Effect of IFN-γ and dexamethasone on TGF-β_1-induced human fetal lung fibroblast-myofibroblast differentiation[1]

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KEY WORDS transforming growth factor beta; interferon type II; dexamethasone; fibroblasts; cell differentiation; collagen; smooth muscle; actins

ABSTRACT

AIM: To study whether Smads signaling pathway was involved in human fetal lung fibroblast-myofibroblast differentiation induced by transforming growth factor (TGF)-β_1 and the role of interferon (IFN)-γ, dexamethasone (DEX) in the fibroblast-myofibroblast differentiation. **METHODS:** α-Smooth muscle actin (α-SMA), Smad2/3, and Smad7 protein were assessed by Western blot. Collagen protein was analyzed by measuring hydroxyproline. α-SMA and collagen III mRNA were assessed by RT-PCR. Myofibroblasts morphology and Smad2/3 nuclear translocation were assessed by immunohistochemistry. The overexpression of Smad7, a negative mediator of Smads signaling pathway, was acquired by transfection of Smad7 vector. **RESULTS:** During fibroblast-myofibroblast differentiation induced by TGF-β_1, IFN-γ 200 μg/L markedly blocked TGF-β_1-induced α-SMA protein expression (P<0.01), collagen protein (P<0.01) and mRNA (P<0.05) expression, and myofibroblasts morphological transformation, but DEX 10 μmol/L augmented TGF-β_1-induced α-SMA expression (P<0.01). For myofibroblasts, both IFN-γ 200 μg/L and DEX 10 μmol/L did not inhibit α-SMA expression (P>0.05) and collagen protein (P>0.05) and mRNA expression (P>0.05) and did not change myofibroblasts morphology. Transient transfection of Smad7 vector resulted in significant inhibition of TGF-β_1-induced α-SMA expression (P<0.01). IFN-γ 200 μg/L did not block TGF-β_1-stimulated Smad2/3 phosphorylation and their nuclear translocation. **CONCLUSION:** TGF-β_1 induced fibroblast-myofibroblast differentiation in a Smad proteins-dependent manner. IFN-γ could block this process but it was not mediated by interrupting smad2/3 phosphorylation and their nuclear translocation and DEX played a synergism with TGF-β_1. Differentiated myofibroblasts, however, were resistant to both IFN-γ and DEX.

INTRODUCTION

Interstitial myofibroblasts, characterized by α-smooth muscle actin (α-SMA) expression, have been found in idiopathic pulmonary fibrosis (IPF) which is a progressive, irreversible, and fatal fibrotic disease[1]. Because they contribute to the increase of extracellular matrix deposition and the decrease of compliance of lung parenchyma associated with pulmonary fibrosis, myofibroblasts are identified as a key participant in abnormal remodeling and progressive destruction of lung parenchyma as seen in IPF[1-3]. However, relatively little is known about the underlying mechanisms that regulate myofibroblasts emergence and disappearance in IPF.

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Several lines of evidence suggested a critical role of peptide growth factor in the regulation of myofibroblasts activation. For instance, transforming growth factor (TGF)-β_1, a well-known fibrogenic cytokine, has been demonstrated to induce fibroblast-myofibroblast differentiation by up-regulating α-SMA expression[4,5]. However, the molecular mechanisms involved in this differentiation process are not fully understood. TGF-β_1 can initiate cellular signals through Smad proteins[6,7]. TGF-β_1 binds receptor serine-threonine kinase and phosphorylates the transcription factors Smad2 and Smad3. The phosphorylated Smad2/3 form heteromeric complexes with the common partner Smad4, then translocate from the cytoplasm into the nucleus where they function as transcriptional regulators. Furthermore, in contrast to receptor-activated Smad2/3, Smad7 can stably bind to TGF-β_1 receptors and interferes with ligand-induced phosphorylation of Smad2/3, so Smad7 appears to serve an autoregulatory negative feedback function in cellular TGF-β_1 signaling[8]. Thus, it needs to further study whether TGF-β_1 Smads signaling pathway is involved in regulation of human fetal lung fibroblast-myofibroblast differentiation

Blockage of TGF-β_1 signaling may prevent myofibroblastic activation. However, negative regulators of fibroblast-myofibroblast differentiation induced by TGF-β_1 are still poorly defined. IFN-γ has been reported as a possible therapeutic agent for IPF[9,10]. It can inhibit fibroblast chemotaxis, proliferation, and production of extracellular matrix[11,12]. These experimental studies focused on its regulation of lung fibroblasts. As the key role of myofibroblasts in fibrotic disease has been noticed, it is necessary to clarify the effect of IFN-γ on lung fibroblast-myofibroblast differentiation induced by TGF-β_1 and differentiated myofibroblasts.

In addition, dexamethasone (DEX) is often used to treat lung fibrotic disorders as its anti-inflammatory role, but frequently shows relatively little benefit in IPF. This therapy is based on the concept that IPF is a chronic inflammatory process. However, recent evidence strongly suggest that a central event in the pathogenesis of IPF is not inflammation, but myofibroblasts persistence[13]. Thus, it raises the question as to how DEX might interact with TGF-β_1 with regard to regulating fibroblast-myofibroblast differentiation. The experimental study of anti-fibrotic effect of DEX in IPF has been lacking until now.

In current study, we investigated whether Smads signaling pathway took part in fibroblast-myofibroblast differentiation induced by TGF-β_1, whether IFN-γ, DEX could inhibit this process and if so, what was its mechanism and whether IFN-γ and DEX also downregulated α-SMA expression and collagen production in myofibroblasts.

MATERIALS AND METHODS

Cell culture Human fetal lung fibroblasts (HFL-F) were purchased from Cell Center, Chinese Academy of Medical Sciences & Peking Union Medical College. Cells were grown in Dulbecco's modified Eagle's medium (DMEM, Life Technologies, Inc), supplemented with 10 % fetal bovine serum(FBS, HyClone) and antibiotics (benzylpenicillin 100 kU/L and streptomycin 100 mg/L) under conditions of humidified 5 % CO_2/95 % air at 37 ºC. Viability of the cells was >90 %. Cells in early passages (passage 5-10) were used in all experiments.

Antibodies and reagents Antibodies and reagents were purchased from the following vendors: active human recombinant TGF-β_1 and IFN-γ from Peprotech; dexamethasone from Sigma; mouse monoclonal anti-α-SMA antibody from DAKO; goat polyclonal anti-Smad2/3, anti-phospho Smad2/3, anti-Smad7 and anti-mouse, goat horseradish peroxidase from Santa Cruz Biotechnology; mouse monoclonal anti-flag M2 antibody and FITC-conjugated goat anti-mouse antibody from Sigma.

Immunohistochemistry To investigate fibroblast-myofibroblast morphological transformation, HFL-F (1×10^4/cm^2) were grown on culture glass cover slides in medium containing 10 % FBS for 12 h, starved for 24 h under medium containing 0.1 % FBS, then treated with various reagents. Cells were fixed with 4 % paraformaldehyde, incubated with anti-α-SMA antibody (1:50) overnight at 4 ºC, then with FITC-conjugated goat anti-mouse antibody for 1 h at room temperature, viewed with an Olympus immunofluorescent microscope. To investigate Smad2/3 nuclear translocation in HFL-F, HFL-F (2×10^4/cm^2) were grown on culture glass cover slides in medium containing 10 % FBS for 12 h, starved for 24 h in 0.1 % FBS medium, and treated with various reagents. At the end of indicated periods, cells were incubated with anti-Smad2/3 antibody (1:100) overnight at 4 ºC and then with biotinylated link secondary antibody and peroxidase-labeled streptavidin followed by a diaminobenzidine (DAB) revelation and a counterstaining with Mayer's hematoxylin. The cells

were viewed with an Olympus microscope.

Western blotting To analyze α-SMA, Smad2/3, and Smad7 protein expression, HFL-F were grown as described in immunohistochemistry respectively. At the end of indicated periods, whole cell lysates were prepared with lysis buffer containing protease inhibitor and nuclear protein was prepared with Nuclear Extract Kit (Active Motif). The protein concentrations were measured by using BCA protein assay reagent kit (Pierce). Equal amounts of protein were electrophoresed on 12 % SDS-polyacrylamide gel, and wet transferred onto polyvinylidene difluoride (PVDF) membrane. The membranes were incubated overnight at 4 ºC with various primary antibodies, then incubated with horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. Signals were visualized with chemiluminescence reagents (Pierce). Quantification of the bands was performed by using densitometric analysis software -Quantity One (Bio-Rad).

Hydroxyproline assay Collagen production was analyzed by measuring the amount of protein-bound hydroxyproline in the cell monolayer and conditioned medium[14]. HFL-F were grown on cell culture plates ($1\times10^4/cm^2$) in medium containing 10 % FBS for 12 h, starved for 24 h in medium containing 0.1 % FBS and then treated with various reagents. At the end of the experiment, the cell monolayer was scraped in the conditioned medium and the medium was mixed with 2 vol of absolute ethanol, precipitated at -20 ºC for 24 h. After precipitation, samples were centrifuged at 30 000×*g* for 30 min, and the pellet was air-dried and resuspended in 1 mL of HCl 6 mol/L. Samples were sealed in glass ampoule and hydrolysis was performed at 120 ºC for 12 h. After hydrolysis, samples were evaporated to dryness, reconstituted with 1 mL water, and solution pH was adjusted to 6-8. Hydroxyproline content was determined with a color-based reaction as described by Stegemann and Stalder[15]. Concentrations of unknown hydroxyproline were quantified according to standard curve for hydroxyproline 0 to 6 mg/L.

RT-PCR HFL-F were cultured as described as above. Total cellular RNA was isolated by using Trizol reagent (GIBCO) and semiquantitative RT-PCR was performed according to standard techniques (Tab 1). Various cycles were performed to ensure that amplification was in the linear range of curve. Equal amounts of the PCR production from both test and control (GAPDH) were mixed and separated by 1.5 % agarose gels and photographed. Band intensities were quantitated and results were expressed as test/ GAPDH ratios.

Transient transfection HFL-F were seeded at a density of 2×10^5 cells/6 well dish. The next day, cells were transfected PcDNA3.0-Flag-Smad7 expression vector or PcDNA3.0 using Fugene 6.0 (Roche) according to the manufacture's protocol. After being transfected for 24 h, cells were incubated in presence or absence of TGF-β_1 5 μg/L for 3 d. Whole cell lysates were analyzed by Western blot with anti-Flag and anti-α-SMA antibody.

Statistical analysis Data were expressed as mean±SD. The differences in mean values were analyzed by one-way ANOVA test and *post hoc* analysis with *Bonferroni* method with SPSS11.0 software. $P<0.05$ was considered statistically significant.

RESULTS

TGF-β_1-induced fibroblast-myofibroblast differentiation TGF-β_1, as expected, increased the expression of α-SMA protein in a dose-dependent (Fig 1A)

Tab 1. The PCR amplification reaction.

Gene	Primers	Cycles	Annealing temperature/ºC	Product /bp
Type I (pro α I) collagen	5′-GTCTTCCTGGCCCCTCTGGTG-3′ 5′-TCGCCCTGTTCGCCTGTCTCA-3′	30	58	391
Type III (pro α III) collagen	5′-CAGGGGCCCCAGGACTTAGAG-3′ 5′-GGGCCAGGAGGACCAATAGGA-3′	30	60.5	250
α-SMA	5′-CCAGCTATGTGAAGAAGAAGAGG-3′ 5′-GTGATCTCCTTCTGCATTCGGT-3′	25	56	965
GAPDH	5′-CCCATCACCATCTTCCAGGA-3′ 5′-TTGTCATACCAGGAAATGAGC-3′	25	56	731

and a time-dependent manner (Fig 1B). TGF-β_1 2.5 to 50 μg/L increased α-SMA protein expression (P<0.05 at 2.5 μg/L, P<0.01 at 5.0 and 50 μg/L). When HFL-F were cultured with TGF-β_1 5 μg/L, the level of α-SMA protein began to increase at 4 d (P<0.05) and 6 d (P<0.01) and the level of α-SMA mRNA began to increase at 24 h, reached the peak at 48 h, and then decreased but were still higher than that of control until 96 h (Fig 1C).

Effect of IFN-γ and DEX on fibroblast-myofibroblasts differentiation IFN-γ blocked α-SMA expression induced by TGF-β_1 in a dose-dependent manner during fibroblast-myofibroblasts differentiation. The blocking effect was maximal at IFN-γ 200 μg/L (P <0.01, Fig 2A). In contrast to IFN-γ, DEX augmented α-SMA expression induced by TGF-β_1 in a dose-dependent manner and DEX 10 μmol/L had a maximal synergistic effect with TGF-β_1 5 μg/L (P<0.01, Fig 2B).

Effect of IFN-γ and DEX on differentiated myofibroblasts HLF-F treated with TGF-β_1 5 μg/L for 4 d markedly induced α-SMA expression (P<0.01, Fig 2C, lane 2). After removal of TGF-β_1 from the medium α-SMA protein level (Fig 2C, lane 3) maintained for another 3 d, so we used these cells pretreated with TGF-β_1 5 μg/L as differentiated myofibroblasts. α-SMA expression did not decrease either in IFN-γ 200 μg/L-treated myofibroblasts (P>0.05, Fig 2C, lane 4) or DEX 10 μmol/L-treated myofibroblasts (P>0.05, Fig 2C, lane 5).

Effect of IFN-γ and DEX on morphological transformation Upon treatment with TGF-β_1 5 μg/L for 4 d, cells appeared abundant bundles of α-actin filament and transformed to a flattened myofibroblast type morphology (Fig 3B). After removal of TGF-β_1 from the medium myofibroblast morphology could maintain for another 3 d (Fig 3C). IFN-γ 200 μg/L almost completely prevented switching of lung fibroblast to myofibroblast morphology induced by TGF-β_1 (Fig 3E). DEX 10 μmol/L had no effect on morphology transformation (Fig 3H). Myofibroblasts treated with IFN-γ or DEX still appeared numerous bundles of α-actin filament (Fig 3F, I).

Effects of IFN-γ and DEX on collagen production During fibroblast-myofibroblast differentiation induced by TGF-β_1, there was an increased collagen production. IFN-γ 200 μg/L inhibited collagen production (P<0.01, Fig 4A and Fig 4D, lane 5; P<0.05, Fig 4C, lane 5). In contrast to IFN-γ, DEX 10 μmol/L had no effect on collagen production (P>0.05, Fig 4B; Fig 4C lane 6; Fig 4D, lane 6). Differentiated myofibroblasts

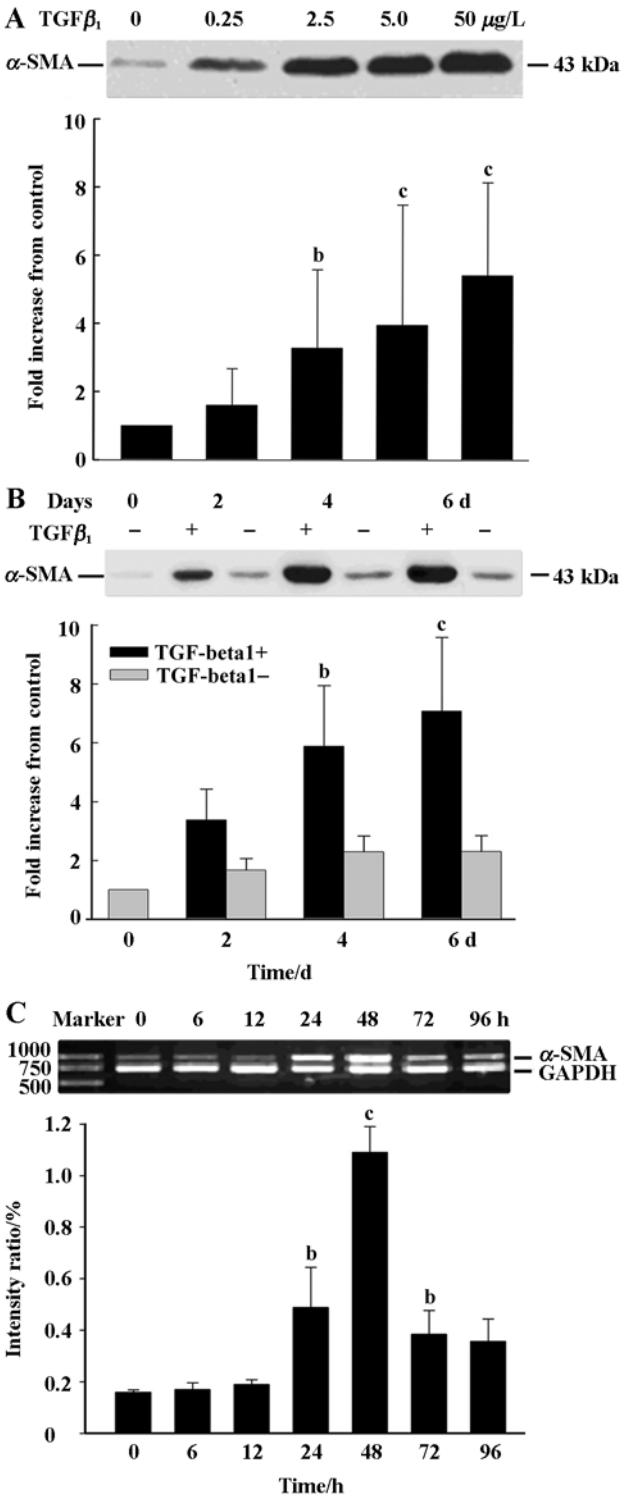

Fig 1. TGF-β_1-induced fibroblast-myofibroblast differentiation. (A) HFL-F were cultured with different concentrations of TGF-β_1 or 0.1 % FBS for 6 d. (B) HFL-F were cultured either with 0.1 % FBS or with TGF-β_1 5 μg/L for 2, 4, and 6 d. The amounts of α-SMA protein expression analyzed by Western blot were represented as relative fold to those in control (lane 1). (C) HFL-F were cultured with 0.1 % FBS or with TGF-β_1 5 μg/L for 6, 12, 24, 48, 72, and 96 h. The levels of α-SMA mRNA were represented by α-SMA/GAPDH ratio. n=3. Mean±SD. ^bP<0.05, ^cP<0.01 *vs* control.

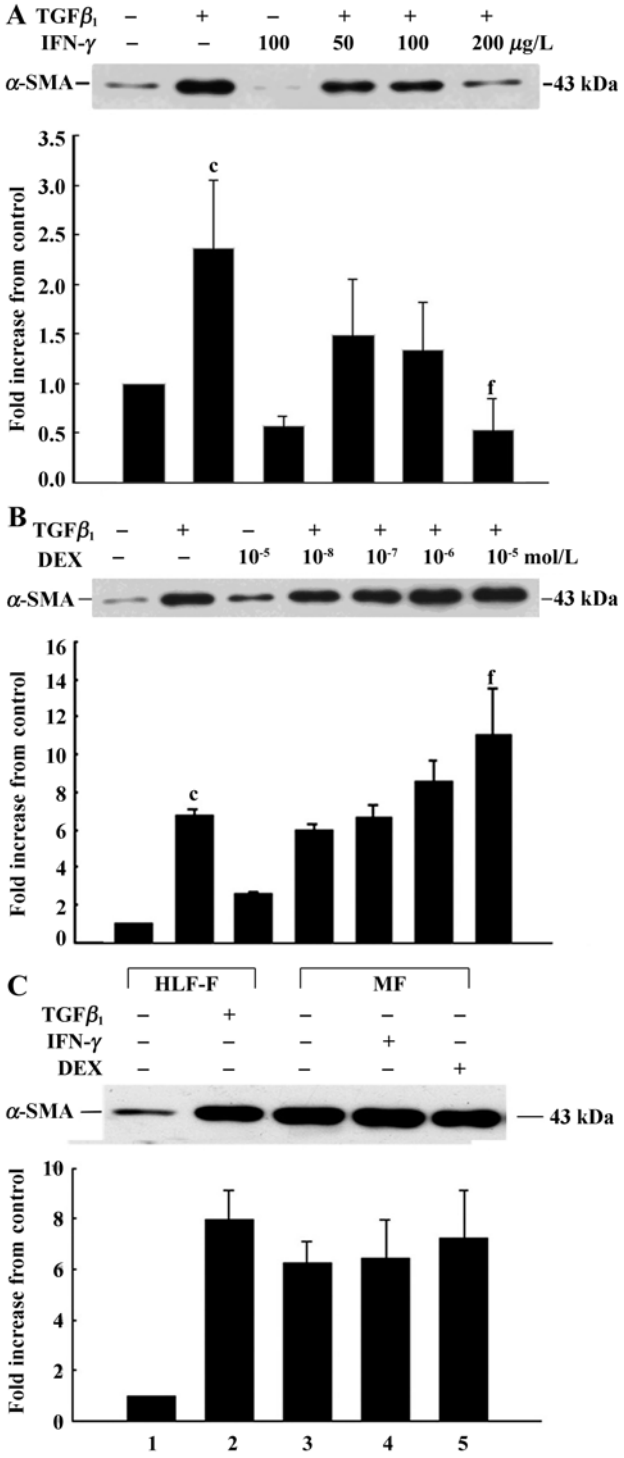

Fig 2. Effects of IFN-γ and DEX on α-SMA expression. (A) HLF-F were cultured with TGF-β 5 μg/L and/or IFN-γ 200 μg/L for 4 d. (B) HLF-F were cultured with TGF-β_1 5 μg/L and/or DEX 10 μmol/L for 4 d. (C) HLF-F were pretreated with TGF-β_1 5 μg/L for 4 d (lane 2), which were used as differentiated myofibroblasts (MF). After removal of TGF-β_1 HLF-F were further cultured for 3 d with 0.1 % FBS (lane 3) or IFN-γ 200 μg/L (lane 4), DEX 10 μmol/L (lane 5). The amounts of α-SMA protein expression analyzed by Western blot were presented as relative fold to those in control HLF-F (lane 1). n=3. Mean±SD. ^cP<0.01 *vs* control HLF-F. ^fP<0.01 *vs* cells treated with TGF-β_1 alone (lane 2).

treated with IFN-γ 200 μg/L (P>0.05, Fig 4A; Fig 4C, lane 8; Fig 4D, lane 8) or DEX 10 μmol/L (P>0.05, Fig 4B; Fig 4C, lane 9; Fig 4D, lane 9) still had higher collagen production. These results indicated that IFN-γ and Dex did not antagonize fibrotic effect of myofibroblasts.

Role of Smads in TGF-β_1-induced α-SMA expression in HFL-F Smad-2/3 phosphorylation reached the peak at 45 min and returned toward baseline after 4 h (Fig 5A). A Smad7 vector transfection resulted in markedly inhibition of α-SMA expression at d 3 compared with TGF-β_1-treated fibroblasts (P<0.01, Fig 5B). In parallel experiments, Western blot assay with anti-Flag antibody confirmed that Smad7 was being expressed in the transfected cells (Fig 5B). The result provided evidence that Smads signaling pathway was involved in fibroblast-myofibroblast differentiation induced by TGF-β_1.

Effect of IFN-γ on endogenous Smad2/3 and Smad7 protein expression Neither TGF-β_1 nor IFN-γ affected endogenous Smad2/3 and Smad7 expression. Simultaneous treatment with both TGF-β_1 and IFN-γ also did not alter Smad2/3 and Smad7 expression (P> 0.05, Fig 6).

Effect of IFN-γ on Smad2/3 phosphorylation and their nuclear translocation IFN-γ might inhibit Smad2/3 phosphorylation and their nuclear translocation to block Smads signaling pathway. But IFN-γ did not affect TGF-β_1-initiated Smad2/3 phosphorylation level either in whole cell proteins (P>0.05, Fig 7A) or in nuclear proteins (P>0.05) by Western blot analysis. To further observe translocation of Smad2/3 in the cells, HFL-F was subjected to immnohistochemistry analysis. TGF-β_1 caused accumulation of Smad2/3 within the nucleus (Fig 7C). IFN-γ pretreatment failed to prevent TGF-β_1-induced Smad2/3 nuclear translocation (Fig 7E). These data correlated with and supported the results of Western blot analysis.

DISCUSSION

In this study, we demonstrated TGF-β_1 could induce human fetal lung fibroblast-myofibroblast differentiation *in vitro* and Smads signaling pathway was involved in the process. The differentiated myofibroblasts could still remain their morphology, α-SMA expression, and higher collagen production for at least three days in medium without TGF-β_1. Thus, drugs that can interfere with fibroblast-myofibroblast differentiation induced by TGF-β_1 or downregulate α-SMA expression and col-

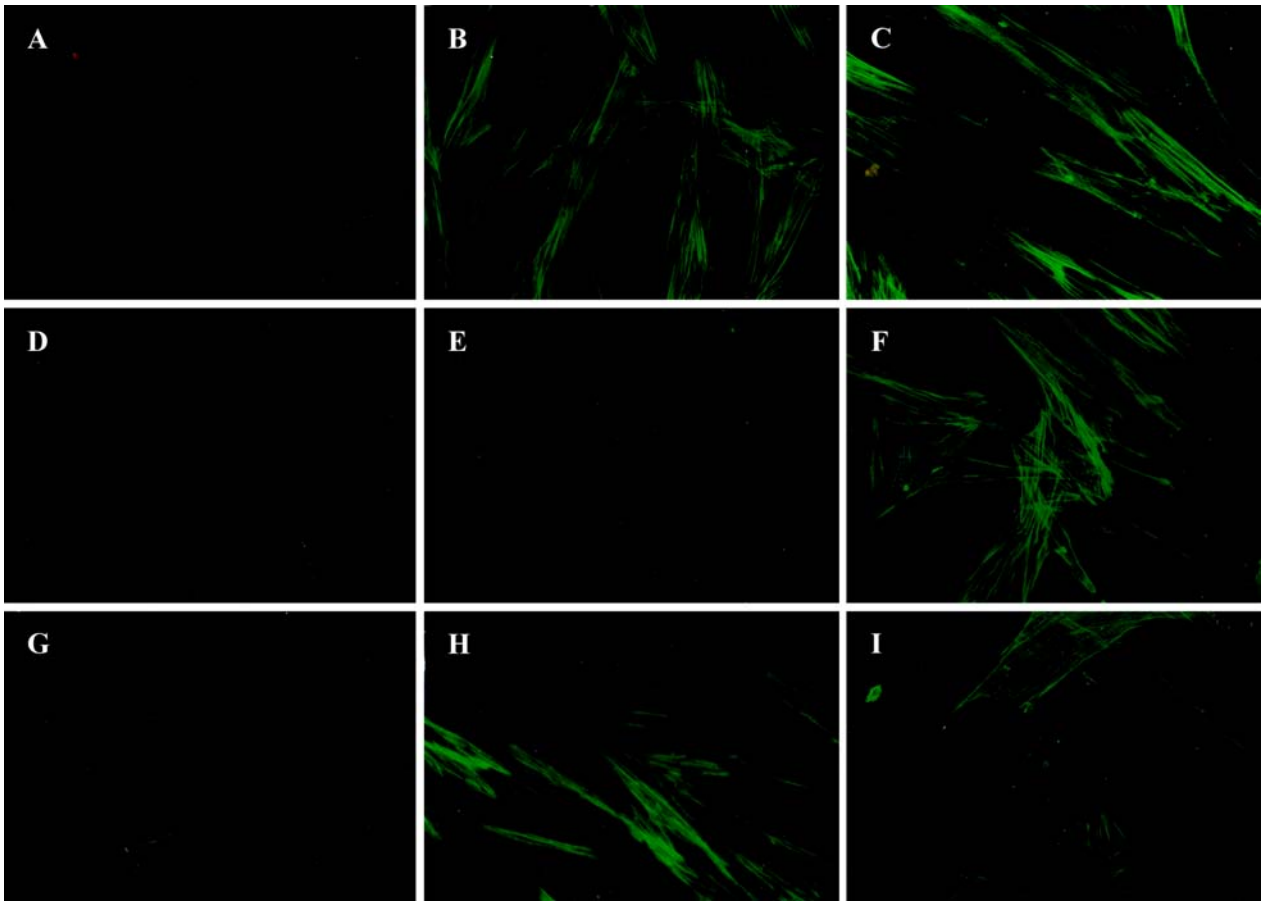

Fig 3. Effects of IFN-γ and DEX on morphologic transformation of myofibroblasts. HFL-F were incubated for 4 d with 0.1 % FBS (A) or TGF-β_1 5 μg/L (B), IFN-γ 200 μg/L (D), DEX 10 μmol/L (G), TGF-β_1 5 μg/L and IFN-γ 200 μg/L (E), TGF-β_1 5 μg/L and DEX 10 μmol/L (H). Myofibroblasts (MF) were further cultured for 3 d with 0.1 % FBS (C), or IFN-γ 200 μg/L (F), DEX 10 μmol/L (I). FITC stain. ×200.

lagen production in myofibroblasts should be therapeutically useful in IPF.

The present study demonstrated that IFN-γ strongly inhibited fibroblast-myofibroblast differentiation induced by TGF-β_1 by down-regulating α-SMA expression and collagen production, but it did not inhibit α-SMA expression and collagen production in myofibroblasts. These results were similar to that recently reported by Hasegawa *et al*[16]. They demonstrated that IFN-γ could inhibit TGF-β_1-induced α-SMA and type I collagen production in normal dermal fibroblasts, but failed to antagonize α-SMA and type I collagen production in keloid dermal fibroblasts which expressed great amount of α-SMA and appeared myofibroblast phenotype. Yokozeki *et al*[17] previously reported that IFN-γ suppressed the myofibroblast phenotype of rat palatal fibroblasts induced by TGF-β_1 pretreatment for 24 h. They used fibroblasts pretreated with TGF-β_1 only for 24 h in 10 % FBS culture medium as myofibroblasts. In our study, however, we found fibroblasts pretreated with TGF-β_1 for 3 d in 0.1 % FBS culture medium expressed α-SMA more strongly and appeared more numerous bundles of α-actin filament than those pretreated with TGF-β_1 for 1 d and 0.1 % FBS culture medium which excluded other cytokines' effect on α-SMA expression, so we suggested that this model of myofibroblasts *in vitro* was more close to myofibroblasts *in vivo*. In the future, we should further investigate whether myofibroblasts isolated from IPF patients can response to IFN-γ. IFN-γ have been noted as a potential antifibrotic agent. Clinical experience was, however, limited to a single clinical trial that showed objective functional improvement in a small number of patients treated with IFN-γ and low-dose corticosteroids[10]. Recent small series of idiopathic pulmonary fibrosis cases treated with IFN-γ did not support the use of IFN-γ therapy for patients with IPF, especially advanced IPF[18,19]. These conflicting results

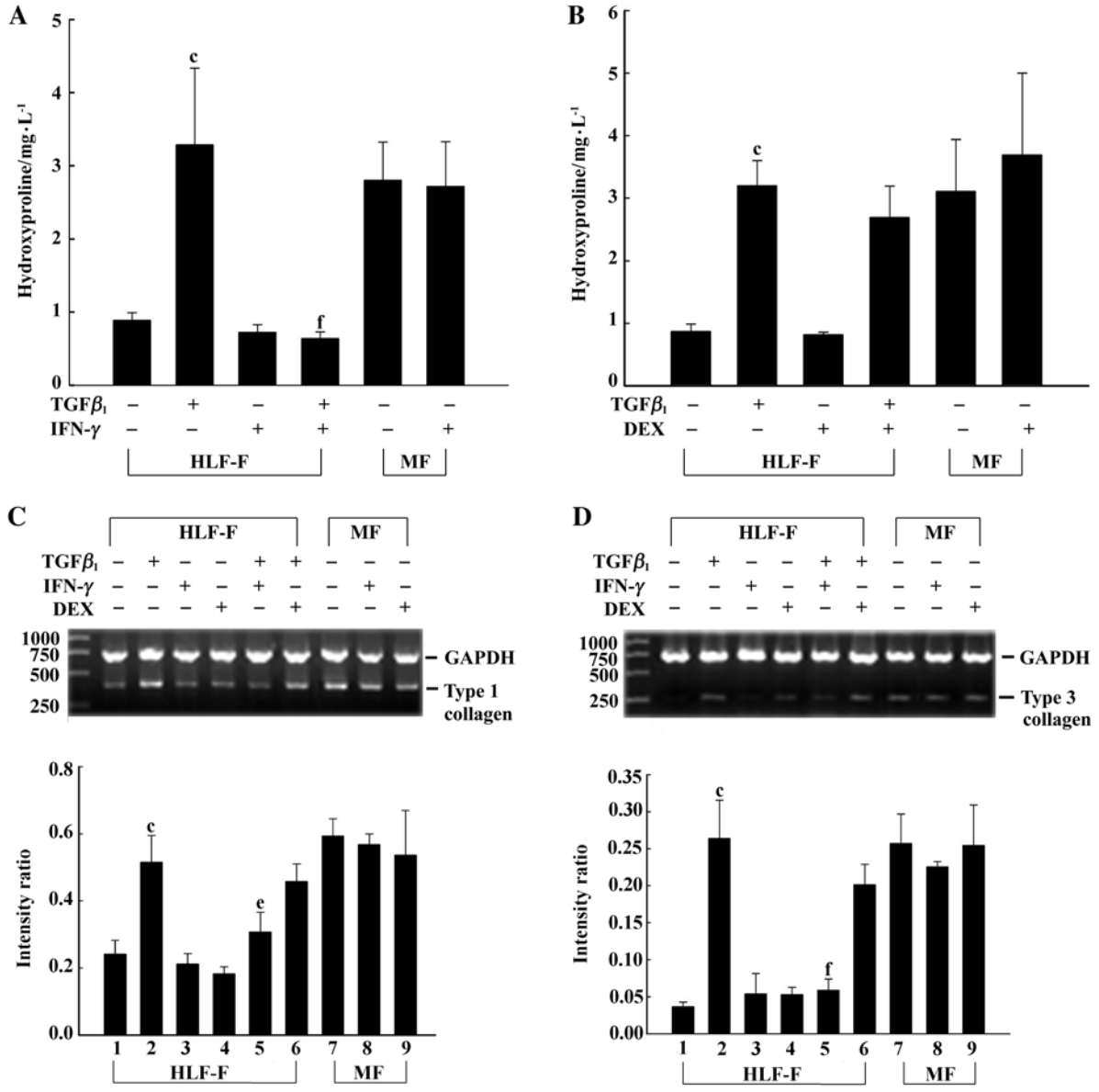

Fig 4. Effects of IFN-γ and DEX on collagen production (A, B) and collagen mRNA expression (C,D). (A) HLF-F were cultured with TGF-β_1 5 μg/L and/or IFN-γ 200 μg/L for 4 d. Myofibroblasts (MF) were further cultured with IFN-γ 200 μg/L for 3 d. (B) HLF-F were cultured with TGF-β_1 5 μg/L and/or DEX 10 μmol/L for 4 d. MF were further cultured with DEX 10 μmol/L for 3 d. The levels of type I (C) and type III (D) collagen mRNA were quantitated as relative ratio to GAPDH. $^cP<0.01$ *vs* control HLF-F (no reagent). n=3. Mean±SD. $^eP<0.05$, $^fP<0.01$ *vs* HLF-F (with TGF-β_1 alone).

suggest that for IFN-γ use in routine clinic, further research is needed to identify this efficacy. The new findings of our study provided *in vitro* evidence that IFN-γ might be effective in the early stage of IPF which strongly expressed TGF-β_1[20,21] due to blockade of fibroblast-myofibroblast differentiation, but in progressive stage, a lot of myofibroblasts appeared and IFN-γ might be little effective.

Blockade of fibroblast-myofibroblast differentiation induced by IFN-γ was likely mediated by antagonizing TGF-β_1 signaling. Although Ulloa *et al*[22] concluded that suppression of Smad-mediated responses by IFN-γ in a epithelium-derived cell was mediated by up-regulation of endogenous Smad7, our study of fibroblasts presented here suggested that this action of IFN-γ was neither dependent on alteration of Smad7 expression, nor on interruption of Smad2/3 phosphorylation and their nuclear translocation, implying that the regulation of Smads singaling was cell type-dependent and there was negative regulation of Smad signal-

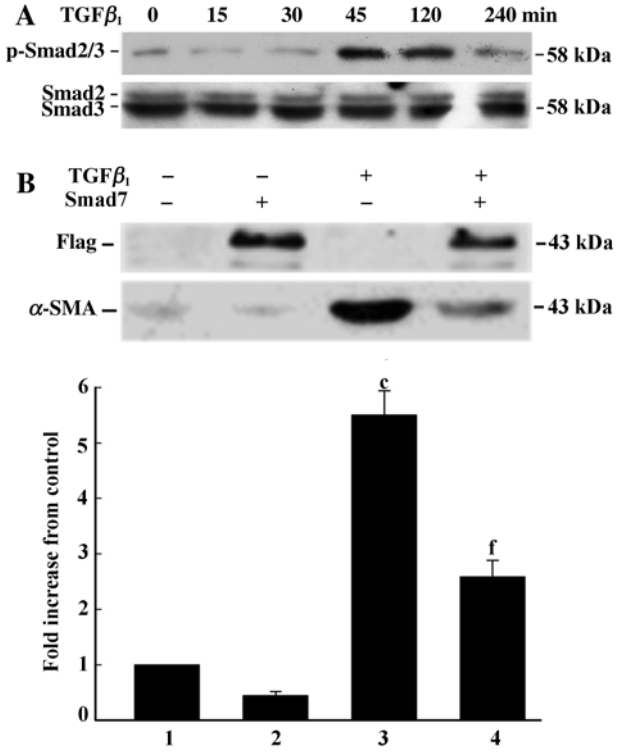

Fig 5. Decreased α-SMA expression by over-expression of Smad7 in HFL-F. (A) Kinetics of Smad2/3 phosphorylation after incubation with TGF-β_1 5 μg/L for different periods. (B) α-SMA expression by over-expression of Smad7 after incubation with or without TGF-β_1 5 μg/L for 3 d. The amounts of α-SMA protein were presented as relative ratio to those in control cells (lane 1). *n*=3. Mean±SD. $^cP<0.01$ *vs* control (lane 1). $^fP<0.01$ *vs* cells treated with TGF-β_1 alone (lane 3).

ing by IFN-γ in HFL-F, which occurred downstream of Smad2/3 nuclear translocation. Recent study demonstrated IFN-γ inhibited TGF-β_1-induced type I collagen production, in part, by antagonistic interaction of Smad and Jak-State pathway at nuclear p300/CBP level [23]. Further investigation should be done to elucidate whether IFN-γ also suppress Smad-mediated α-SMA expression during fibroblast-myofibroblast differentiation by this manner. The investigation on molecular mechanisms of blocking effects of IFN-γ during fibroblast-myofibroblast differentiation induced by TGF-β_1 and resistance of myofibroblasts will facilitate future design of specific drug for management of this lethal illness.

In contrast to IFN-γ, present study showed DEX actually could synergize with TGF-β_1 to induce fibroblast-myofibroblast differentiation and also did not inhibit α-SMA expression and collagen production in myofibroblasts. Our results and a recent study that DEX treatment did not inhibit but rather stimulated collagen synthesis by human fetal lung fibroblasts[24] did not support the use of DEX to prevent pulmonary fibrosis and even speculated that DEX treatment enhanced the fibrotic response because DEX could synergize with TGF-β_1. Theoretically, therefore, DEX did not block fibrotic process. Such interactions between DEX and TGF-β_1 may represent a major impediment to the use of glucocorticoids in the treatment of IPF.

In conclusion, the present results demonstrated that TGF-β_1 induced fibroblast-myofibroblast differentiation in a Smad proteins-dependent manner and that IFN-γ could block this process but it was not mediated by interruption of smad2/3 phosphorylation and their nuclear translocation and DEX played a synergism with TGF-β_1. However, differentiated myofibroblasts were resistant to both IFN-γ and DEX. The present findings do not support the use of Dex and IFN-γ for controlling the progression of undesirable fibrosis.

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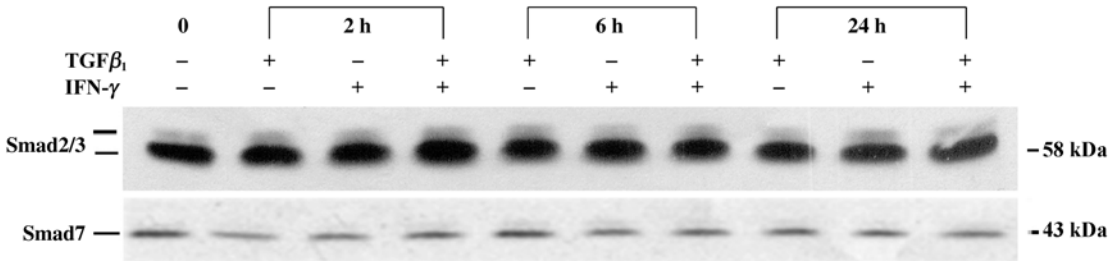

Fig 6. Effect of IFN-γ 200 μg/L on Smad2/3 and Smad7 expression induced by TGF-β_1 5 μg/L in HLF-F. *n*=3 separate experiments.

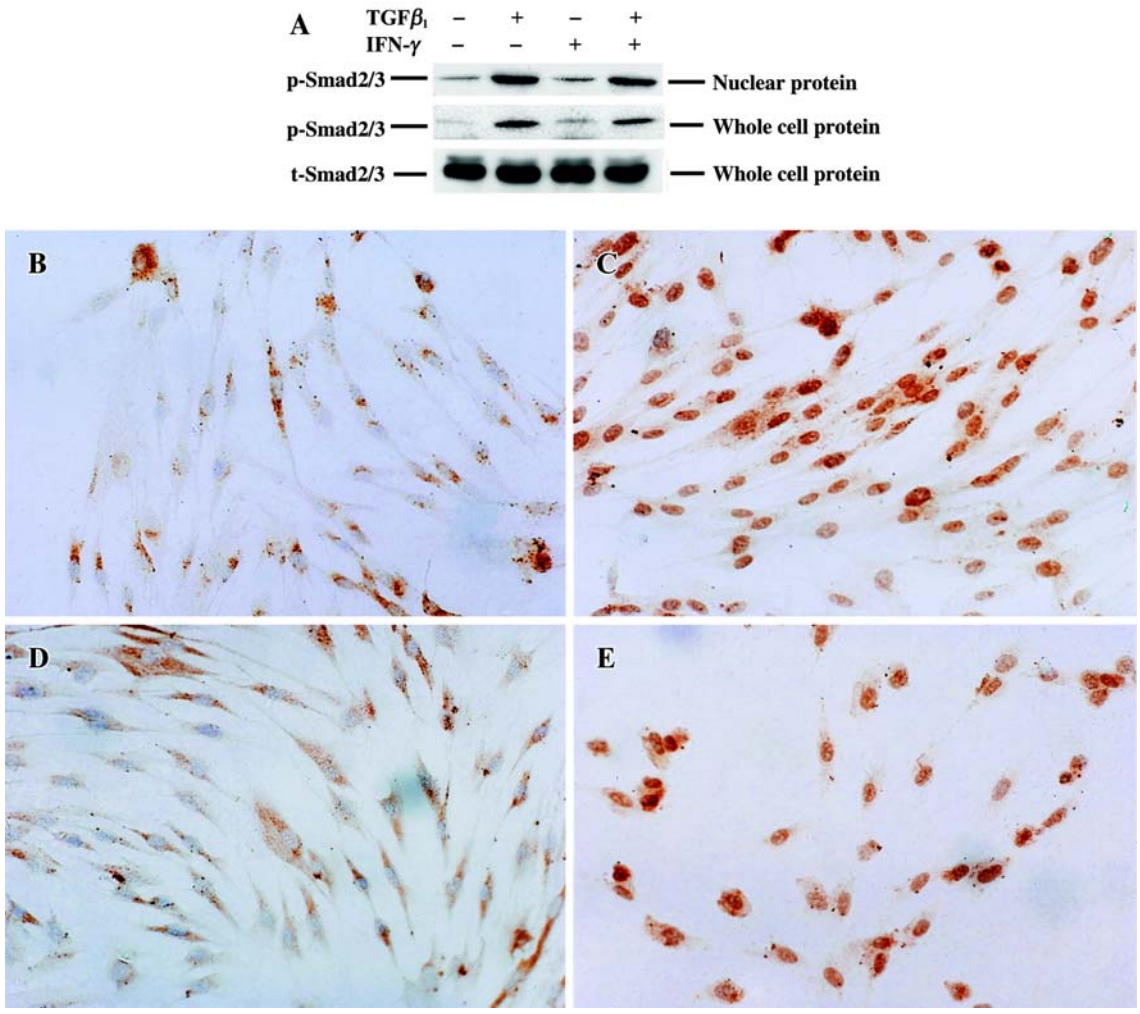

Fig 7. Effect of IFN-γ 200 μg/L on TGF-β_1-initiated Smad2/3 phosphorylation or phosphorylated Smad2/3 nuclear translocation in HLF-F. (A) HLF-F were pretreated with IFN-γ 200 μg/L for 30 min and followed by incubation with TGF-β_1 5 μg/L for an additional 45 min. (B) No reagent. (C) TGF-β_1 5 μg/L. (D) IFN-γ 200 μg/L. (E)TGF-β_1 plus IFN-γ. *n*=3 separate experiments. DAB stain. ×200.

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