European Journal of Pharmacology 749 (2015) 89–97

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Pulmonary, gastrointestinal and urogenital pharmacology

Role of eukaryotic translation initiation factor 3a in bleomycin-induced pulmonary fibrosis

Xian-Wei Li [a,b,1], Yue-Han Wu [a,1], Xiao-Hui Li [a], Dai Li [c], Jie Du [a], Chang-Ping Hu [a], Yuan-Jian Li [a,*]

[a] Department of Pharmacology, School of Pharmaceutical Sciences, Central South University, Changsha 410078, China
[b] Department of Pharmacology, Wannan Medical College, Wuhu 241002, China
[c] National Institution of Drug Clinical Trial, Xiangya Hospital, Central South University, Changsha 410078, China

ARTICLE INFO

Article history:
Received 25 November 2014
Received in revised form
4 January 2015
Accepted 6 January 2015
Available online 12 January 2015

Keywords:
Pulmonary fibrosis
Pulmonary fibroblasts
Eukaryotic translation initiation factor 3a
TGF-β_1
ERK1/2

ABSTRACT

Eukaryotic translation initiation factor 3a (eIF3a) is a multifunctional protein and plays an important role in regulation of cellular function including proliferation and differentiation. In the present study, we tested the function of eIF3a in pulmonary fibrosis. Pulmonary fibrosis was induced by intratracheal instillation of bleomycin (5 mg/kg) in rats. Primary pulmonary fibroblasts were cultured for proliferation investigation by BrdU incorporation method and flow cytometry. The expression/level of eIF3a, TGF-β_1, ERK1/2 and α-SMA were analyzed by ELISA, real-time PCR or western blot. Results showed that the expression of eIF3a was obviously increased in lungs of pulmonary fibrosis rats accompanied by up-regulation of α-SMA and collagens. In cultured pulmonary fibroblasts, application of exogenous TGF-β_1 induced cell proliferation and differentiation concomitantly with up-regulation of eIF3a expression and ERK1/2 phosphorylation. The effects of TGF-β_1-induced proliferation of fibroblasts and up-regulation of α-SMA were abolished by eIF3a siRNA. TGF-β_1-induced eIF3a expression was reversed in the presence of PD98059, an inhibitor of ERK1/2. These findings suggest that eIF3a plays an important role in bleomycin-induced pulmonary fibrosis by regulating pulmonary fibroblasts' function, and up-regulation of eIF3a induced by TGF-β_1 is mediated via the ERK1/2 pathway.

1. Introduction

Pulmonary fibrosis (PF) is a serious interstitial lung disease with high mortality because of the poor response to available medication (Cook et al., 2002). It has been demonstrated that several factors contribute to the pathogenesis of PF, including inflammation, epithelial mesenchymal transition, oxidative stress and immune dysfunction, which results in alveolar epithelial cell injury and fibroblast proliferation that consequently leads to abnormal deposition of extracellular matrix and tissue remodeling (Gross and Hunninghake, 2001).

Fibroblasts have been implicated as a major participant in pulmonary fibrosis and are currently being studied as new therapeutical targets (Wynn, 2011). Histologic sections of diseased lung from patients with PF show clusters of proliferating fibroblasts termed "fibroblastic foci" (Scotton and Chambers, 2007). These clusters of fibroblasts are composed primarily of myofibroblasts, contractile cells that express both fibroblast and smooth muscle cell markers such as α-smooth muscle actin (α-SMA) (Hinz et al., 2007). Myofibroblasts are largely responsible for the excess production of extracellular matrix components, such as collagen and fibronectin (Phan, 2002). Unfortunately, the underlying molecular mechanisms responsible for fibroblast proliferation and excessive deposition of collagen in fibrotic lesions are not fully understood.

Translational control plays a major role in regulating protein expression and occurs primarily at the initiation step which is controlled by multiple eukaryotic translation initiation factors (eIFs) (Maitra et al., 1982). It has been indicated that eIF2α is involved in the process of mouse embryonic fibroblast proliferation and differentiation (Kazemi et al., 2007), which suggests a role of eIFs in fibrosis. Among the eIFs family, eIF3 is the largest and most complex initiation factor and contains 13 different subunits designated eIF3a-m (Damoc et al., 2007; Saletta et al., 2010). eIF3a (also known as p170), the largest subunit of eIF3 complex, has been suggested to play roles in tumorigenesis (Dong et al., 2009; Dong and Zhang, 2006). Suppressing endogenous eIF3a expression has been shown to reverse malignant phenotype of human cancer cells while over-expression of ectopic eIF3a promotes malignant transformation of mammalian cells (Dong et al., 2004; Zhang et al., 2007). It has been demonstrated that eIF3a expression is

* Correspondence to: Department of Pharmacology, School of Pharmaceutical Science, Central South University, Xiang-Ya Road 110#, Changsha 410078, China. Tel./fax: +86 731 82355078.
E-mail address: yuan_jianli@yahoo.com (Y.-J. Li).
[1] Equal contribution to the work.

http://dx.doi.org/10.1016/j.ejphar.2015.01.004
0014-2999/

correlated with better prognosis of human cancer patients and eIF3a up-regulation in lung cancer patients correlates with their response to platinum-based chemotherapy and contributes to increased cisplatin sensitivity (Yin et al., 2011). Researchers also demonstrated that eIF3a may play as a regulator of a subset of mRNAs and regulate the expression of p27, tyrosinated α-tubulin and ribonucleotide reductase M2 subunit. These molecules have a critical role in the regulation of the cell cycle (Shen et al., 2014).

Based on the facts that eIF3a plays an important role in cell cycle regulation and tumorigenesis including lung cancer, we speculate that eIF3a may be involved in the regulation of fibroblasts proliferation in pulmonary fibrosis. The present experiment was designed to investigate the role of eIF3a in bleomycin-induced pulmonary fibrosis in vivo and in vitro. Considering the fact that increased TGF-β_1 level is a hallmark in bleomycin-induced pulmonary fibrosis and TGF-β_1 plays a critical role in fibroblast proliferation regulation, we also investigated the possible effect and mechanism of TGF-β_1 on eIF3a expression in cultured pulmonary fibroblasts.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats weighing 180–220 g were obtained from Laboratory Animal Center, Xiangya School of Medicine, Central South University (Changsha, China). All experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and the experimental protocol was approved by the Medicine Animal Welfare Committee of Xiangya School of Medicine, Central South University.

2.2. Animal experiments

The pulmonary fibrosis model was prepared as previously described (Serrano-Mollar et al., 2003). Briefly, rats were anesthetized with sodium pentobarbital (P3761, 30 mg/kg; Sigma) by intraperitoneal injection followed by intratracheal instillation of 5 mg/kg bleomycin (Nippon Kayaku, Tokyo, Japan) in 1 mL of saline. Control animals received the same volume of intratracheal saline instead of bleomycin. Pulmonary fibrosis was assessed by lung histology as described in the following section (Ashcroft et al., 1988). The rats were sacrificed 28 days after bleomycin injection.

2.3. Histological analysis

For light microscopic investigation, lung tissues were fixed by inflation with freshly prepared 4% paraformaldehyde in PBS (pH 7.4) for 24 h and embedded in paraffin. Tissue sections (5 μm) were stained with hematoxylin and eosin (H&E) and Masson's trichrome stain to enable histological evaluation of lung fibrosis. Masson's trichrome stain was used to demonstration collagen deposition, and collagen fiber is stained blue, nuclei are stained dark red/purple, and cytoplasm is stained red/pink. The procedure is according to the manufacturer's instructions (KeyGEN Biotech, Nanjing, China).

2.4. ELISA

Plasma concentration of TGF-β_1 was measured by ELISA kits (R&D Systems Inc., Hong Kong, China) as previously described (Kang et al., 2007), and the procedure according to the manufacturer's instructions.

2.5. Cell experiments

Primary rat pulmonary fibroblasts were prepared from the lung tissue of male 10-week-old healthy SD rats using trypsin digestion method as described previously (Zhang et al., 2010). The cells were cultured at 37 °C under 5% CO_2 in Dulbecco's modified Eagle's medium containing 20% fetal bovine serum, 100 U/mL penicillin and 100 g/mL streptomycin. Fibroblasts were identified by immunofluorescence staining with the antibody of Vimentin (ab8978, 1:50; Abcam, Hong Kong, China). The cells between passages 3 and 6 were used for the experiments. Two series of experiments were designed. The first series of experiments were to explore the effect

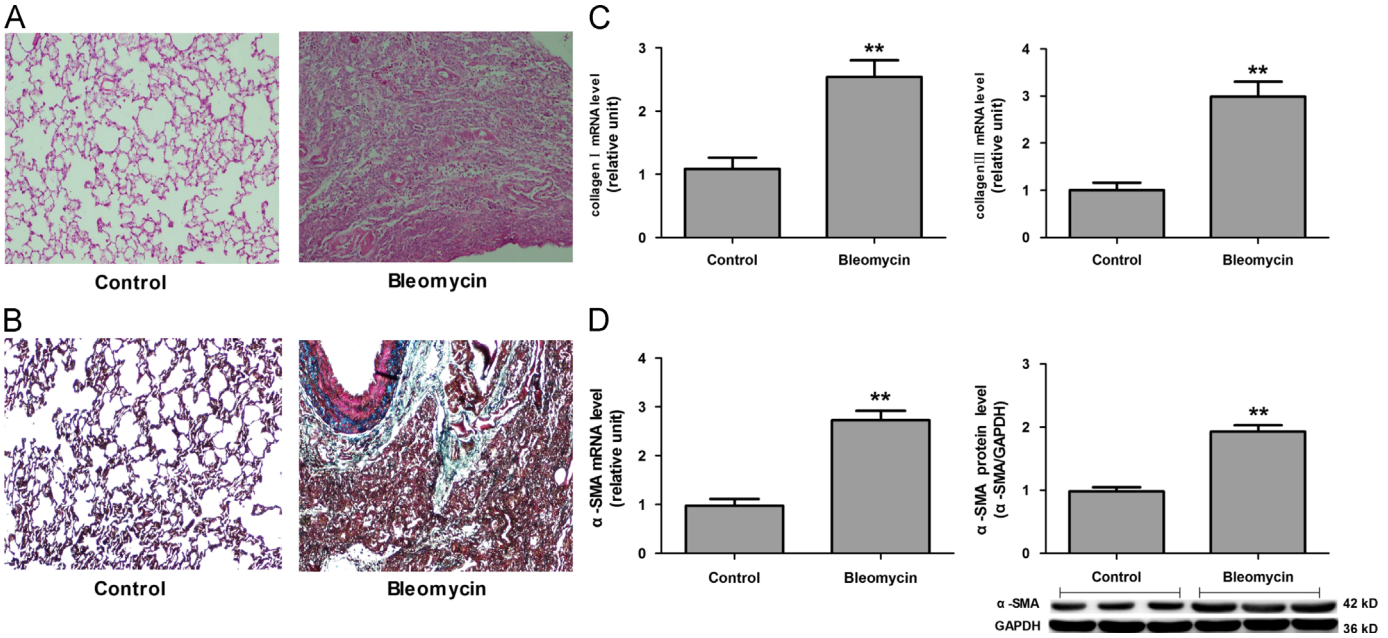

Fig. 1. Lung histology and collagen expression in bleomycin-induced pulmonary fibrosis of rats. (A) Hematoxylin-eosin staining of lung tissue. (B) Masson's trichrome staining of lung tissue. (C) The expression of collagen I and collagen III mRNA was determined by real-time PCR. (D) The expression of α-SMA mRNA and protein was determined by real-time PCR and Western blot. Data are means ± S.E.M. $n=8$. $^{**}P<0.01$ vs. Control.