Sitagliptin Inhibits Extracellular Matrix Accumulation and Proliferation in Lung Fibroblasts

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Fibroblasts activation-induced fibrosis can cause idiopathic pulmonary fibrosis (IPF). Excessive activation of fibroblasts contributes to poor healing or severe visceral fibrosis and even organ dysfunction. Sitagliptin acts as a dipeptidyl peptidase 4 inhibitor to reduce glucose level in type 2 diabetes, but its role in fibrosis of lung fibroblasts is elusive. We investigated the mechanism of sitagliptin in TGF-β-activated lung fibroblasts and evaluated the efficacy of sitagliptin in extracellular matrix accumulation and fibroblasts proliferation.

Material/Methods: By in vitro lung fibroblasts culture, we assessed the expression of lung fibroblasts biomarker (α-SMA) and extracellular matrix (Col-1, Col-3, fibronectin) following TGF-β stimulation and treatment with sitagliptin. Mechanistically, the phosphorylation level of Smad-3 protein in cells was analyzed using Western blotting, and the apoptosis level was assessed by Western blotting and flow cytometry. The degree of proliferation was determined using immunofluorescence and scratch-healing assay.

Results: We found that treatment with sitagliptin attenuates fibroblasts activation following TGF-β stimulation. Furthermore, the extracellular matrix was decreased by sitagliptin treatment by suppressing the phosphorylation level of Smad-3 protein. We found that sitagliptin does not affect apoptosis in fibroblasts, but it does affect the degree of proliferation of lung fibroblasts, thus ameliorating fibrosis after TGF-β stimulation.

Conclusions: Sitagliptin inhibits fibrosis in TGF-β-induced lung fibroblasts activation, which restrains extracellular matrix formation and cell proliferation in fibroblasts. Therefore, sitagliptin appears to have promise as a treatment of fibroproliferative disease caused by activation and proliferation of fibroblasts.

MeSH Keywords: Extracellular Matrix • Fibroblasts • Fibrosis

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Background

Fibrosis is a chronically exacerbating pathology that contributes to organic disease and scar hyperplasia of wounds. Excessive fibrosis can be caused by chronic inflammatory stimuli, increasing lipidosis, infection, and immunity [1,2]. Despite increased understanding of the pathophysiology of fibrosis, treatments for idiopathic pulmonary fibrosis (IPF) are lacking. Given the etiology of IPF, parenchymal tissue replaced by extensive fibrous connective tissue is a major characteristic in the lungs, which provokes organic structural destruction, lung hypofunction, and even respiratory failure [3]. Earlier studies confirmed that effective suppression of fibroblast bioactivity in multiple fibrotic diseases attenuated lesions [4,5], indicating that the modulation of fibroblasts at an early stage can inhibit the development of fibrotic diseases. Acting as a physiological adaptive response, fibrosis participates in tissue injury repair, while repeated damage to lung tissue triggers a mass of fibroblasts to proliferate to repair histological damage and ultimately leads to pathological changes of fibrosis, especially in alveolus tissue [6]. To control fibrosis in IPF, decreasing fibroblast activation is considered to be a promising strategy aspect. Fibroblasts are differentiated from mesenchymal cells during the embryonic period and serve as a main cellular component of loose connective tissue, which proliferate through mitosis and synthesize and secret collagens and matrix components [7]. However, over-activated fibroblasts proliferate and secrete a large amount of extracellular matrix (ECM) via stimulation of multiple growth factors such as transforming growth factor (TGF), connective tissue growth factor (CTGF), and fibroblast growth factors (FGF) [8,9]. TGF-β is a recognized tissue factor that stimulates fibroblasts differentiation and has been widely used to induce activation of fibroblasts *in vitro* [10]. Numerous studies have indicated that activation of the TGF-β pathway is associated with fibrosis and related histologic lesions [11, 12]. Hence, much research has concentrated on understanding TGF-β pathway regulation to control fibrosis and disease progression. Sitagliptin, a dipeptidase-4 (DPP-4) inhibitor, modulates hyperglycemia and related complications by protecting endogenous incretins and enhancing their action in type 2 diabetes [13]. Several studies reported that DPP-4 inhibitor plays a protective role in various fibrosis-induced diseases. Particularly, DPP-4 inhibitor modulated kidney fibrosis in streptozotocin-induced diabetic mice [14], potently inhibited fibrosis and inflammation in experimental autoimmune myocarditis [15], and attenuated hepatic fibrosis via suppression of activated hepatic stellate cells [16]. From past studies, we recognize that DPP-4 inhibitor can change the fibrosis process of IPF through modulating several pathogenic factors. However, the effect and mechanism of DPP-4 inhibitor in lung fibroblasts activation via TGF-β induction are unclear. We hypothesized that DPP-4 inhibitor can mediate the TGF-β pathway and cell proliferation or apoptosis to play an anti-fibrosis role in lung fibroblasts. To test this *in vitro*, we used sitagliptin in TGF-β-induced lung fibroblasts and explored the effect and mechanism of the DPP-4 inhibitor sitagliptin on fibrotic regulation.

Material and Methods

Cell culture and treatment

Human lung fibroblasts were purchased from American Type Culture Collection (ATCC) (Manassas, VA, USA). Primary fibroblasts were digested with 0.25% trypsin (Gibco, Rockville, MD, USA) inoculated into a 75-cm² cell flask with Dulbecco's modified Eagle's medium containing 1% penicillin and streptomycin (DMEM, KeyGEN, Nanjing, China) with 15% fetal bovine serum (FBS, Gibco, Rockville, MD, USA). When cells grew to 90% confluence, we performed cell passage into 25-cm² flasks. After starvation with serum-free DMEM for 24 h, sitagliptin (10 nM and 20 nM, Abcam, Cambridge, MA, USA) was used to pretreat fibroblasts for 24 h, then TGF-β (10 ng/mL, Sigma, St. Louis, MO, USA) was used to activate fibroblasts for 24 h.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Total RNA was extracted from fibroblasts using TRIzol reagent (Beyotime Biotechnology, Shanghai, China) according to the manufacturer's protocol. Reverse transcription was conducted to synthesize complementary deoxyribose nucleic acid (cDNA) using a qScript Flex cDNA Synthesis Kit (Quanta Biosciences, Beverly, MA, USA). A total of 20 μL reaction mixture was used to quantify the RNAs using Perfecta SYBR Green Supermix (Quanta Biosciences, Beverly, MA, USA). We determined the specificity of every reaction by using melting curve analysis. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used for normalization. Relative mRNA expression levels were quantified by the $2^{-\Delta\Delta Ct}$ method and data were collected. Table 1 provides a full list of primers used. All reactions were performed in triplicate using a real-time PCR system.

Western blot (WB) analysis

Fibroblasts were treated with lysis buffer. Total proteins of the cells were extracted using a Total Protein Extraction Kit (Keygen, Nanjing, China) supplemented with phenylmethylsulfonyl fluoride (PMSF), phosphatase inhibitors, and proteinase inhibitor. We measured protein concentration using an Enhanced Bicinchoninic Acid (BCA) Protein Assay Kit (Beyotime, Shanghai, China) and balanced them. After electrophoretic separation, transfer onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA), and blocking procedures, proteins were incubated overnight with anti-α-SMA (Abcam, Cambridge, MA, USA, 1: 1000), anti-Col-1 (Abcam, Cambridge, MA, USA, 1: 1000), anti-Col-3 (Abcam, Cambridge, MA, USA, 1: 1000), anti-fibronectin (Abcam,

Table 1. Primer sequences of quantitative reverse transcription-
polymerase chain reaction.

Genes		Sequences
Col-1	Forward	GAGGGCCAAGACGAAGACATC
	Reverse	CAGATCACGTCATCGCACAAC
Col-3	Forward	GAGCCAAAGGATCTGCTGGT
	Reverse	TTGGGGCCTTGTTCACCTTT
Fibronectin	Forward	CGGTGGCTGTCAGTCAAAG
	Reverse	AAACCTCGGCTTCCTCCATAA
GAPDH	Forward	AGGTCGGTGTGAACGGATTTG
	Reverse	GGGGTCGTTGATGGCAACA

Cambridge, MA, USA, 1: 1000), and anti-GAPDH (Proteintech, Rosemont, IL, USA, 1: 10 000). After washing with Tris-buffered saline-tween (TBST), the proteins were incubated with secondary antibody (YiFeiXue, Nanjing, China, 1: 10 000) for 1 h at room temperature. Proteins were then visualized and detected using an electrochemiluminescence (ECL) system.

Immunofluorescence (IF)

Fibroblasts were fixed with 4% PFA and assessed by IF with α-SMA (Abcam, Cambridge, MA, USA, 1: 200) and Ki67 (Abcam, Cambridge, MA, USA, 1: 300) overnight at 4°C. Cells were then

washed with phosphate-buffered saline (PBS) and combined with Alexa Fluor 488 or 594-conjugated AffiniPure F(ab')2 Fragment Goat Anti-Rabbit IgG (H+L) (Jackson, West Grove, PA, USA, 1: 500) for 1 h at room temperature. Counterstaining of nuclei and mounting were performed with 4',6-diamidino-2-phenylindole (DAPI)-Fluoromount-G (Southern Biotech, Birmingham, AL, USA). Then, cells were imaged using a microscope.

Flow cytometry assay

Apoptosis was determined using a 70-AP101-30 Annexin V-FITC/PI Apoptosis Kit (MultiSciences, Hangzhou, China) following the manufacturer's procedures. Fibroblasts were incubated with Annexin V-FITC and propidium iodide (PI) in the dark for 30 min. Apoptotic cells and viable cells were sorted using a fluorescence-activated cell-sorting flow cytometer (BD FACSCalibur, BD Biosciences, Detroit, MI, USA). Then, we used FlowJo software to collect the cytometry images.

Scratch assay

Fibroblasts were seeded into a 12-well plate and underwent starvation treatment for 24 h, then we added 2 mL DMEM with 10% FBS into each well and used 10 nM and 20 nM sitagliptin to pretreat cells for 24 h. After TGF-β (10 ng/mL) treatment for 24 h, we used a 10-μL pipette tip to scratch the cell layer. After 24 h, we collected images using a microscope.
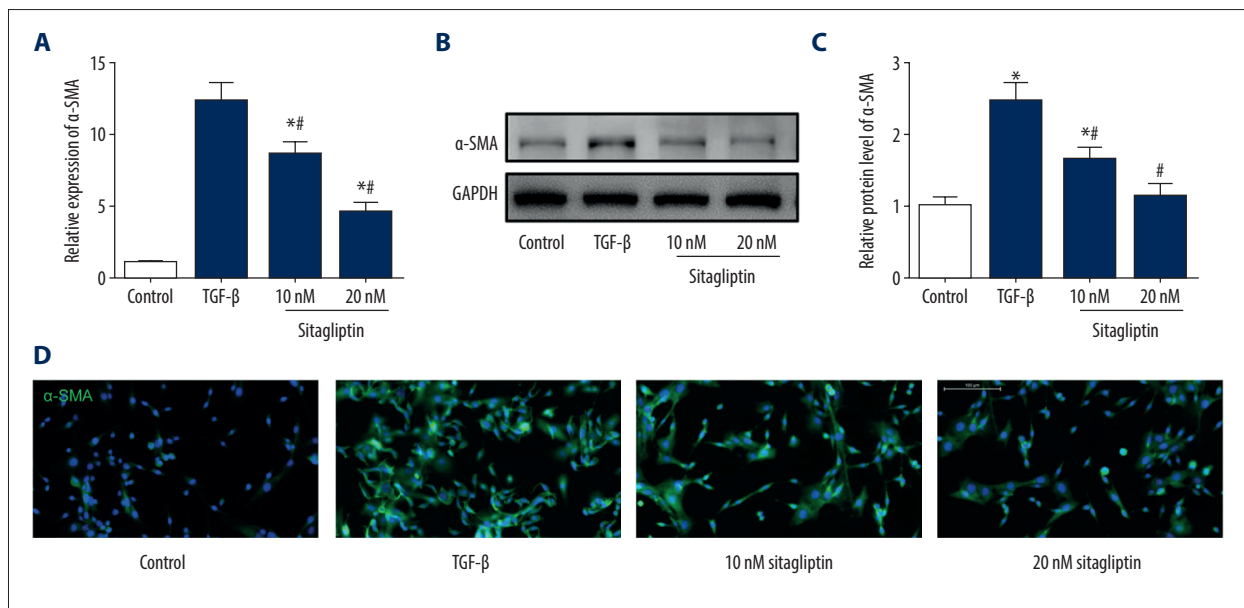

Figure 1. Concentration-dependent effect of sitagliptin in inhibiting lung fibroblasts from differentiating into myofibroblasts. (**A**) Representative α-SMA RNA levels in control, TGF-β, 10-nM sitagliptin, and 20-nM sitagliptin groups. (**B**) Representative α-SMA protein in control, TGF-β, 10-nM sitagliptin, and 20-nM sitagliptin groups. (**C**) Representative quantitative analysis of α-SMA in control, TGF-β, 10-nM sitagliptin, and 20-nM sitagliptin groups. (**D**) Representative immunofluorescence of α-SMA (green) in control, LPS, 10-nM Liraglutide, and 20-nM Liraglutide groups (200×); scale bar=100 μm. * Means *vs.* control group; # means *vs.* TGF-β group with statistical significance.
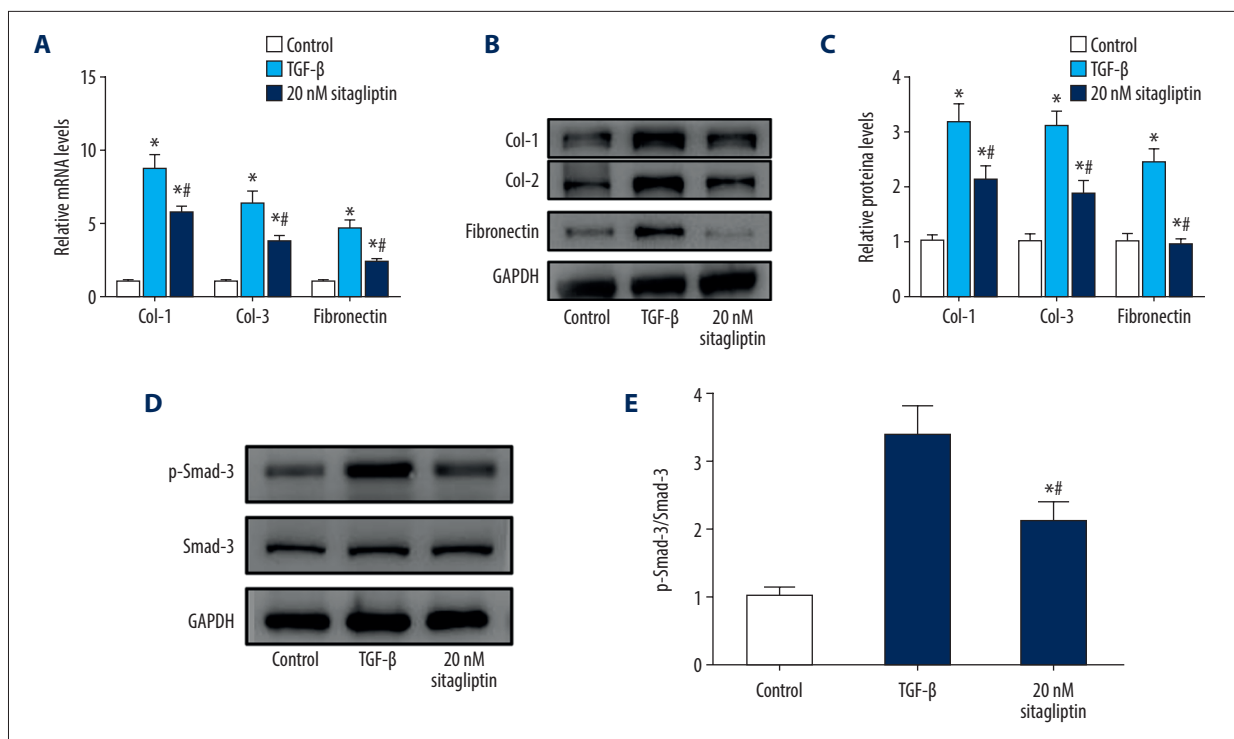
Figure 2. Sitagliptin attenuates ECM accumulation via suppressing the TGF-β/Smad-3 pathway. (**A**) Representative RNA levels of Col-1, Col-3, and fibronectin in control, TGF-β, and 20-nM sitagliptin groups. (**B**) Representative proteins level of Col-1, Col-3, and fibronectin in control, TGF-β, and 20-nM sitagliptin groups. (**C**) Representative quantitative analysis of Col-1, Col-3, and fibronectin in control, TGF-β, and 20-nM sitagliptin groups. (**D**) Representative proteins level of p-Smad-3 and Smad-3 in control, TGF-β, and 20-nM sitagliptin groups. (**E**) Representative quantitative analysis of p- Smad-3 and Smad-3 ratio in control, TGF-β, and 20-nM sitagliptin groups. * Means *vs.* control group with statistical significance; # means *vs.* TGF-β group with statistical significance.

Statistical analysis

Data are displayed as means±SD (standard deviations). Difference between the 2 groups were analyzed using the *t* test. Comparisons between multiple groups were done using one-way ANOVA test followed by a post hoc test (least significant difference). Data were collected and assessed using Statistical Product and Service Solutions (SPSS) 18.0 software (SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered statistically significant.

Results

Sitagliptin inhibits lung fibroblasts differentiating to myofibroblasts in a concentration-dependent manner

To examine whether sitagliptin inhibits lung fibroblasts differentiation, we used TGF-β to stimulate fibroblasts and different concentrations of sitagliptin-treated cells. Myofibroblasts are differentiated from fibroblasts, which specifically expresses α-smooth muscle actin (α-SMA) at high levels. Hence, we

assessed the RNA level of α-SMA in each group, finding that sitagliptin inhibited the RNA level of α-SMA, especially in the 20-nM group (Figure 1A). Consistent with results at the transcriptional level, the protein level of α-SMA also decreased after sitagliptin treatment (Figure 1B, 1C). Moreover, immunofluorescence showed that 10 nM sitagliptin slightly decreased the expression of α-SMA (green), while 20 nM sitagliptin significantly reduced the expression of α-SMA (green) in fibroblasts/myofibroblasts (Figure 1D). The above results show that sitagliptin inhibits fibroblasts differentiation in a concentration-dependent manner.

Sitagliptin attenuates ECM accumulation by suppressing the TGF-β/Smad-3 pathway

Next, we investigated the effect of sitagliptin on ECM expression in lung fibroblasts. Type 1 collagen (Col-1), type 3 collagen (Col-3), and fibronectin were measured at the transcriptional and translational levels. We found that sitagliptin treatment remarkably decreased the RNAs expression of Col-1, Col-3, and fibronectin compared with the TGF-β group (Figure 2A). Consistently, the protein levels of Col-1, Col-3, and fibronectin
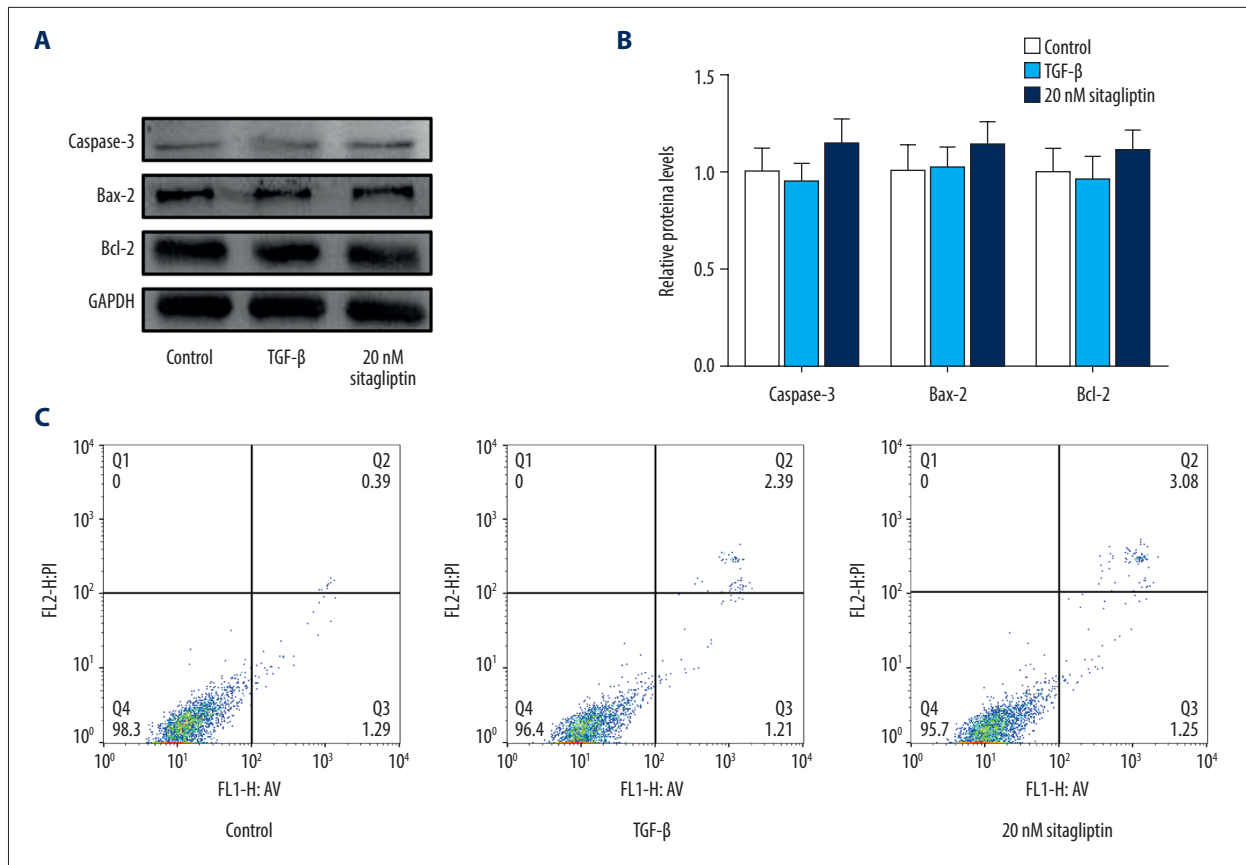
Figure 3. Administration of Sitagliptin did not affect apoptosis of lung fibroblasts. (**A**) Representative proteins level of Caspase-3, Bax-2, and Bcl-2 in control, TGF-β, and 20-nM sitagliptin groups. (**B**) Representative quantitative analysis of Caspase-3, Bax-2, and Bcl-2 in control, TGF-β, and 20-nM sitagliptin groups. (**C**) Representative flow cytometry results of apoptosis in control, TGF-β, and 20-nM sitagliptin groups. * Means *vs.* control group with statistical significance; # means *vs.* ALI group with statistical significance.

decreased after sitagliptin administration (Figure 2B, 2C). To determine the mechanism by which sitagliptin inhibits ECM production, we examined the phosphorylated level of Smad-3, which is a critical factor in the TGF-β pathway. Western blotting showed that TGF-β induction provoked strong phosphorylation of Smad-3, but sitagliptin reduced phosphorylation of Smad-3 (Figure 2D, 2E). Therefore, sitagliptin alleviates ECM deposition through downregulating the TGF-β/Smad-3 pathway.

Administration of sitagliptin did not affect apoptosis of lung fibroblasts

We also speculated that sitagliptin can affect apoptosis or proliferation in lung fibroblasts. Hence, we further assessed levels of the apoptosis-related factors Bax-2, Bcl-2, and Caspase-3. Western blotting showed that there was no significant difference in these proteins between groups (Figure 3A, 3B). We also visualized the apoptosis ratio of cells in each group using cytometry assay, finding that sitagliptin and TGF-β did not affect the number of apoptotic fibroblasts (Figure 3C). These

results confirm that sitagliptin administration does not inhibit fibrosis by promoting apoptosis.

Sitagliptin restrains proliferation of lung fibroblasts by downregulating Ki-67 expression

Because sitagliptin did not affect the apoptosis of lung fibroblasts, we further examined the proliferation level of lung fibroblasts. Ki-67, a representative biomarker of cell proliferation, was used to reflect the proliferation level of fibroblasts by using immunofluorescence. We found that TGF-β treatment remarkably promoted Ki-67 expression in fibroblasts, but sitagliptin administration significantly inhibited the Ki-67 level compared with the TGF-β treatment group (Figure 4A). We performed the scratch assay to assess the proliferation of fibroblasts in 24 h, showing that the number of cells in the scratch greatly increased compared with the control group, but sitagliptin intervention reduced the number of cells in the scratch after TGF-β stimulation (Figure 4B). Hence, the results indicate that sitagliptin reduces Ki-67 expression and inhibits proliferation of fibroblasts.
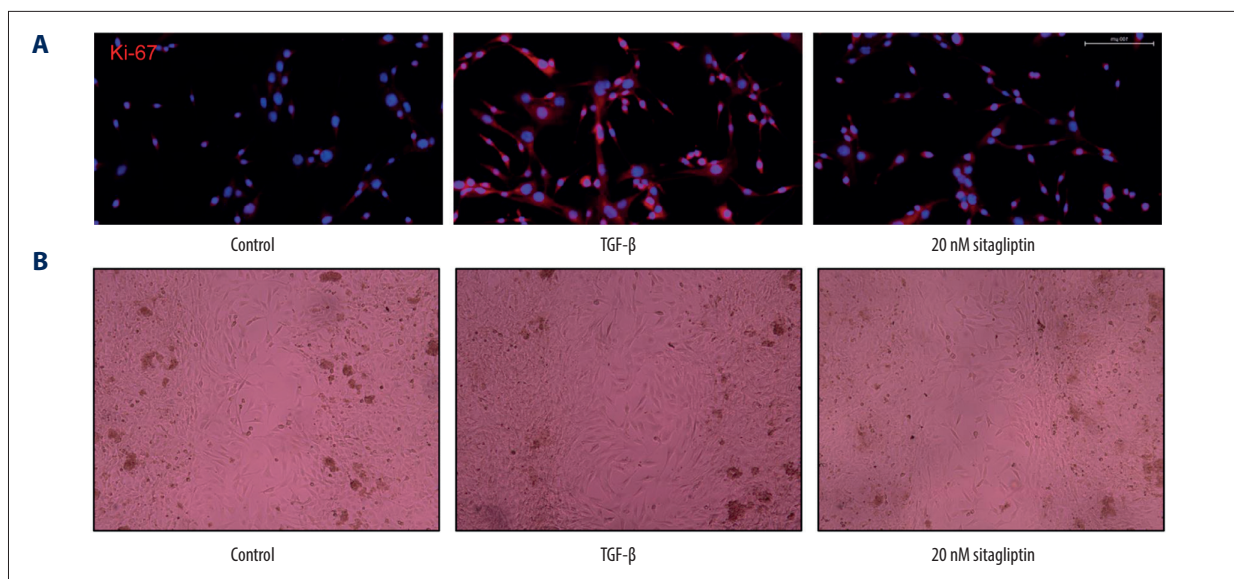
Figure 4. Sitagliptin restrains proliferation of lung fibroblasts via downregulating Ki-67 expression. (**A**) Representative IF staining of Ki-67 (red) in control, TGF-β, and 20-nM sitagliptin groups (200×); scale bar=100 μm. (**B**) Representative scratch assay result at 24 h in control, TGF-β, and 20-nM sitagliptin groups (40×).

Discussion

The activation of lung fibroblasts in IPF is a complex process involving pathogenic factors in various organic diseases. Deteriorative fibrosis in IPF induces extensive interstitial hyperplasia, systemic dysfunction, and even life-threating events [17–19]. Repeated injury provokes proliferation and differentiation of lung fibroblasts in lesions to repair the injured site. However, chronic fibrosis in lung tissue can trigger parenchymal cells to transform into fibroblasts, ultimately forming scars and impairing physiological functions [20]. Therefore, finding an effective method to control fibroblasts at the preliminary stage might reduce IPF exacerbation. Lung fibroblasts are important interstitial cells regulating tissue repair response in the lungs. When activated, fibroblasts turn into myofibroblasts and secrete a variety of collagens, fibrous proteins, and growth factors to induce more extensive fibrosis [21]. The overexpression of pro-fibrotic factors becomes the driving force in the deterioration caused by fibrosis. DDP-4 inhibition has been shown to ameliorate fibrosis in various tissues. Interestingly, a recent study reported that sitagliptin reduced inflammation and fibrosis and improved cardiac function [22]. Therefore, we assumed that sitagliptin can attenuate IPF by targeting lung fibroblasts after TGF-β treatment. In the present study, we found that sitagliptin downregulated α-SMA expression in lung fibroblasts after TGF-β stimulation by inhibiting differentiation of myofibroblasts in response to fibrosis. Sitagliptin treatment also caused a clear decrease in the ECMs Col-1, Col-3, and fibronectin. Mechanically, the TGF-β/Smad-3 pathway is a critical regulatory signaling loop that plays a key role in regulating the expression of fibrosis, including fibroblasts activation,

secretion, and proliferation. In several fibrotic diseases, effectively inhibiting the canonical TGF-β/Smad-3 pathway activation in fibroblasts can attenuate scarring in repairing tissue. Hence, we suspected that sitagliptin serves as a potent antidiabetic and can alleviate fibrosis by restraining TGF-β/Smad-3 pathway activation in fibroblasts. We found that sitagliptin clearly reduced the level of phosphorylated Smad-3 protein in activated lung fibroblasts, indicating that sitagliptin exerts an anti-fibrotic effect on the TGF-β-induced lung fibroblasts by inhibiting the Smad-3 signaling pathway. DDP-4 inhibition negatively regulates non-canonical TGF-β pathways such as ERK, and P38 signaling also attenuated cultured fibroblasts activation as well as experimental fibrosis. It was reported that DPP-4 inhibition ameliorated fibroblasts activation and acted as a potential target for systemic sclerosis treatment via specifically suppressing ERK signaling [23]. Another study demonstrated that sitagliptin decreased p38 phosphorylation in activated hepatic stellate cells and exerted an anti-fibrotic effect on hepatic fibrosis [24]. We proved that sitagliptin inhibits the Smad pathway to mitigate lung fibroblasts activation, but the regulatory effect of sitagliptin on other non-canonical TGF-β pathways needs to be explored in further research. We also assessed the function of sitagliptin in cell apoptosis and proliferation after TGF-β stimuli. Our assessment of apoptosis-related proteins in lung fibroblasts showed that sitagliptin and TGF-β did not affect apoptosis of cells, and flow cytometry showed that the numbers of apoptotic fibroblasts in each group were not significantly different. These data indicate that sitagliptin does not exert a pro-apoptosis effect in fibroblasts. We also assessed the effect of sitagliptin on cell proliferation through immunofluorescence staining of Ki-67, showing that

fibroblasts can be activated to the fibrogenic phenotype and promotes proliferation via TGF-β induction. Previous studies showed that TGF-B/Smad pathway signaling increased fibroblast proliferation and Ki-67 expression [25,26]. In the present study, we found that sitagliptin inhibited Ki-67 expression in lung fibroblasts, possibly by altering the TGF-B/Smad pathway. Our scratch assay results showed that sitagliptin reduced the number of proliferative cells in the scratch, indicating that sitagliptin could be useful in inhibiting fibroblast proliferation. Although we proved that sitagliptin possesses a promising anti-fibrotic effect on fibroblasts *in vitro*, *in vivo* studies using an IPF model are needed, and further exploration of the mechanism regulating IPF is warranted.

Conclusions

Our results suggest that sitagliptin treatment reduces fibrosis by inhibiting the TGF-β/Smad-3 pathway in lung fibroblasts, resulting in decreased ECMs and limited proliferation, but does not affect apoptosis. Thus, sitagliptin may be a promising therapeutic agent to inhibit IPF.

Conflict of interest

None.

References:

1. Sorensen RU, Waller RL, Klinger JD: Cystic fibrosis. Infection and immunity to pseudomonas. Clin Rev Allergy, 1991; 9: 47–74

2. Hsu D, Taylor P, Fletcher D et al: Interleukin-17 pathophysiology and therapeutic intervention in cystic fibrosis lung infection and inflammation. Infect Immun, 2016; 84: 2410–21

3. Selman M, King TE, Pardo A: Idiopathic pulmonary fibrosis: Prevailing and evolving hypotheses about its pathogenesis and implications for therapy. Ann Intern Med, 2001; 134: 136–51

4. Wei J, Zhu H, Komura K et al: A synthetic PPAR-gamma agonist triterpenoid ameliorates experimental fibrosis: PPAR-gamma-independent suppression of fibrotic responses. Ann Rheum Dis, 2014; 73: 446–54

5. Marudamuthu AS, Shetty SK, Bhandary YP et al: Plasminogen activator inhibitor-1 suppresses profibrotic responses in fibroblasts from fibrotic lungs. J Biol Chem, 2015; 290: 9428–41

6. Rand TG, White K, Logan A, Gregory L: Histological, immunohistochemical and morphometric changes in lung tissue in juvenile mice experimentally exposed to *Stachybotrys chartarum* spores. Mycopathologia, 2003; 156: 119–31

7. Alt E, Yan Y, Gehmert S et al: Fibroblasts share mesenchymal phenotypes with stem cells, but lack their differentiation and colony-forming potential. Biol Cell, 2011; 103: 197–208

8. Munoz-Felix JM, Fuentes-Calvo I, Cuesta C et al: Absence of K-Ras reduces proliferation and migration but increases extracellular matrix synthesis in fibroblasts. J Cell Physiol, 2016; 231: 2224–35

9. Tipton DA, Howell KJ, Dabbous MK: Increased proliferation, collagen, and fibronectin production by hereditary gingival fibromatosis fibroblasts. J Periodontol, 1997; 68: 524–30

10. Carthy JM, Sundqvist A, Heldin A et al: Tamoxifen Inhibits TGF-beta-mediated activation of myofibroblasts by blocking non-Smad signaling through ERK1/2. J Cell Physiol, 2015; 230: 3084–92

11. Sonnylal S, Denton CP, Zheng B et al: Postnatal induction of transforming growth factor beta signaling in fibroblasts of mice recapitulates clinical, histologic, and biochemical features of scleroderma. Arthritis Rheum, 2007; 56: 334–44

12. Azuma M, Tamatani T, Yuki T et al: Increased matrix metalloproteinase-2 activity induced by TGF-beta 1 in duct cells of human salivary gland is associated with the development of cyst formation *in vivo*. J Oral Pathol Med, 1996; 25: 467–73

13. Aso Y, Ozeki N, Terasawa T et al: Serum level of soluble CD26/dipeptidyl peptidase-4 (DPP-4) predicts the response to sitagliptin, a DPP-4 inhibitor, in patients with type 2 diabetes controlled inadequately by metformin and/or sulfonylurea. Transl Res, 2012; 159: 25–31

14. Kanasaki K, Shi S, Kanasaki M et al: Linagliptin-mediated DPP-4 inhibition ameliorates kidney fibrosis in streptozotocin-induced diabetic mice by inhibiting endothelial-to-mesenchymal transition in a therapeutic regimen. Diabetes, 2014; 63: 2120–31

15. Hirakawa H, Zempo H, Ogawa M et al: A DPP-4 inhibitor suppresses fibrosis and inflammation on experimental autoimmune myocarditis in mice. PLoS One, 2015; 10: e119360

16. Kaji K, Yoshiji H, Ikenaka Y et al: Dipeptidyl peptidase-4 inhibitor attenuates hepatic fibrosis via suppression of activated hepatic stellate cell in rats. J Gastroenterol, 2014; 49: 481–91

17. Gribbin J, Hubbard RB, Le Jeune I et al: Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax, 2006; 61: 980–85

18. Lettieri CJ, Nathan SD, Barnett SD et al: Prevalence and outcomes of pulmonary arterial hypertension in advanced idiopathic pulmonary fibrosis. Chest, 2006; 129: 746–52

19. Kolb M, Kirschner J, Riedel W et al: Cyclophosphamide pulse therapy in idiopathic pulmonary fibrosis. Eur Respir J, 1998; 12: 1409–14

20. Kazufumi M, Sonoko N, Masanori K et al: Expression of bcl-2 protein and APO-1 (Fas antigen) in the lung tissue from patients with idiopathic pulmonary fibrosis. Microsc Res Tech, 1997; 38: 480–87

21. Nedeau AE, Bauer RJ, Gallagher K et al: A CXCL5- and bFGF-dependent effect of PDGF-B-activated fibroblasts in promoting trafficking and differentiation of bone marrow-derived mesenchymal stem cells. Exp Cell Res, 2008; 314: 2176–86

22. Esposito G, Cappetta D, Russo R et al: Sitagliptin reduces inflammation, fibrosis and preserves diastolic function in a rat model of heart failure with preserved ejection fraction. Br J Pharmacol, 2017; 174: 4070–86

23. Soare A, Gyorfi HA, Matei AE et al: Dipeptidylpeptidase 4 as a marker of activated fibroblasts and a potential target for the treatment of fibrosis in systemic sclerosis. Arthritis Rheumatol, 2020; 72: 137–49

24. Shimozato N, Namisaki T, Kaji K et al: Combined effect of a farnesoid X receptor agonist and dipeptidyl peptidase-4 inhibitor on hepatic fibrosis. Hepatol Res, 2019; 49: 1147–61

25. Mamalis A, Jagdeo J: High-fluence light-emitting diode-generated red light modulates the transforming growth factor-beta pathway in human skin fibroblasts. Dermatol Surg, 2018; 44: 1317–22

26. Wang X, Qu M, Li J et al: Induction of fibroblast senescence during mouse corneal wound healing. Invest Ophthalmol Vis Sci, 2019; 60: 3669–79