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SOCS3 was induced by hypoxia and suppressed STAT3 phosphorylation in pulmonary arterial smooth muscle cells

Li Bai [a], Zubin Yu [b], Guisheng Qian [a], Pin Qian [c], Jinjun Jiang [d], Guansong Wang [a], Chunxue Bai [d,*]

[a] *Institute of Respiratory Diseases, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, PR China*
[b] *Department of Thoracic Surgery, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, PR China*
[c] *Department of Ultrasound Diagnosis, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, PR China*
[d] *Department of Pulmonary Medicine, Research Institute of Respiratory Disease, Fudan University, Zhongshan Hospital, No. 180 Fenglin Road, Shanghai, 200032, PR China*

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Abstract

Recently identified suppressors of cytokine signaling (SOCS) have been proposed as negative regulators of cytokine signaling, which have distinct mechanisms of inhibiting JAK-STAT pathway. In this study, using cultures of rat primary pulmonary vascular smooth muscle cells (PASMC), we found that hypoxia induced strongly STAT3 phosphorylation by up to four-fold. At the same time, mRNA for the endogenous cytokine signaling repressor SOCS3, but not SOCS1, was markedly induced in PASMC as early as 2 h following hypoxic stimulation. Furthermore, forced expression of SOCS3 gene suppressed tyrosine phosphorylation of STAT3 and transcription of c-myc gene by more than 70% and 60% in PASMC under hypoxic conditions, respectively. Additionally, we showed here that hypoxia enhanced nearly two-fold increase of PASMC proliferation and overexpression of SOCS3 gene downregulated hypoxia-induced PASMC proliferation by about 50%. The finding suggest that STAT3-dependent pathway is involved in the activation and proliferation of PASMC stimulated by hypoxia, and SOCS3 is a rapidly hypoxia-inducible gene and acts to inhibit activation of cellular signaling pathway in a classical negative feedback loop.

Keywords: Hypoxia; Pulmonary artery; Muscle; Smooth; Vascular; SOCS; Signal transduction

1. Introduction

Janus kinase-signal transducers and activators of transcription (JAK-STAT) signaling plays a critical role in regulating biologic responses to cytokine stimulation, including cellular proliferation, differentiation, and survival (Cooney, 2002). Eight members, identified suppressors of cytokine signaling (SOCS) family (CIS and SOCS1 to SOCS7), could be induced by a variety of cytokines or growth factors, which directly inhibit JAK-STAT signaling as part of a classic feedback loop (Krebs and Hilton, 2000;

* Corresponding author. Tel.: +86 21 6404 1990 2445; fax: +86 21 5496 1729.
E-mail address: cxbai@zshospital.com (C. Bai).

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Aaronson and Horvath, 2002). They are characterized by a central SH2 domain, a conserved C-terminal SOCS-box and a unique N terminus (Hilton et al., 1998).

It is now well known that JAKs and STAT proteins transmit the intracellular signaling elicited by various of cytokines and growth factors in a wide variety of cell types (Kisseleva et al., 2002). As shown by recent research, JAKs and STAT proteins are expressed constitutively in cultured vascular smooth muscle cells (VSMC) and AngII could induce proliferation of cultured VSMC via STAT3 activation. Moreover, AngII-induced proliferation of cultured VSMC is inhibited by either pretreatment with JAK2-specific inhibitor AG490 or electroporation of anti-STAT3 antibody (Marrero et al., 1997). Activated STAT3 has been found in vascular remodeling after vascular injury accompanied by VSMC proliferation (Calo et al., 2003; Seki et al., 2000). JAK2 and STAT3-dependent expression of cytosolic phospholipase A2 is required for PDGF-BB-induced arachidonic acid release and growth in VSMC (Yellaturu and Rao, 2003). These findings suggest JAKs and STAT proteins might play an essential role in VSMC under physiological and pathological conditions.

Although abnormalities of JAK-STAT pathway is implicated in activation and proliferation of VSMC, little is known regarding the relevance of JAK-STAT signaling and its negatively regulating machinery to the process of hypoxia-associated vascular remodeling. Our earlier experiments found that overexpression of SOCS3 suppressed the growth of pulmonary arterial smooth muscle cells (PASMC) under hypoxia, accompanied by inhibiting transcription of c-fos and c-jun (Bai et al., 2005). Since SOCS3 gene has been demonstrated to exhibit a negative regulatory feedback on cellular signal cascade by limiting the activation of STATs, necessary for its translocation to the nucleus and regulation of target genes, we further sought to determine the expression and modulation of SOCS3 gene in PASMC exposed to hypoxia. In this study, we demonstrated STAT3 activation was enhanced in cultured rat PASMC exposed to hypoxia. Since STAT3 is implicated in regulating the expression of its intrinsic negative regulators – SOCS1 and SOCS3 gene (Auernhammer et al., 1999; Brender et al., 2001), we showed here that SOCS3 mRNA, but not SOCS1, was indeed significantly induced in PASMC by hypoxia. Furthermore, overexpression of SOCS3 gene suppressed tyrosine phosphorylation of STAT3 and transcription of c-myc gene in PASMC exposed to hypoxia. In addition, we found SOCS3 protein inhibited hypoxia-induced PASMC proliferation. All these data raised the possibility that STAT3-dependent pathway was involved in the process of hypoxia-induced PASMC proliferation, and activation of SOCS3 gene might represent a general negative feedback loop that keeps a balance of activated cellular signaling network.

2. Materials and methods

2.1. Cell culture

The primary cell lines of PASMC were obtained from explants of rat pulmonary artery. After being washed in Dulbecco's modified Eagle's medium (DMEM) (HyClone USA) without serum, the artery was stripped off the intimal cell layer and adventitial tissue by forceps. The resting media of vessel was cut into small pieces and transferred into prewetted cell culture flasks, and cultured in DMEM supplemented with 10% fetal calf serum (FCS) (HyClone USA), 100 IU/ml penicillin and 100 μg/ml streptomycin. After 10 days, cells were passaged using 0.25% trypsin–0.02% EDTA and used for experiments between passage 4 and 8. PASMC were identified by immunocytochemical staining using monoclonal antibody for α-smooth muscle cell actin.

2.2. Cell culture conditions

Normoxic culture conditions were defined as follows: 21% O_2 and 5% CO_2. Hypoxic conditions were induced in a constant 3% O_2 and 5% CO_2 in an oxygen-regulated cell culture incubator (Heraeus, Germany). Subconfluent cells were starved in DMEM with 0.4% FCS for 24 h and then incubated either under hypoxic or normoxic conditions for various time points (0, 2, 6, 12, 24, 48 h).

2.3. Immunocytochemistry

PASMC were plated on coverslips in 24-well plates (approximately 2×10^4 cells/well), and then exposed