWWP2A (Fig. 1D), which was then utilized in subsequent experiments. In MLg2908 cells treated with different concentrations of TGF-β (0, 5, 10 ng/ml), circ_PWWP2A was increased in a concentration-dependent manner (Fig. 1A), indicating that circ_PWWP2A is promoted during lung fibroblast activation. MLg2908 cells were transfected with circ_PWWP2A siRNA and then induced by 5 ng/ml TGF-β, and it was found that circ_PWWP2A in the cells was decreased (Fig. 1B). After 48 h of TGF-β treatment accompanied by a decrease in proliferation activity (Fig. 1C). It indicated that the low expression of circ_PWWP2A could inhibit the proliferation of lung fibroblasts induced by TGF-β.

**circ_PWWP2A can adsorb miR-27b-3p**

Bioinformatics analysis ENCORI found that circ_PWWP2A has a potential binding site with miR-27b-3p (Fig. 2A). Next, RIP assays in MLg2908 cells further verified the interaction between circ_PWWP2A and miR-27b-3p; (C) dual luciferase reporter experiment to verify the targeting relationship between circ_PWWP2A and miR-27b-3p; *vs. other three groups, P<0.05.

**Consumping miR-27b-3p enhances the proliferation of TGF-β-induced MLg2908 cells**

Figure 3A showed that 5 ng/ml TGF-β can inhibit miR-27b-3p expression in MLg2908 cells, and after co-treatment with miR-27b-3p inhibitors, miR-27b-3p expression was further reduced, and the accompanying cell proliferation was further activated. circ_PWWP2A siRNA could reduce TGF-β-regulated inhibition of miR-27b-3p, that is, circ_PWWP2A siRNA augmented miR-27b-3p expression in TGF-β-treated MLg2908 cells.

**Silencing miR-27b-3p affects fibrosis-related genes in lung fibroblasts**

α-SMA, collagen I, and collagen III in cells were determined by RT-qPCR and immunoblot analysis (Fig. 4A, B), demonstrating an increase α-SMA, collagen I, and collagen III increased in MLg2908 after TGF-β induction, which was promoted α-SMA, collagen I, and collagen III express after co-treatment with miR-27b-3p inhibitors, but alleviated α-SMA, collagen I, and collagen express III after circ_PWWP2A siRNA co-treatment. miR-27b-3p inhibitor co-treatment lessened circ_PWWP2A siRNA-regulated suppression of these fibrosis-related genes.

**circ_PWWP2A can regulate GATA3 expression through miR-27b-3p**

ENCORI database showed that GATA3 was a direct target gene of miR-27b-3p (Fig. 5A). The interaction between the two was further verified by luciferase experiments, as the results showed that miR-27b-3p mimic reduced the luciferase activity of GATA3 WT without affecting that of GATA3 MUT (Fig. 5B). Our test results also found that circ_PWWP2A siRNA could inhibit the increase of GATA3 in MLg2908 cells induced by TGF-β, while miR-27b-3p inhibitors did oppositely. GATA3 was down-regulated in MLg2908 cells co-treated with TGF-β and circ_PWWP2A siRNA, but this phenomenon was reversed by miR-27b-3p inhibitors (Fig. 5C–E).

**Therapeutic effect of circ_PWWP2A siRNA on IPF rats**

HE staining and Masson staining showed (Fig. 6A): in rats receiving sham operation, lung structure was normal, the alveolar interval was uniform, no obvious exudation was seen in the cavity, and a small number of collagen fibers dyed in light blue can be seen in the alveoli, but without obvious pathological change. However, BLM injection destroyed the alveolar structure, reduced the alveolar cavity, exfoliated alveolar epithelium and protein exudate, and caused obvious collagen deposition and fibrosis areas. circ_PWWP2A siRNA-treated rats consti-
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tivated mild hyperplasia of fibroblasts, deposition of collagen fibers in the pleura and alveolar septa, and mild to moderate pulmonary fibrosis. Ashcroft scores indicated that BLM-induced rats had higher Ashcroft scores, but circ_PWWP2A siRNA reduced Ashcroft scores in BLM-treated rats (Fig. 6B). BLM-induced increased expression of circ_PWWP2A and GATA3 in rat lung tissue, while decreased expression of miR-27b-3p, was reversed by circ_PWWP2A siRNA treatment (Fig. 6C). BLM rats had enhanced immunohistochemical staining results of α-SMA, collagen I and collagen III in lung tissue, but the staining degree was reduced after circ_PWWP2A siRNA injection (Fig. 6D).

DISCUSSION

In humans, IPF is a progressive disease associated with aging caused by uncontrolled proliferation and differentiation of fibroblasts resulting from sustained damage to the alveolar epithelium. These myofibroblasts are responsible for hyperproliferation, EMT, ECM production, and contribute to collagen deposition in the affected organ (King et al., 2011). Transforming growth factor-β1 (TGF-β1) is a key member of the TGF-β superfamily. It was discovered in 1983 for its ability to stimulate the growth of rat fibroblasts cultured in soft AGAR and has been identified as a master cytokine in liver fibrosis (Xu et al., 2016). In fibrosis, increased TGF-β signaling significantly affects the behavior of the fibroblast population, which represents the majority of cells in fibrotic lesions. In addition, low levels of TGF-β promoted fibroblast proliferation (Zhang et al., 2017). These activities may explain the number of fibroblasts in fibrotic lesions.
tion in fibrotic diseases, which has been revealed in a mouse model of fibrosis (Katsuno et al., 2021).

With the rapid development of deep transcriptome sequencing technology, the research on noncoding RNAs is growing exponentially (Matsui et al., 2017). Although in some cases ncRNAs lack protein-coding capabilities and appear to lack biological functions, increasing evidence confirms that they play a critical role in regulating gene expression through multiple mechanisms, such as targeting transcripts (Liu et al., 2012). Reports have demonstrated that several lncRNAs and miRNAs play critical roles in the progression of IPF. For instance, Li and others (Li et al., 2020) reported that circTADA2A could repress fibroblasts activation and proliferation via miR-526b/Cav1 and miR-203/Cav2 pathway, thus alleviating IPF. Another hsa_circ_0044226 was markedly higher in lung tissues from IPF patients than from healthy controls. which RLE-6TN cells and in a bleomycin-induced mouse model of IPA and diminished TGF-β1-induced fibrosis. These findings indicate that downregulation of hsa_circ_0044226 attenuates pulmonary fibrosis in vitro and in vivo by inhibiting CDC27, which in turn suppresses EMT. This suggests hsa_circ_0044226 may be a useful therapeutic target for the treatment of IPF (Qi et al., 2020). In addition, lncRNA H19 (Lu et al., 2018), MEG3 (Gokey et al., 2018) have been also confirmed to play critical roles in IPF.

At present, previous studies have found that circ_PWLP2A plays a role in various diseases. For example, in OB-6 osteoblasts and primary human osteoblasts, overexpression of circ_PWLP2A can effectively inhibit dexamethasone-induced cell death and apoptosis (Hong et al., 2019). circ_PWLP2A is up-regulated in mouse fibrotic liver tissue and is positively correlated with HSC activation and proliferation (Liu et al., 2019). Similarly, MLg2908 cells were treated with TGF-β (0, 5, 10 ng/ml) and circ_PWLP2A expression was analyzed to be increased in a concentration-dependent manner. Animal experiments also found that circ_PWLP2A was also up-regulated in BLM-induced rat lung tissue, suggesting that circ_PWLP2A involves the activation of lung fibroblasts and may be a pathogenic factor for IPF. After inhibiting circ_PWLP2A expression in TGF-β-induced MLg2908 cells, cell proliferation ability was reduced, which verified our speculation.

The most important function of circRNA is to play as miRNA “sponge” (Shi et al., 2020). The present study proved that circ_PWLP2A could adsorb miR-27b-3p. In this study, miR-27b-3p expression in MLg2908 cells induced by TGF-β was decreased, which is consistent with previous reports: for example, exosomal miR-27b derived from human umbilical cord mesenchymal stem cells can ameliorate subretinal fibrosis (Li et al., 2021). Also, miR-27b-3p knockout can reduce cardiac hypertrophy, fibrosis, and inflammation induced by a pathological cardiac hypertrophy model (Li et al., 2021). Furthermore, miR-27b-3p overexpression attenuates renal fibrosis by downregulating α-SMA and collagen III (Bai et al., 2021). All of the studies indicate that miR-27b-3p is a fibrosis-related gene, and its overexpression the role can be anti-fibrotic. Our data further described that low expression of miR-27b-3p could reverse the effect of circ_PWLP2A siRNA on TGF-β-induced mouse lung fibroblast proliferation, and circ_PWLP2A siRNA could reduce the expression of miR-27b-3p in lung tissue of IPF rats, indicating that circ_PWLP2A could adsorb miR-27b-3p thus promoting TGF-β-induced proliferation and activating MLg2908 cells. α-SMA, collagen I and collagen III, as fibrosis-related genes are overexpressed in various tissue fibrosis, such as experimental pulmonary fibrosis (Huang et al., 2020), cardiac fibrosis (Zeng et al., 2019), liver fibrosis (Cheng et al., 2019), and kidney fibrosis (Zheng et al., 2019). Here, the expression patterns of α-SMA, collagen I, and collagen III in MLg2908 cells induced by TGF-β were increased, which were similar to the results of previous studies (Cheng et al., 2019; Liu et al., 2019).
al., 2017), but circ_PWWP2A siRNA can inhibit its increase. circ-PWWP2A depletion can alleviate mouse liver fibrosis, accompanied by decreased α-SMA and collagen I expression (Liu et al., 2019). In vivo experiments from the present study clarify that low expression of circ_PWWP2A can improve BLM-induced pulmonary fibrosis in rats, accompanied by decreased expression of α-SMA, collagen I, and collagen III, indicating that the inhibitory effect of circ_PWWP2A on IPF may be through the reduction of fibrosis-related genes.

miRNAs are endogenous small non-coding RNAs (Chen et al., 2019), which can bind to the 3’UTR of target gene mRNA (Bartel, 2009). miR-27b-3p can target the regulation of GATA3 located at 10p14 (Enciso-Mora et al., 2010). T cells regulate the activation of skin fibroblasts, which is in part achieved by GATA3-mediated unique tissue-distinctive transcriptional program (Kalekar et al., 2019). GATA3 is also involved in pulmonary fibrosis development (Itrura et al., 2018).

On the whole, the study only discusses the mechanism from experimental tests, and future clinical experiments are required to verify our results; how GATA3 is involved in circ_PWWP2A-mediated adsortion of miR-27b-3p regulating IPF needs to be done as funds and time allow.

CONCLUSION
circ_PWWP2A expression in M1g2908 cells induced by TGF-β decreases in a concentration-dependent manner. Silencing circ_PWWP2A can inhibit the role of TGF-β in the activation and proliferation of lung fibroblasts through the miR-27b-3p/GATA3 axis, and reduce BLM-induced IPF in rats, potentially renewing therapeutic targets for IPF.

Declarations
Competing interests. The authors declare that they have no competing interests.

Ethical statement. All animal experiments were performed in accordance with the ARRIVE guidelines and performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The experiments were approved by the Affiliated Hospital of Inner Mongolia Medical University.

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