Original article

Expression of suppressor of cytokine signaling 1 in the peripheral blood of patients with idiopathic pulmonary fibrosis

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***Keywords**: idiopathic pulmonary fibrosis; suppressor of cytokine signaling; interleukin 4*

Background Idiopathic pulmonary fibrosis (IPF) is a progressive diffuse parenchymal disease with a poor prognosis. A variety of cytokines and chemokines are involved in its pathophysiology. The aim of this study was to evaluate the clinical features in IPF patients with the expression of suppressor of cytokine signaling 1 (SOCS-1), which acts as a negative regulator of cytokine signaling.

Methods IPF patients (*n*=20) and healthy controls (*n*=16) were included in this study. The expression of SOCS-1 was analyzed in peripheral blood mononuclear cells (PBMC) of subjects using RT-PCR. Interleukin 4 (IL-4), transforming growth factor β1 (TGF-β1) and type I collagen expression were also analyzed in each individual using enzyme-linked immunosorbent assay (ELISA). The clinical characteristics of IPF patients were delineated. These results were analyzed by SPSS13.0 statistics software.

Results SOCS-1 mRNA expression was significantly decreased in the PBMC of IPF patients compared with healthy controls; serum levels of IL-4 and TGF-β1 were higher in IPF patients. The patients with lower expression of SOCS-1 developed lower percentage of forced vital capacity (FVC%) and DLCO/VA. A patients' SOCS-1 mRNA level was negatively correlated with serum levels of IL-4, and negatively correlated with their high-resolution computed tomography (HRCT) scores.

Conclusions SOCS-1 mRNA can be detected in PBMC, and it is down-regulated in IPF patients. The expression of SOCS-1 is associated with the severity of IPF patients' symptoms, so it might be the predictor of disease severity. SOCS-1 might play an important role in IPF by reducing the expression of the T helper type 2 (Th2) cell-related cytokine IL-4.

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Idiopathic pulmonary fibrosis (IPF) is defined as a specific form of chronic, progressive fibrosing interstitial pneumonia of unknown cause, occurring primarily in old adults, and limited to the lungs.[1] It is characterized by progressive worsening of dyspnea and lung function and is associated with a poor prognosis. The median survival of IPF is less than 3–5 years following diagnosis.[2] A variety of cytokines and chemokines are involved in its pathophysiology. T helper type 2 (Th2) cell-related cytokines are considered to be dominant, and they can promote fibroblast activation and fibrosis.[3]

The suppressor of cytokine signaling (SOCS) family, which consists of SOCS-1 to 7 and cytokine-inducible SH2-containing protein (CIS), has been shown to participate in a negative feedback loop to attenuate cytokine signaling.[4,5] Further studies of mechanisms have shown that SOCS proteins inhibit cytokine signals by regulating the Janus kinase-signal transducer and activators of transcription (JAK-STAT) pathway, which plays an important part in the initiation and activation of inflammation.[6] Recent data demonstrated that the SOCS-1 mRNA expression in fibroblasts from the lungs of IPF patients was significantly lower than controls, and it was also an inhibitor of profibrotic cytokines, such as interleukin 4 (IL-4).[7]

The objective of this study was to explore whether and how, SOCS-1 expression in the peripheral blood mononuclear cells (PBMC) of IPF patients is related with the serum concentration of cytokines IL-4 and transforming growth factor β1 (TGF-β1) and with type I collagen. We further correlated the clinical features of these patients with the expression of SOCS-1 and these cytokines.

METHODS

Patients

Serum samples were obtained from 20 Chinese adult patients with IPF who was admitted to Shanghai Ruijin Hospital from December 2011 to December 2012. Sixteen

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health examination patients were also included as healthy control subjects. IPF was diagnosed based on the American Thoracic Society (ATS).[8] IPF patients' clinical data including medical history, physical examination results, high-resolution computed tomography (HRCT), and pulmonary function test were collected upon admission. Informed consent from study participants was obtained and the study was approved by institutional review board.

Measurement of SOCS-1 mRNA, IL-4, TGF-β1 and type I collagen levels

SOCS-1 expression was examined by real-time quantitative reverse transcription-based polymerase chain reaction in IPF patients and healthy control subjects. Total RNAs were extracted from PBMC by TRIzol reagent (Invitrogen, California, USA). RNA was treated with DNase and complementary DNA was synthesized using a cDNA synthesis kit (Fermentas, Canada) according to manufacturer's instructions. Real-time PCR was performed on an ABI Prism 7900HT sequence detection system and SDS analysis software (Applied Biosystems, Foster City, California, USA). Using the ΔCt method, GAPDH was co-amplified to normalize the amount of RNA added to the reaction and the data were subjected to cycling threshold analysis. The primers used in this study were as follows: SOCS-1 forward 5′-ACCCCGTCCTCCGCGACTAC-3′ and reverse 5′-GCCGGGGAGGGTACCCACAT-3′, GAPDH forward 5′-TTGTCAAGCTCATTTCCTGGT-3′ and reverse 5′-TTACTCCTTGGAGGCCATGTA-3′.

To determine the levels of IL-4, TGF-β1 and type I collagen, serum samples were collected at the first visit. Aliquots of serum were frozen at −80ºC until assayed. All samples were assayed at the same time by enzyme-linked immunosorbent assay (ELISA) (BioTNT, Shanghai, China) according to the kit instructions.

Lung HRCT scoring

Two expert respiratory physicians, with more than 10 years of experience in CT interpretation who were blinded to the patient's clinical course, independently reviewed the IPF patients' lung HRCT. The observers assessed the presence and extent of areas of ground-glass attenuation, reticulation, honeycombing, decreased attenuation, centrilobular nodules, other nodules, consolidation and emphysema. Honeycombing[8] is manifested on HRCT as clustered cystic airspaces, typically of comparable diameters on the order of 3–10 mm but occasionally as large as 2.5 cm. The HRCT findings were graded on a scale of 0–4[9,10] on the basis of the overall extent of fibrosis (i.e., the extent of reticulation and honeycombing): 0=no involvement, 1=1%–25% involvement, 2=26%–50% involvement, 3=51%–75% involvement, and 4=76%–100% involvement.

Statistical analysis

Data are shown as mean±standard deviation (SD). Differences between groups were analyzed using *t* test. Multiple linear correlation analysis was performed to assess the correlation between SOCS-1 expression and clinical data, and the serum concentration of cytokines. Data management and analysis were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA.). A P <0.05 indicated statistical significance.

RESULTS

Clinical characteristic of IPF patients

Twenty IPF patients, including 14 males and six females with a mean age of 64 years (ranging from 38 to 87 years), were enrolled in the present study. Their lung HRCT score was 2.62±1.02, and the pulmonary function results were as follows: forced vital capacity (FVC) was 1.59±0.34 (L), FVC% was (47.84±13.09)%, DLCO/VA was 55.31±13.14.

Expression of SOCS-1 mRNA in PBMC

Analysis of RT-PCR data of SOCS-1 mRNA, corrected with GAPDH as an internal control, showed that SOCS-1 mRNA expression was significantly decreased in the PBMC of IPF patients compared with healthy controls (P=0.001) (Figure 1).

Serum levels of IL-4, TGF-β1 and type I collagen

The amounts of IL-4 and TGF-β1 in IPF patients' serum was higher than in the serum of healthy people, and the difference was statistically significant (P <0.05). But the serum levels of type I collagen in the two groups were similar, and there was no significant difference (Table 1).

Association between SOCS-1 expression and severity of clinical characteristics in patients with IPF

A linear correlation analysis was used to assess the relationship between the expression of SOCS-1 mRNA and the IPF patients' clinical characteristics. The results showed that the SOCS-1 mRNA was positively correlated with the pulmonary function (FVC% and DLCO/VA) of IPF patients

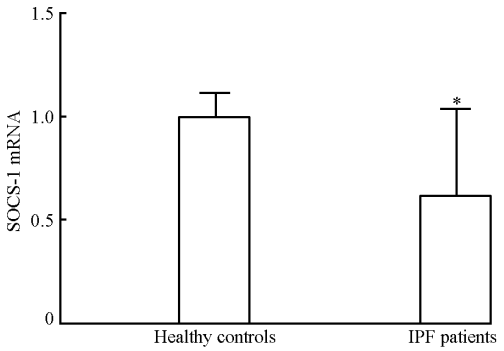

Figure 1. PBMC from IPF patients expressed lower levels of SOCS-1 mRNA than those from healthy controls. *P <0.05, compared with the healthy controls group.

Table 1. The serum levels of IL-4, TGF-β1 and type I collagen in two groups (mean±SD)

Groups	Serum levels of cytokines		
	IL-4 (pg/ml)	TGF-β1 (ng/ml)	Type I collagen (ng/ml)
IPF patients	6.51±3.26*	39.67±16.75*	5.25±2.33
Healthy controls	4.87±0.72	26.53±15.02	5.53±2.18

*P <0.05, compared with the healthy controls group.

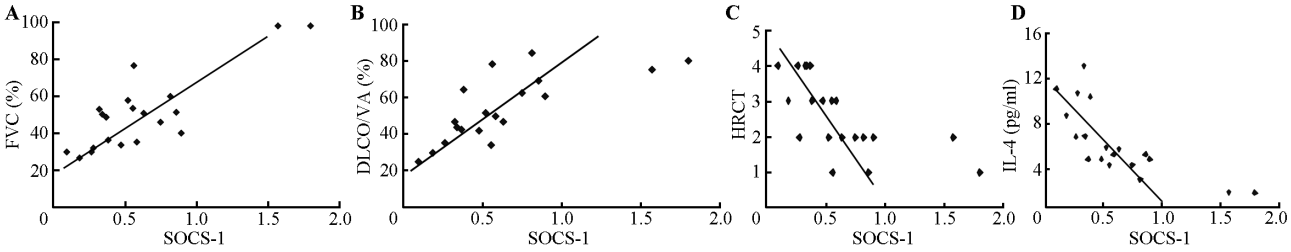

Figure 2. The association between SOCS-1 mRNA and patients' clinical data. The results showed that the SOCS-1 mRNA was positively correlated with the pulmonary function (**A, B**), negatively correlated with their HRCT scores (**C**) and serum levels of IL-4 (**D**) (P <0.05).

Table 2. Correlation between the expression of SOCS-1 and clinical characteristics

Items	FVC%	DLCO/VA	HRCT scores	IL-4
n	20	20	20	20
Correlation (*r*)	0.016	0.018	−0.269	−0.05
P values	0.01	0.03	<0.05	0.005

(P=0.01 and P=0.03), and negatively correlated with their HRCT scores (P <0.05) (Figure 2 and Table 2).

Association between SOCS-1 expression and concentration of serum cytokines in patients with IPF

The association between the expression of SOCS-1 and cytokines was analyzed by linear correlation analysis. The results showed that SOCS-1 mRNA was negatively correlated with serum levels of IL-4 (P=0.005) (Figure 2 and Table 2). However, no correlation was observed between SOCS-1 mRNA and TGF-β1 and type I collagen.

DISCUSSION

The exact mechanism of IPF has not been completely clarified. We know that Th2 cell-related cytokines have been seen in IPF patients and may play a vital role in the stimulation of the fibrosis that is a hallmark of the disease. Epithelial-mesenchymal transition (EMT) may also be an important factor in the pathogenesis, as it may lead to the accumulation of fibroblasts in the lung and a disruption of normal tissue structure.[11]

SOCS-1 has been confirmed to interrupt Th2 cell-related cytokine IL-4 signaling by inhibiting phosphorylation of STAT6 and suppression of collagen production by inhibiting Th2 cytokine signaling. To date, there are few reports showing the role of SOCS-1 in pulmonary fibrosis. Recent articles[7,12] suggested that enhanced collagen production by lung fibroblasts from patients with IPF was causally related to diminished expression of SOCS-1: SOCS-1 haplodeficient mice treated with bleomycin showed markedly enhanced pulmonary inflammation and fibrosis compared with wild-type mice. However, typical IPF patients can be diagnosed without lung biopsy, and some patients cannot tolerate bronchoscopy. Our current study used a more accessible sample, peripheral blood, to detect SOCS-1 mRNA and related cytokines, a less invasive technique. The results here demonstrated that SOCS-1 mRNA can be detected in PBMC, and it was lower in IPF patients than in healthy controls. The results also suggest that the lower the SOCS-1 mRNA level, the worse the pulmonary function (FVC% and DLCO/VA) and the higher the HRCT score. This indicated that the expression of SOCS-1 in PBMC was inversely correlated with the severity of the disease.

Experiments have shown that many cytokines, such as IL-4, IL-5, IL-13 and TGF-β1, play important roles in the pathogenesis of IPF. Th2 cell-related cytokines, IL-4, IL-5 and IL-13, were proven to have a distinct role in the regulation of tissue remodeling and fibrosis.[13] It has been proposed that inhibition of Th2 cytokine signaling could be used therapeutically to reduce fibrosis.[14,15] Nakashima et al[12] further found that the expression of IL-4 in bronchoalveolar lavage fluid (BALF) was suppressed in the SOCS-1 gene-transfected mice. TGF-β1 was shown to induce cultured primary rat alveolar epithelial cells and a rat alveolar epithelial cell line to undergo EMT.[16] Hisatomi and colleagues[17] also reported that TGF-β1 enhanced the expression of type I collagen mRNA and protein in A549 cells, which was considered to be one of the useful parameters for recognizing EMT. In the present study we demonstrated increased expression of IL-4 and TGF-β1 in IPF patients' PBMC; and with the decrease of SOCS-1 in PBMC, the IL-4 levels increased. However, there was no difference in collagen type I levels between IPF patients and healthy controls, and no correlation between SOCS-1 and TGF-β1 and collagen type I levels. This might suggest that the collagen type I level in PBMC cannot reflect the degree of lung fibrosis; its level in lung tissues may better reflect the severity of the disease.

In conclusion, our results strongly suggest that a decrease in SOCS-1 mRNA expression in PBMC is involved in IPF. It is easy to be detect and might be a predictive factor for the disease prognosis. In our previous studies,[18] we have already constructed a lentiviral vector for RNA interference targeting the SOCS-1 gene. With this tool we can do further studies to investigate whether SOCS-1 could be a novel target for treating IPF.

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