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Expression and significance of GRP78 and CXCL12 protein in idiopathic pulmonary fibrosis.

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Abstract

This study was to investigate the expression and significance of ER stress related protein GRP78 and CXCL12 in idiopathic pulmonary fibrosis. 55 biopsy or surgical specimens from idiopathic pulmonary fibrosis patients were collected in Jingzhou Central Hospital from 2009 to 2017, 10 cases of normal lung tissue as control. Western blotting was used to detect GRP78 and CXCL12 protein expression, and RT-PCR to detect GRP78 and CXCL12 expression in mRNA level. Results showed that compared with the control group, the expression of GRP78 and CXCL12 proteins in the alveolar epithelium of lung fibrosis patients were up-regulated, also the expressions of GRP78 and CXCL12 in mRNA level were up-regulated. And regressive analysis revealed that there was a positive relationship between GRP78 and CXCL12 in protein and mRNA level statistically. This suggested that GRP78/CXCL12 axis may be involved in the pathogenesis and development of idiopathic pulmonary fibrosis.

Keywords: Idiopathic pulmonary fibrosis, Endoplasmic reticulum stress, GRP78, CXCL12.

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Introduction

Idiopathic Pulmonary Fibrosis (IPF) is a type of chronic lung disease caused by various pathogenic factors, also the most common type of idiopathic interstitial. Its incidence increases significantly with the age increasing, and poor response to traditional therapies, most patients with a median survival of only 3 y, patients often died of progressive dyspnea and respiratory failure [1-3]. Recent studies have shown that oxidative stress, gene mutation, epithelial mesenchymal transition and TGF-beta activation may contribute to the pathogenesis of pulmonary fibrosis [4-6], but its pathogenesis need to be very clear.

Endoplasmic Reticulum (ER) is a very important organelle which was involved in the formation, folding and packaging of secretory proteins and membrane proteins together with Golgi apparatus. When the cell of protein requirement and endoplasmic reticulum protein synthesis ability was imbalance, the endoplasmic reticulum stress (endoplasmic reticulum, stress, ERS) will happen. If this lasts for a long time, cells will eventually start the program caspase12 dependent apoptosis. Previous studies have shown that ERS played an important role in the development and progression of cardiovascular, liver and cancer diseases, but there are few studies fuscous on ERS in IPF [7]. Glucose-regulated protein 78 kDa (GRP78), also referred to as BiP (heavy chain immunoglobulin binding protein), is the major endoplasmic reticulum (ER) chaperone regulating ER stress signaling processes.GRP78 has been described in different intracellular compartments of malignant cells, e.g. in the ER or the mitochondria, and even on the cell surface [8,9]. Also found that fibrotic response to bleomycin is dependent on GRP78-mediated events and provides evidence that macrophage polarization and apoptosis may play a role in this process [10]. CXCL12 is a cytokine of small molecules, belongs to the CXC chemokine family, fiber cells can express cytokine receptors CXCR4 and CCR2, CXCL12 is the ligand of CXCR4, the ligand binding may raise the fiber cells and participate in the process of fibrosis [11]. In this study, the clinical and pathological specimens of IPF patients were collected to observe the expression of GRP78 and CXCL12,

and to investigate its role in the pathogenesis and development of idiopathic pulmonary fibrosis.

Materials and Methods

Patients

Samples were collected from the department of pathology of Jingzhou Central Hospital from 2009 to 2017 including 55 cases of IPF patients diagnosed clinically and pathologically and 10 cases of normal lung tissues as controls. The diagnostic results were single blind and checked by two senior pathologists. The experimental materials were safe and reliable. This study was approved by the ethics committee of Yangtze University (Jingzhou, China). All patients provided informed written consent to participate in this study.

Reagents

GRP78 and CXCL12 monoclonal antibody were purchased from Wuhan boster Biological Engineering Co., Ltd., and corresponding second-antibody was purchased from Beijing Golden Bridge Biotechnology Co. ltd. Paraffin tissue protein extraction kit was purchased from Shanghai Xinyu biotechnology company, ECL kit was purchased from GE company, mRNA Extraction Kit was purchased from Invitrogen Life Technology.

Western blot analysis

Total tissue protein was extracted by paraffin tissue protein extraction kit and then the protein concentration was determined by the Bradford method [12,13]. SDS sample buffer (6X; 50 mmol/l Tris-HCL (pH 6.8), 100 mmol/l DTT, 2% SDS, 0.1% bromophenol blue and 10% glycerol) was added to the extracts prior to denaturing by boiling for 10 min. Equal aliquots (80 μg) of protein were analysed by 12% SDS-PAGE, followed by electrotransfer onto nitrocellulose membranes. Following blocking with 10% skimmed milk in Tris-buffered saline containing 0.1% Tween 20, the blots were incubated overnight with the primary mouse anti- GRP78 or CXCL12 monoclonal antibody at 4°C. Secondary horseradish peroxidase-conjugated goat anti-mouse immunoglobulin G was applied at room temperature for 45 min. The reactions were developed with the 3, 3'-diaminobenzidine kit detection system. GAPDH was detected using mouse anti- GAPDH monoclonal antibody as the internal control.

PCR

After routine cleaning and baking in the oven at 180°Cfor 8 h, plastic products in 0.1% DEPC solution for 12 h after high-pressure sterilization (at 121°C, 30 min) to remove the interference of RNA enzyme. Slicing machine with 0.1% DEPC solution fully clean, use disposable blade 0.1% DEPC solution, according to a 5 μm thick cut 10 paraffin sections in RNA enzyme inactivated by 1.5 ml EP tube; adding 1.2 ml xylene dewaxing 3 times, each time after centrifugation (9000 r/min, 10 min) were abandoned to the upper layer of the liquid. With 1.2 ml alcohol washed 3 times (centrifugal 9000 r/min, 10 min), discarding the supernatant liquid after opening the cover on the 37°C drying box 20 min. The tissue lysate was mixed with 250 μL and mixed with protease K (final concentration 300 mg/L) and digested in 58°C water. The digested tissue inactivated proteinase K (10 min) at a temperature of 95°C. After the completion of the tissue treatment, the total RNA was extracted according to the kit. The concentration of RNA was determined and the reverse ratio was amplified by ultraviolet spectrophotometer. The concentration of RNA was determined. The total RNA of 1 μg was used as RT-PCR, and GAPDH as the internal control. The reverse transcription process was carried out according to the kit. The primer sequences are as follows, GRP78 sense strand 5-'AAG GTG AAC GAC CCC TAA CAA A-3', antisense strand 5'-GTC ACT CGG AGA ATA CCA TTA ACA TCT-3'; CXCL12 sense strand 5'-CAC CAT TGA GAG GTC GGA AG-3', antisense strand 5'-AAT GAG ACC CGT CTT TGC AG-3'; GAPDH sense strand 5'-GGC ATG GACTGT GGT CAT GA-3', antisense strand 5'-TTC ACC ACC ATG GAG AAG GC-3'. The product of 2% agarose gel electrophoresis after ethidium bromide staining observation system in gel imaging analysis.

Statistical analysis

SPSS 16 statistical software was used for data processing and analysis. All test data were expressed as means ± SEM ($\bar{x} \pm s$). The difference between the control group and the lung fibrosis group was analysed by t test. $P<0.05$ is statistically significant.

Results

GRP78 and CXCL12 expression in protein level

Compared with normal lung tissues, GRP78 and CXCL12 protein from pulmonary fibrosis patients were higher expressed, and the difference was very significant ($P<0.05$, Figure 1).

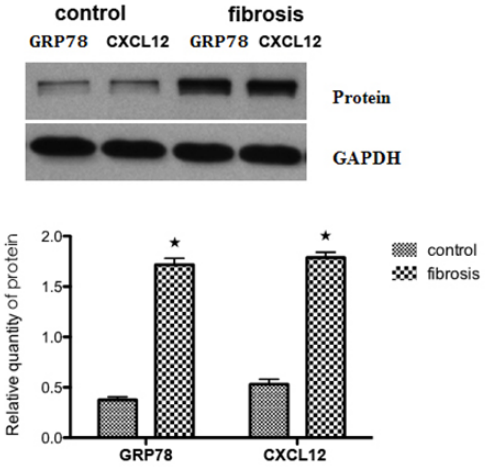

Figure 1. GRP78 and CXCL12 expression by Western blotting.

GRP78 and CXCL12 expression in mRNA level

Compared with normal lung tissues, GRP78 mRNA and CXCL12 mRNA from pulmonary fibrosis patients were higher expressed, and the difference was very significant (P<0.05, Figure 2).

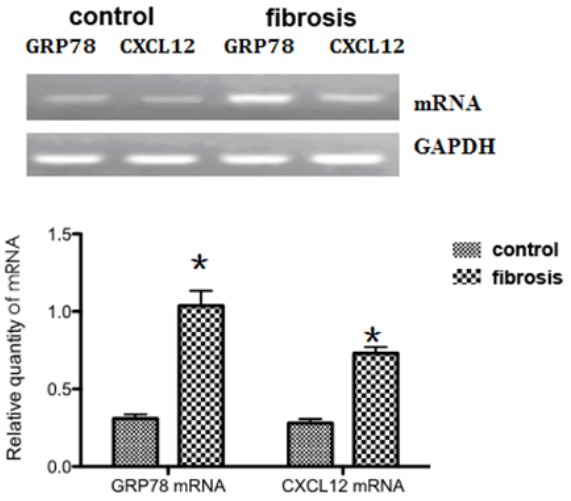

Figure 2. *GRP78 mRNA and CXCL12mRNA expression.*

The relationship between GRP78 and CXCL12

All the samples were performed to detect the relationship between GRP78 and CXCL12 by regressive analysis, and found that in protein level as the GRP78 increased, the CXCL12 also increased. Statistically, there was a positive relationship between GRP78 and CXCL12; also was confirmed in mRNA level (P<0.05, Figure 3).

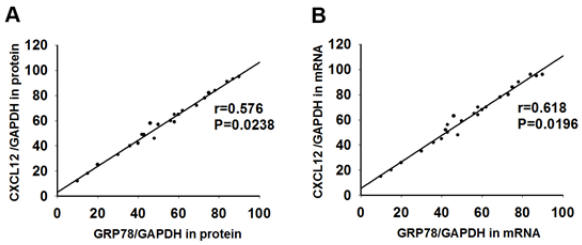

Figure 3. *Relationship between GRP78 and CXCL12. A. The regressive analysis of CXCL12 and GRP78 in protein level. B. The regressive analysis of CXCL12 and GRP78 in mRNA level.*

Discussion

Idiopathic pulmonary fibrosis is a group of interstitial lung diseases characterized by alveolar epithelial damage, the formation of fibroblast foci and accumulation of extracellular matrix. The pathogenesis is not clear, may be related to infection, drug, physical and chemical stimulation and autoimmune disease. There is no specific treatment for idiopathic pulmonary fibrosis, and the prognosis is very poor. Recent studies have shown that the endoplasmic reticulum stress response of alveolar epithelium is increased in the focal area of patients with idiopathic pulmonary fibrosis. The apoptotic pathway initiated by ER stress is a novel apoptotic pathway, which is also confirmed by the hypothesis of epithelial/mesenchymal cells in the pathogenesis of idiopathic pulmonary fibrosis [14]. The results of this study also showed that compared with normal controls, the expression GRP78 in alveolar epithelial endoplasmic reticulum stress related proteins was significantly increased in patients with idiopathic pulmonary fibrosis, and the difference was statistically significant. It also suggested that endoplasmic reticulum stress plays a very important role in the pathogenesis of idiopathic pulmonary fibrosis.

In the process of the pathogenesis of pulmonary fibrosis, fibroblasts play a more important role, fibroblasts can directly produce extracellular matrix (such as type I collagen and type III collagen), can differentiate into fibroblasts and myofibroblasts, and can produce cytokines that can promote collagen deposition, which are involved in the pathogenesis of fibrosis. Fibroblasts can be found in the blood circulation and lung parenchyma in patients with idiopathic pulmonary fibrosis. The number of fibroblasts in the blood circulation is correlated with the prognosis of the patients. Alveolar epithelial cells may play a key role in the recruitment of fibroblasts. Fibroblast cell surface expresses cytokine receptor CXCR4, and CXCL12, as a ligand of CXCR4, can be involved in the recruitment by fibroblasts [15]. Previous studies focused on endoplasmic reticulum stress, apoptosis, tissue damage and pulmonary fibrosis. This study explored the relationship between CXCL12 expression and endoplasmic reticulum stress related protein expression. The results showed that compared with the control group, the CXCL12 expression was up-regulated in the idiopathic alveolar epithelial lesions of patients with pulmonary fibrosis, suggested that alveolar epithelial cells may be through the CXCR4/CXCL12 axis to raise fiber cells involved in the pathogenesis of idiopathic pulmonary fibrosis.

In addition, co-up-regulated expressions of GRP78 and CXCL12 in protein level suggested that ER stress may stimulate the expression of chemokines and further promote the development of pulmonary fibrosis. Studies have shown that endoplasmic reticulum stress can increase the expression of CXCL12, but the specific mechanism is still unknown [16]. Also, GRP78 and CXCL12 were increased in mRNA level compared with the normal group; this consisted with the results in protein level. Regressive analysis showed that there was a positive relationship between GRP78 and CXCL12 in protein and mRNA level statistically, this confirmed that ER stress promoted the development of idiopathic pulmonary fibrosis by activating GRP78/ CXCL12 axis.

In summary, the results showed that compared with the control group, GRP78 expression were up-regulated consistent with CXCL12 expression in idiopathic pulmonary fibrosis patients, suggesting that endoplasmic reticulum stress may stimulate chemokine CXCL12 expression in the pathogenesis of idiopathic pulmonary lung fibrosis. Also some cellular and animal experiments should to be process to further confirm this idea.

Conflict of Interest

None declared.

Acknowledgements

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