Acta Biochim Biophys Sin 2010, 42: 483–488 | © The Author 2010. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmq041.
Advance Access Publication 10 June 2010

Original Article

Activation of hypoxia-inducible factor-1α via nuclear factor-κB in rats with chronic obstructive pulmonary disease

Hong Jiang†, Yesen Zhu†, Hui Xu, Yu Sun, and Qifang Li*

Department of Anesthesiology, Shanghai Ninth People's Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China
†These authors contributed equally to this work.
*Correspondence address. Tel: +86-21-23271699; Fax: +86-21-53591385; E-mail: jiuyuanpaper@yahoo.cn

Accumulating data suggested that hypoxia inducible factor (HIF)-1α plays an important role in the evolution and propagation of the inflammatory process. To characterize the activation of HIF-1α in rats with chronic obstructive pulmonary disease (COPD) and examine the possible role of nuclear factor (NF)-κB in this process, rats were challenged by introtracheal instillation of lipopolysaccharide (LPS) and exposure to cigarette smoke. Pyrrolidine dithiocarbamate (PDTC) was administered via the oral route 1 h before LPS or cigarettes administration. Four weeks later, pulmonary function and histology were tested; bronchoalveolar lavage fluid (BALF) and arterial blood gases were assayed. Activation of pulmonary NF-κB was assessed by quantitative PCR, immunoblot analysis, and electrophoretic mobility shift assay, respectively. Results showed that LPS and smog induced the characteristics of COPD seen in human. PDTC alleviated the development of COPD and the levels of cytokines in BALF of PDTC + COPD group were significantly decreased compared with that of COPD group. The activation of pulmonary NF-κB was inhibited by PDTC and the accumulation of *HIF-1α* gene expression in the COPD group was attenuated by PDTC pretreatment. Furthermore, the mRNA levels of HIF-1α target genes heme oxygenase-1 (HO-1) and vascular endothelial growth factor (VEGF) were parallel to the attenuation of HIF-1α by PDTC. These findings indicated that the activation of HIF-1α pathway via NF-κB contributes to the development of COPD, and administration of NF-κB inhibitor may attenuate the development of COPD.

Keywords chronic obstructive pulmonary disease; inflammation; hypoxia; nuclear factor-κB

Received: January 17, 2010 Accepted: April 20, 2010

Introduction

Chronic obstructive pulmonary disease (COPD) is a complex, multifactorial airway disease associated with significant morbidity and mortality worldwide. Although the development of COPD is known to be linked to cigarette smoke, little is known about the pathogenesis of this disease, but it is believed that inflammatory component plays a pivotal role in the pathogenesis of the disease. Nuclear factor (NF)-κB is a transcription factor expressed in numerous cell types, which plays a key role in the expression of many pro-inflammatory genes, leading to the synthesis of cytokines, adhesion molecules, chemokines, growth factors and enzymes [1]. Increased expression of NF-κB has been found in bronchial biopsies from smokers and patients with COPD and accumulating data suggested targeting the NF-κB pathway in the COPD therapy [2,3]. Very recently, sites of chronic inflammation demonstrating distinct microenvironmental features including decreased oxygen availability have been documented [4]. Hypoxia inducible factor (HIF)-1, a master regulator of oxygen homeostasis, plays critical roles both during development and in response to physiologic and pathophysiologic stimuli in organisms. This heterodimeric transcriptional factor consists of two subunits, HIF-1α and HIF-1β. HIF-1α is the specific and oxygen-regulated subunit of the HIF-1 complex and determines the level of HIF-1 activity, whereas HIF-1β is constitutively expressed [5]. However, unequivocal evidences were recently established to implicate HIF-1 as a regulator of the evolution and propagation of the inflammatory process [6,7]. Previously, we revealed the activation of HIF-1α in hypoxia-induced pulmonary hypertension [8]. Furthermore, HIF-1α and NF-κB showed an intimate interdependence at several mechanistic levels [4]. Nonetheless,

the real role of NF-κB on HIF-1α expression in the development of COPD is still unknown. Pyrrolidine dithiocarbamate (PDTC), an antioxidant, is a potent inhibitor of NF-κB [9]. So we were prompted to establish a rat COPD model to investigate the role of NF-κB on HIF-1α activation in the development of COPD by using PDTC.

Materials and Methods

Establishment of rat model

Sixty male Sprague–Dawley rats (280–320 g) were purchased from the Animal Center of Shanghai Jiao Tong University School of Medicine (Shanghai, China), and housed in air-filtered, temperature-controlled units with free access to food and water. The experimental protocols were approved by the institutional animal care committee and all experiments were done in conformity with the Guiding Principles for Research Involving Animals of Shanghai Jiao Tong University School of Medicine. Rats were randomly divided into five groups ($n = 12$ per group): (i) control group, rats without any intervention; (ii) sham group, rats were instilled intratracheally with LPS-free sterile saline; (iii) PDTC group, PDTC was administered via the oral route with the dose of 200 mg/kg every day for 28 days; (iv) COPD group, 350 μg/200 μl of lipopolysaccharide (LPS) was administrated by intratracheal instillation on days 1 and 14; the rats were then exposed to 10 cigarettes for 2 h per day from days 2 to 3 and from days 15 to 28 as described previously [10]; (v) PDTC + COPD group, PDTC was administered 1 h before LPS or cigarettes administration and every day thereafter for 28 days.

Pulmonary function test

Half of the rats in each group were randomly chosen for lung function test as described by Xu *et al*. [11]. After anesthesia with intraperitoneal administration of chloral hydrate (3 ml/kg), a 'Y' type endotracheal cannula was connected to a flow transducer (Beijing Yue Hong Da Co., Beijing, China) for measurement of forced expiratory volume in 0.3 s (FEV0.3)/forced vital capacity (FVC) and peak expiratory flow (PEF).

Arterial blood gas and bronchoalveolar lavage fluid assay

Under anesthesia, celiotomy and thoracotomy were performed to obtain blood samples from abdominal aorta for arterial blood gas (ABG) assay and to obtain bronchoalveolar lavage fluid (BALF), respectively. The lungs of rats were instilled with 3 ml of phosphate-buffered saline (PBS) and the fluid was gently aspirated back. This procedure was repeated twice. The BALF was then centrifuged at 4°C for 10 min. The cellular pellet was resuspended in 200 μl of PBS. Total cell number was counted with a hemocytometer and a differential analysis is conducted after cytospin preparation and Quik Diff staining of the cellular pellet prepared on a glass slide as described previously [12].

Cytokines analysis in BALF

The levels of tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, interleukin (IL)-1β and IL-6 in BALF were measured by enzyme-linked immunosorbant assay according to manufactures' instruction, and corrected by total protein in BALF using the Bio-Rad protein assay.

Determination of mRNA levels by quantitative real-time PCR

Whole-lung tissue samples were extracted as described previously [13]. Quantitative real-time PCR estimation of mRNA levels was performed as described previously [14]. The mRNA levels of genes were normalized to levels of β-actin. Genes and PCR primers are listed as follows: *HIF-1*α, (forward) 5′-TACTGGGGTTCATGATGATTATTGTGG-3′, (reverse) 5′-ACTTCAGGAACCGGCGTGGATTTA-3′; heme oxygenase-1 (*HO-1*), (forward) 5′-TCTATCGTGCTCGCATGAAC-3′, (reverse) 5′-CAGCTCCTCAAACAGCTCAA-3′; vascular endothelial growth factor (*VEGF*), (forward) 5′-TTACTGCTGTACCTCCAC-3′, (reverse) 5′-ACAGGACGGCTTGAAGATA-3′; and β-*actin*, (forward) 5′-CTTTCTACAATGAGCTGCGTG-3′, (reverse) 5′-TCATGAGGTAGTCTGTCAGG-3′. Amplification and detection were carried out by using an ABI PRISM 7700 detection system (Applied Biosystems, Foster City, USA).

Histology, immunoblot analysis and electrophoretic mobility shift assay

The middle lobe of right lung, not lavaged, was immersed in 4% phosphate-buffered paraformaldehyde to complete fixation and then embedded in paraffin, sectioned, and finally stained with hematoxylin and eosin to evaluate the morphological changes of lungs. A total of 100 μg of protein was loaded on to a 10% sodium dodecyl sulfate (SDS)/polyacrylamide gel and after electrophoresis it was blotted on to nitrocellulose membranes. The primary rabbit anti-rat HIF-1α and p65 (Upstate, USA) polyclonal antibodies were used at 1:400 and anti-β-actin monoclonal antibody (Sigma, St. Louis, USA) was used at 1:4 000 dilution. The anti-rabbit IgG secondary antibody (KPL, Gaithersburg, USA) was used at 1:2 000 dilution, and the signal was analyzed by enhanced chemiluminescence. The abundance of HIF-1α was normalized to β-actin using software Quantity One-4.2.3. NF-κB activity was detected by the LightShift Chemiluminescent electrophoretic mobility shift assay (EMSA) kit (Pierce, Rockford, USA) that uses a non-isotopic method to detect DNA–protein interactions according to the manufacturer's instruction.

Statistical analysis

The SPSS 13.0 statistical package was used for statistical analyses. Data were expressed as the mean ± SD and differences were compared using the analysis of variance. Differences within one group were compared using least significant difference (LSD) test. P value <0.05 was considered statistically significant.

Results

Pulmonary function test, histology, ABG and BALF analysis

Pulmonary function test (PFT) showed that there was a significant increase in airway resistance (PEF) and significantly reduced flow ($FEV_{0.3}$/FVC) in the COPD group compared with control rats (**Table 1**). Likewise, in histologic analysis, a portion of the airway epithelium and some cilia had been shed and air spaces were enlarged in an irregular manner in the COPD group. In the control group, the airway epithelium was intact, ciliary arrangement was regular, and the structure of the alveoli was normal. In the PDTC + COPD group, the shedding of the airway epithelium and cilia was less severe. However, air spaces were still enlarged (**Fig. 1**). ABG analysis demonstrated that rats in the COPD group developed hypoxia and hypercapnia to various degrees (**Table 1**). Parameters of PFT and ABG were significantly milder in the PDTC + COPD group than that of the COPD group (all P values < 0.05) (**Table 1**). The total cell count in BALF was determined to assess the degree of inflammation in the lung. A sharp increase in BALF cells was observed in the COPD group (**Table 2**). In terms of the composition of the inflammatory cell influx in BALF, monocytes/macrophages formed the main fraction of BALF cells; lymphocyte and polymorphonuclear

Table 1 Pulmonary function test and ABG assay

Group	pH	PO_2 (mmHg)	PCO_2 (mmHg)	$FEV_{0.3}$/FVC (%)	PEF (ml/s)
Control	7.33 ± 0.08	103.00 ± 2.53	40.21 ± 3.46	96.54 ± 0.53	56.32 ± 8.16
Sham	7.36 ± 0.15	95.00 ± 3.65	38.42 ± 2.18	90.76 ± 0.85	51.31 ± 4.62
PDTC	7.30 ± 0.06	106.00 ± 3.23	42.13 ± 2.26	98.71 ± 0.62	58.42 ± 7.36
COPD	7.21 ± 0.24*	68.00 ± 4.41*	55.63 ± 4.62*	75.45 ± 1.24*	25.63 ± 2.72*
PDTC + COPD	7.29 ± 0.31	89.00 ± 4.17*,**	45.12 ± 3.16**	88.72 ± 1.43*,**	38.68 ± 1.79*,**

*$P < 0.05$ vs. control, **$P < 0.05$ vs. COPD. Data are expressed as the mean ± SD, $n = 6$.

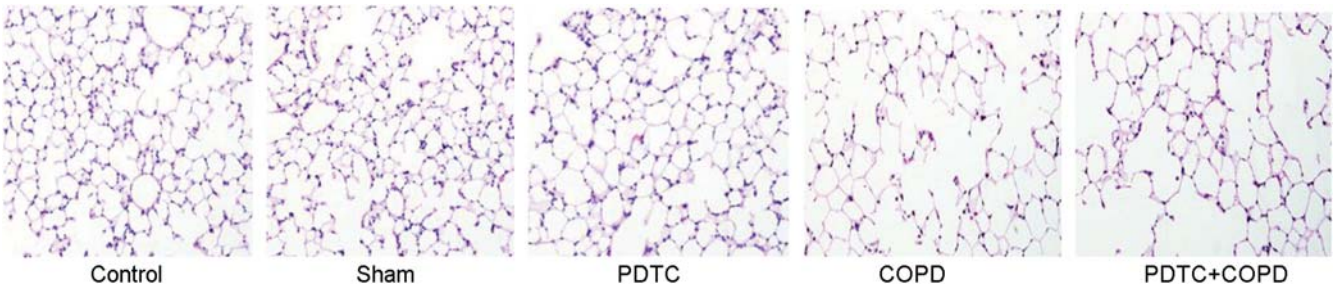

Figure 1 Lung tissue sections The irregular airspace enlargement was observed in the COPD group and PDTC + COPD group. PDTC, pyrrolidine dithiocarbamate; COPD, chronic obstructive pulmonary disease. Magnification, 100×.

Table 2 Total cell count and classification in bronchoalveolar lavage fluid

Group	Total cell ($\times 10^6$/L)	Monocytes/ macrophages	Lymphocytes ($\times 10^6$/L)	Polymorphonuclear leukocytes ($\times 10^6$/L)
Control	187 ± 64	109.41 ± 27.34	51.72 ± 14.34	27.47 ± 7.19
Sham	249 ± 75*	132.48 ± 36.23	84.81 ± 26.25*	33.71 ± 6.21
PDTC	213 ± 43	148.41 ± 30.54	46.25 ± 10.14	18.34 ± 4.12
COPD	943 ± 126*	572.71 ± 73.53*	227.51 ± 51.61*	144.28 ± 44.67*
PDTC + COPD	621 ± 88*,**	346.35 ± 51.27*,**	167.25 ± 48.32*,**	108.40 ± 38.72*,**

*$P < 0.05$ vs. control, **$P < 0.05$ vs. COPD. Data are expressed as the mean ± SD, $n = 12$.

Table 3 Cytokine levels in bronchoalveolar lavage fluid

Group	TNF-α	TGF-β	IL-1β	IL-6
Control	81.32 ± 16.42	139.81 ± 47.32	55.75 ± 14.64	67.47 ± 7.19
Sham	94.29 ± 7.45	152.43 ± 36.73	64.81 ± 16.25	73.21 ± 9.31
PDTC	88.47 ± 18.32	159.31 ± 43.72	58.25 ± 16.34	64.37 ± 9.27
COPD	243.56 ± 32.16*	242.31 ± 43.33*	127.61 ± 21.61*	154.18 ± 24.67*
PDTC + COPD	181.00 ± 18.28*,**	186.35 ± 21.27*,**	87.35 ± 18.32*,**	138.60 ± 28.32*

*$P < 0.05$ vs. control, **$P < 0.05$ vs. COPD. Data are expressed as the mean ± SD, $n = 12$. Unit used in table is pg/mg of total proteins.

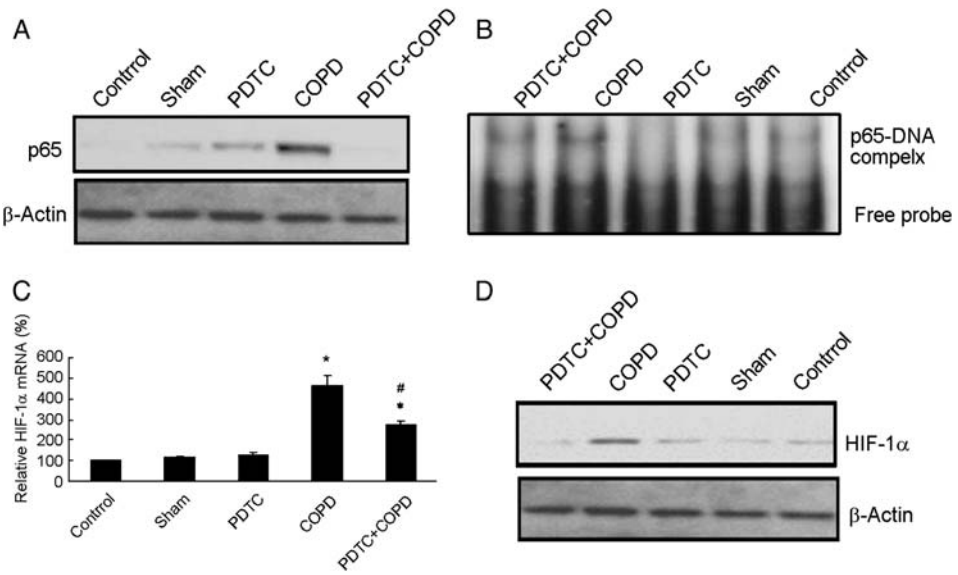

Figure 2 The effects of PDTC treatment on p65 protein level (A), p65 activity (B), *HIF-1α* mRNA (C), and HIF-1α protein (D) levels in the lungs of rats The normalized *HIF-1α* mRNA level of control was assigned to value 1. The level of *HIF-1α* in the remaining of the samples was represented as fold over control. Data were expressed as the means ± SD, and assessed by LSD test. *$P < 0.05$ vs. control group, #$P < 0.05$ vs. COPD group, $n = 4$. Immunoblot and EMSA results are representative of three separate results. PDTC, pyrrolidine dithiocarbamate; COPD, chronic obstructive pulmonary disease.

leukocyte (PMN) in the COPD group were increased in BALF. Compared with COPD group, the total number of cells and differential count were both decreased in the PDTC + COPD group (**Table 2**). This increase in inflammatory cell number was accompanied by a significant increase in TNF-α, TGF-β, IL-1β and IL-6 in BALF, and PDTC attenuated the increase of all the cytokines except IL-6 compared with COPD group (**Table 3**).

Role of NF-κB in HIF-1α pathway activation in the development of COPD

To determine the molecular mechanisms involved in the development of COPD, we assessed the level of NF-κB p65, the active subunit of NF-κB complex, in the lungs of rats. There was a significant accumulation of p65 protein in the COPD group as compared with the control and sham group. As expected, treatment with PDTC significantly attenuated the induction of p65 protein [**Fig. 2(A)**]. To further confirm the activation of NF-κB, EMSA shift assays were performed to determine p65 DNA-binding capacity. The EMSA analysis showed that significantly increased p65 DNA-binding capacity in COPD group, while PDTC attenuated the increase of p65 DNA-binding capacity compared with rats in COPD group [**Fig. 2(B)**]. Accordingly, PDTC attenuated the accumulation of *HIF-1α* gene expression in COPD group [**Fig. 2(C,D)**]. To confirm the result observed with the HIF-1α assay, the mRNA levels of HIF-1α target genes *HO-1* and *VEGF*, which have been suggested involved in the pathogenesis of COPD [15,16], were also measured by real-time PCR. The results concur with the HIF-1α assay inasmuch as PDTC diminished the accumulation of the *HO-1* mRNA and *VEGF* mRNA in comparison with the COPD group [**Fig. 3(A,B)**].

Discussion

Chronic exposure to cigarette smog and recurrent airway infection play important roles in the development of COPD

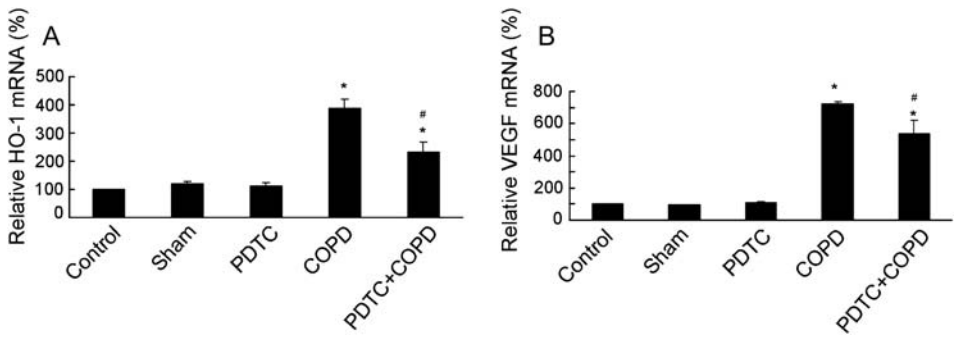

Figure 3 The effects of PDTC treatment on HIF-1α target genes *HO-1* (A) and *VEGF* mRNA (B) levels in the lungs of rats The normalized mRNA level of control was assigned to value 1. The level of the remaining of the samples is represented as fold over control. Data were expressed as the means ± SD and assessed by LSD test, $n = 4$. $^{*}P < 0.05$ vs. control group, $^{\#}P < 0.05$ vs. COPD group. PDTC, pyrrolidine dithiocarbamate; COPD, chronic obstructive pulmonary disease.

in human [17]. The present study showed that exposure of cigarette smog and LPS to rats induced characteristics similar to what observed in COPD patients. More importantly, PDTC, a potent inhibitor of NF-κB, can alleviate the degree of COPD abnormities as compared with COPD groups. PDTC is the most effective and specific NF-κB inhibitor as a result of its ability to traverse the cell membrane and its prolonged stability at physiological pH. PDTC and other dithiocarbamates are members of a group of antioxidants which inhibit the activation of NF-κB. It has been reported that NF-κB activity is controlled by the intracellular redox state. It is possible that the antioxidant properties of PDTC may result in inhibition of reactive oxygen species that activate the upstream kinase, IκBα kinase, causing the degradation of IκBα and nuclear translocation of NF-κB [18]. Enhanced expression of NF-κB, or the p65 subunit as described here, can overcome the inhibitory effects of IκB thus leading to nuclear translocation and the increased inflammatory gene expression seen in the airways of smokers and subjects with COPD. As shown in the present study, NF-κB activation, evidenced by the increased p65 protein levels and DNA-binding capacity was found in the COPD group.

To determine the signals downstream of NF-κB activation, we measured HIF-1α activation by determining the mRNA and protein levels of HIF-1α. Exposure to hypoxia resulted in the induction of HIF-1α expression in the lung tissues [19]. Furthermore, our group has previously shown an activation of HIF-1α pathway during hypoxia-induced pulmonary hypertension in rat lung [8,20]. It is now clear that HIF-1α, as well as being responsive to hypoxia, can also be activated in response to a number of non-hypoxic stimuli including LPS, microtubule disruption, TNF-α, reactive oxygen species and so on. A common factor for all of these non-hypoxic stimuli of HIF-1α is that the mechanism involves NF-κB-dependent up-regulation of HIF-1α [4]. Pretreatment with PDTC attenuated the p65 protein levels and p65 DNA-binding capacity in the lungs of COPD rat. Furthermore, PDTC alleviated the induction of HIF-1α in the COPD group both at the mRNA and protein levels, indicating activation of HIF-1α in the COPD group is via the NF-κB pathway. This suggestion is further strengthened by the accumulation of HO-1 and VEGF in the lungs of COPD rats. COPD is associated with structural and functional changes in the pulmonary circulation that commence at an early stage, as they have been shown also in patients with mild COPD without arterial hypoxemia and even in smokers with normal lung function [21]. VEGF, as a key angiogenic growth factor, has been found that blockade of VEGF receptor results in emphysema [22]. Chronic exposure of mice to cigarette smoke inhibits ischemia-induced angiogenesis that is associated with inhibition of ischemia-induced HIF-1α and VEGF protein expression [23]. However, an increased expression of VEGF in pulmonary muscular arteries of patients with moderate COPD and also in smokers with normal lung function has been shown, and its expression was associated with the enlargement of the arterial wall. In contrast, in patients with severe emphysema, VEGF content tended to be low, despite intense vascular remodeling [24]. Furthermore, it has been lately suggested that VEGF might be involved in the pathogenesis of emphysema through apoptotic mechanisms [25]. Given the importance of HIF-1α in regulating VEGF gene expression [26], the finding that simultaneous attenuation of HIF-1α and VEGF by PDTC suggests that inhibition of HIF-1α gene expression in smokers may lead to VEGF production that is insufficient to maintain normal alveolar structure and thus play a major role in the pathogenesis of emphysema. HO-1 is a ubiquitous and redox-sensitive inducible stress protein that is strongly induced by various stimuli [27]. Studies have shown that HO-1 and its metabolic pathway have anti-inflammatory properties [28]. It has been supported by the fact that HO-1-deficient mice develop a chronic inflammatory state that progresses with age [29], and that the only human reported to lack HO-1 enzymatic activity died of an inflammatory syndrome [30]. Consistent with these

observations, PDTC also alleviated the accumulation of HO-1 in the COPD group.

Taken together, by using a rat model of COPD induced by LPS and smog, we confirmed that the activation of HIF-1α pathway via NF-κB contributes to the development of COPD, and that the administration of NF-κB inhibitor may attenuate the development of COPD.

Funding

This work was supported by grants from the Nature Science Foundation of Shanghai Science Committee (no. 074119626) and Shanghai Rising-Star Program (no. 08QA14044).

References

1 Baldwin AS, Jr. Series introduction: the transcription factor NF-kappaB and human disease. J Clin Invest 2001, 107: 3–6.
2 Di Stefano A, Caramori G, Oates T, Capelli A, Lusuardi M, Gnemmi I and Ioli F, *et al*. Increased expression of nuclear factor-kappaB in bronchial biopsies from smokers and patients with COPD. Eur Respir J 2002, 20: 556–563.
3 Edwards MR, Bartlett NW, Clarke D, Birrell M, Belvisi M and Johnston SL. Targeting the NF-kappaB pathway in asthma and chronic obstructive pulmonary disease. Pharmacol Ther 2009, 121: 1–13.
4 Taylor CT. Interdependent roles for hypoxia inducible factor and nuclear factor-kappaB in hypoxic inflammation. J Physiol 2008, 586: 4055–4059.
5 Huang LE, Gu J, Schau M and Bunn HF. Regulation of hypoxia-inducible factor 1alpha is mediated by an O_2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci USA 1998, 95: 7987–7992.
6 Hellwig-Burgel T, Rutkowski K, Metzen E, Fandrey J and Jelkmann W. Interleukin-1beta and tumor necrosis factor-alpha stimulate DNA binding of hypoxia-inducible factor-1. Blood 1999, 94: 1561–1567.
7 Scharte M, Han X, Bertges DJ, Fink MP and Delude RL. Cytokines induce HIF-1 DNA binding and the expression of HIF-1-dependent genes in cultured rat enterocytes. Am J Physiol Gastrointest Liver Physiol 2003, 284: 373–384.
8 Li QF and Dai AG. Hypoxia inducible factor-1 alpha correlates the expression of heme oxygenase 1 gene in pulmonary arteries of rat with hypoxia-induced pulmonary hypertension. Acta Biochim Biophys Sin 2004, 36: 133–140.
9 Tamada S, Nakatani T, Asai T, Tashiro K, Komiya T, Sumi T and Okamura M, *et al*. Inhibition of nuclear factor-kappaB activation by pyrrolidine dithiocarbamate prevents chronic FK506 nephropathy. Kidney Int 2003, 63: 306–314.
10 Miller LM, Foster WM, Dambach DM, Doebler D, McKinnon M, Killar L and Longphre M. A murine model of cigarette smoke-induced pulmonary inflammation using intranasally administered smoke-conditioned medium. Exp Lung Res 2002, 28: 435–455.
11 Xu L, Cai BQ and Zhu YJ. Pathogenesis of cigarette smoke-induced chronic obstructive pulmonary disease and therapeutic effects of glucocorticoids and N-acetylcysteine in rats. Chin Med J 2004, 117: 1611–1619.
12 Ju CR, Xia XZ and Chen RC. Expressions of tumor necrosis factor-converting enzyme and ErbB3 in rats with chronic obstructive pulmonary disease. Chin Med J 2007, 120: 1505–1510.
13 Heidbreder M, Fröhlich F, Jöhren O, Dendorfer A, Qadri F and Dominiak P. Hypoxia rapidly activates HIF-3alpha mRNA expression. FASEB J 2003, 17: 1541–1543.
14 Li QF, Wang XR, Yang YW and Lin H. Hypoxia upregulates hypoxia inducible factor (HIF)-3alpha expression in lung epithelial cells: characterization and comparison with HIF-1alpha. Cell Res 2006, 16: 548–558.
15 Semenza GL. Pulmonary vascular responses to chronic hypoxia mediated by hypoxia-inducible factor 1. Proc Am Thorac Soc 2005, 2: 68–70.
16 Shinohara T, Kaneko T, Nagashima Y, Ueda A, Tagawa A and Ishigatsubo Y. Adenovirus-mediated transfer and overexpression of heme oxygenase 1 cDNA in lungs attenuates elastase-induced pulmonary emphysema in mice. Hum Gene Ther 2005, 16: 318–327.
17 Stevenson CS, Coote K, Webster R, Johnston H, Atherton HC, Nicholls A and Giddings J, *et al*. Characterization of cigarette smoke-induced inflammatory and mucus hypersecretory changes in rat lung and the role of CXCR2 ligands in mediating this effect. Am J Physiol Lung Cell Mol Physiol 2005, 288: 514–522.
18 Chen F, Castranova V, Shi X and Demers LM. New insights into the role of nuclear factor-kappaB, a ubiquitous transcription factor in the initiation of diseases. Clin Chem 1999, 45: 7–17.
19 Yu AY, Frid MG, Shimoda LA, Wiener CM, Stenmark K and Semenza GL. Temporal, spatial, and oxygen-regulated expression of hypoxia-inducible factor-1 in the lung. Am J Physiol 1998, 275: 818–826.
20 Li QF and Dai AG. Hypoxia-inducible factor-1alpha regulates the role of vascular endothelial growth factor on pulmonary arteries of rats with hypoxia-induced pulmonary hypertension. Chin Med J 2004, 117: 1023–1028.
21 Kranenburg AR, de Boer WI, Alagappan VK, Sterk PJ and Sharma HS. Enhanced bronchial expression of vascular endothelial growth factor and receptors (Flk-1 and Flt-1) in patients with chronic obstructive pulmonary disease. Thorax 2005, 60: 106–113.
22 Kasahara Y, Tuder RM, Taraseviciene-Stewart L, Le Cras TD, Abman S, Hirth PK and Waltenberger J, *et al*. Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. J Clin Invest 2000, 106: 1311–1319.
23 Michaud SE, Menard C, Guy LG, Gennaro G and Rivard A. Inhibition of hypoxia-induced angiogenesis by cigarette smoke exposure: impairment of the HIF-1alpha/VEGF pathway. FASEB J 2003, 17: 1150–1152.
24 Peinado VI, Barberá JA, Abate P, Ramírez J, Roca J, Santos S and Rodriguez-Roisin R. Inflammatory reaction in pulmonary muscular arteries of patients with mild chronic obstructive pulmonary disease. Am J Respir Crit Care Med 1999, 159: 1605–1611.
25 Siafakas NM, Antoniou KM and Tzortzaki EG. Role of angiogenesis and vascular remodeling in chronic obstructive pulmonary disease. Int J Chron Obstruct Pulmon Dis 2007, 2: 453–462.
26 Iyer NV, Kotch LE, Agani F, Leung SW, Laughner E, Wenger RH and Gassmann M, *et al*. Cellular and developmental control of O_2 homeostasis by hypoxia-inducible factor-1alpha. Genes Dev 1998, 12: 149–162.
27 Foresti R and Motterlini R. The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. Free Radic Res 1999, 31: 459–475.
28 Willis D, Moore AR, Frederick R and Willoughby DA. Heme oxygenase: a novel target for the modulation of the inflammatory response. Nat Med 1996, 2: 87–90.
29 Poss KD and Tonegawa S. Heme oxygenase 1 is required for mammalian iron reutilization. Proc Natl Acad Sci USA 1997, 94: 10919–10924.
30 Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T and Ohta K, *et al*. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. J Clin Invest 1999, 103: 129–135.