Int J Clin Exp Pathol 2015;8(7):7752-7761
www.ijcep.com /ISSN:1936-2625/IJCEP0009924

Original Article

Bone marrow mesenchymal stem cells protect against bleomycin-induced pulmonary fibrosis in rat by activating Nrf2 signaling

Shirong Ni[1,2], Dexuan Wang[3], Xiaoxiao Qiu[2], Lingxia Pang[4], Zhangjuan Song[2], Kunyuan Guo[1]

[1]Department of Hematology, Zhujiang Hospital, Southern Medical University, Guangzhou, China; [2]Department of Pathophysiology, Wenzhou Medical University, Wenzhou, Zhejiang Province, China; [3]Department of Pediatrics, The Second Affiliated & Yuying Children's Hospital of Wenzhou Medical University, Wenzhou, Zhejiang Province, China; [4]Teaching Center of Medical Functional Experiment, Wenzhou Medical University, Wenzhou, Zhejiang Province, China

Received May 5, 2015; Accepted June 23, 2015; Epub July 1, 2015; Published July 15, 2015

Abstract: Pulmonary fibrosis is a progressive and lethal disorder. Although the precise mechanisms of pulmonary fibrosis are not fully understood, oxidant/antioxidant may play an important role in many of the processes of inflammation and fibrosis. Keap1-Nrf2-ARE pathway represents one of the most important cellular defense mechanisms against oxidative stress. Mesenchymal stem cells (MSC) are in clinical trials for widespread indications including musculoskeletal, neurological, cardiac and haematological disorders. One emerging concept is that MSCs may have paracrine, rather than a functional, roles in lung injury repair and regeneration. In the present study, we investigated bone marrow mesenchymal stem cells (BMSCs) for the treatment of bleomycin-induced pulmonary fibrosis. Our results showed that BMSCs administration significantly ameliorated the bleomycin mediated histological alterations and blocked collagen deposition with parallel reduction in the hydroxyproline level. The gene expression levels of NAD(P)H: quinine oxidoreductase 1 (NQO1), gama-glutamylcysteine synthetase (γ-GCS), heme oxygenase-1 (HO-1) and nuclear factor erythroid 2-related factor 2 (Nrf2), attenuated by bleomycin, were increased up to basal levels after BMSCs transplantation. BMSCs significantly increased superoxide dismutase (SOD) activity and inhibited malondialdehyde (MDA) production in the injured lung. The present study provides evidence that BMSCs may be a potential therapeutic reagent for the treatment of lung fibrosis.

Keywords: Pulmonary fibrosis, BMSCs, Nrf2, oxidative stress, antioxidant enzymes

Introduction

Pulmonary fibrosis is a chronic progressive disorder characterized by the excessive proliferation of fibroblasts and deposition of extracellular matrix, which destroy the architecture and function of normal lung tissue [1, 2]. The precise pathologic mechanisms of pulmonary fibrosis are not fully understood, and the current managements for it are not satisfactory [3-5]. Therefore, it is crucial to find new therapeutic strategies for pulmonary fibrosis.

Oxidative stress is an important molecular mechanism underlying fibrosis in a variety of organs, including the lungs [6-8]. Oxidative takes part into the process of fibrosis by damaging cellular macromolecules such as DNAs, lipids, and proteins via oxidative stress-induced tissue injury which is caused by excessive levels of reactive oxygen species (ROS) [4, 5]. Nrf2-ARE (Nuclear factor E2-related factor 2-Antioxidant response element) pathway plays an important role in the defense against oxidative stress. Nrf2 belongs to the cap'n'collar (CNC) family of basic leucine zipper proteins, and Nrf2 regulates the expression of phase II detoxifying and antioxidant genes by binding to the ARE sequence. Under unstimulated conditions, Nrf2 is sequestered in cytosol, where it is associated with Kelch-like ECH-associated protein 1 (Keap1). When under oxidative stress, Nrf2 escapes from Keap1 and then translocates to the nucleus where it binds to ARE and

induces the phase II antioxidant genes. Recently, the functions of Nrf2 and its downstream genes have been shown to be important for protection against oxidative stress and chemical-induced cellular damage in various type cells, tissues and organs. Therefore, we hypothesized that the Nrf2-ARE pathway may be involved in the mechanism of cytoprotection in bleomycin-mediated oxidative stress of pulmonary fibrosis, and the activation of Nrf2 can decrease the sensitivity of bleomycin-induced oxidative damage in pulmonary fibrosis.

Bone marrow mesenchymal stem cells (BMSCs) are multipotent stem cells which are capable of self-renewal and can differentiate into a variety of lineages under suitable conditions. BMSCs are now extensively applied in the fields of regenerative medicine, gene therapy and tissue repair. Therefore, BMSCs are thought to be an ideal candidate seeding cells for cell therapy and represent an excellent clinical implication prospect. In the present study, we used in vivo bleomycin-induced lung fibrosis model to investigate the protective role of Nrf2 against the development of bleomycin-induced pulmonary inflammation and fibrosis and assess the potential therapeutic effect of BMSCs.

Materials and methods

Animal and maintenance

Forty healthy Sprague-Dawley rats (180-250 g) were obtained from the Experimental Animal Center of Wenzhou Medical University, and were housed in separate cages under standard temperature (25 ± 2°C) and 12 h light/dark photoperiod. The rats were acclimatized for three days before the start of the experiment and provided food and water ad libitum. The experiments were designed and conducted according to the guidelines for the Care and Use of Laboratory Animals issued by the Chinese Council on Animal Research and the Guidelines of Animal Care [9]. All animal experimentation protocols were approved by the Ethical Committee of Wenzhou Medical University.

Experimental protocol

An animal model of bleomycin induced pulmonary fibrosis as reported earlier, was used in this study [10]. Briefly, after recording the body weights, the rats were anesthetized via an intraperitoneal injection of sodium pentobarbital (40 mg/kg). The skin and subcutaneous tissue overlying the proximal portion of the trachea were exposed by blunt dissection. A single intratracheal instillation of 5 mg/kg of bleomycin (Takasaki plant, NIPPON KAYAKU CO. LTD, JAPAN) in sterile 0.9% NaCl was administered to the rats to develop the model for pulmonary fibrosis. The rats in the control group and the BMSCs (bone marrow mesenchymal stem cells) group were given a single intratracheal dose of sterile saline alone.

Forty rats were randomly divided into four groups with ten animals in each. The control group animals received an intratracheal injection of normal saline alone. The bleomycin group animals were subjected to a single intratracheal instillation of bleomycin as previously mentioned. The bleomycin plus BMSCs group of animals received the same dosage of bleomycin as that of bleomycin group animal and were treated with the suspension of BMSCs (2×10^6/1.5 ml) via the tail vein for 3 days. The BMSCs alone group received an intratracheal injection of normal saline and were then treated with BMSCs (2×10^6/1.5 ml) via the tail vein for 3 days. Twenty-one days after the bleomycin treatment, the animals were sacrificed, and their body weights and lung weights were recorded. The lung tissues were sliced into pieces, one part was immersed in 10% formalin solution for histopathological examination, one part was immersed in liquid nitrogen for Western blot analysis, and the remaining part was immersed in 0.9% saline to obtain the tissue homogenate. The lung tissue homogenates were prepared in appropriate homogenizing buffer and stored at -80°C as aliquots for further assay.

Wet/dry weight ratio assay

The wet/dry (W/D) method was used to measure pulmonary edema. After a thoracotomy, the lungs were collected and weighed before and after drying in the incubator at 60°C for 72 h.

Histological examination and Masson's trichrome staining

After sacrificing the animals, one part of the lungs was carefully excised and fixed for one

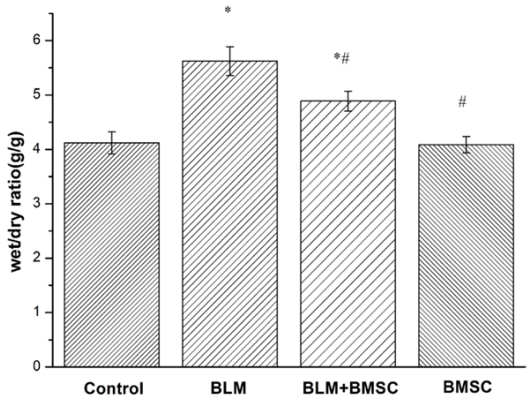

Figure 1. Effect of BMSCs on Wet/Dry ratio of bleomycin-induced pulmonary fibrosis rats. Values are given as mean ± SD for groups of ten rats each. $^{*}P < 0.05$ vs. the control group, respectively; $^{\#}P < 0.05$ vs. the BLM group, respectively.

week in 10% PBS-buffered formaldehyde solution at room temperature, dehydrated using graded ethanol and embedded in paraffin. The paraffin-embedded tissues were cut to 5 μm thicknesses with a microtome (RM-2135, Leica Microsystems, Bensheim, Germany). To evaluate the histopathological changes, the sections were subjected to haematoxylin and eosin staining. To indentify the density of the accumulated collagen fibers, Masson's trichrome staining was performed.

Hydroxyproline assay

The collagen content in the lung homogenates was examined by a hydroxyproline (HYP) assay (HYP kit from Nanjing Jiancheng Bioengineering Company, China). All steps of the HYP assay were performed according to the manufacturer's instructions. The absorbance of each sample at 550 nm wavelength was read by a microplate reader (Thermo Fisher Scientific, USA).

Western blot analysis

The lung tissues were homogenised in RIPA lysis buffer (Beyotime Biotech, Haimen, China) containing 1 mM phenylmethylsulphonyl fluoride. The lysates were then centrifuged at 12,000 rpm for 15 min at 4°C, and the supernatants, which contained the tissue protein extracts, were collected and stored at -80°C. The total protein concentrations were determined using the Bicinchoninic acid protein assay kit (Beyotime Biotech, Haimen, China). The samples (30 ìg) were mixed with sample buffer, denaturated by heating at 95°C for 5 min, resolved via SDS-PAGE and transferred to nitrocellulose membranes. The membranes were blocked with 5% non-fat dried milk in Tris-buffered saline with Tween (TTBS) for 1 h and probed with primary antibodies against NRF2 (1:400 dilution, Boster, Wuhan, China), KEAP1 (1:400 dilution, Boster, Wuhan, China), γ-GCS (1:400 dilution; Boster, Wuhan, China), HO-1 (1:400 dilution; Bioss Biotech, Beijing, China), and NQO1 (1:1000 dilution; Proteintech, Wuhan, China) overnight at 4°C. Then, they were washed with TTBS and incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000; Cowin Biotech, Beijing, China) for 1 h. Finally, the blots were developed with ECL-Plus reagent (Millipore, Bedford, MA, USA) and the graphs were analyzed by the Gel-Pro Analyzer (Media Cybernetics, Bethesda, MD, USA).

Measurements of malondialdehyde (MDA) and superoxide dismutase (SOD) level

SOD activity was measured using superoxide dismutase (SOD) typed assay kit (Hydroxylamine method) (Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The absorbance was determined at 550 nm on a microplate reader (Thermo Fisher Scientific, USA). The MDA content in the serum was measured using, Malondialdehyde (MDA) assay kit (TBA method) (Jiancheng Bioengineering Institute, Nanjing, China), based on the formation of a red complex when MDA reacts with thiobarbituric acid. The absorbance was measured spectrophotometrically at 532 nm.

Cell line

Rat mesenchymal stem cells (H4320-1) were obtained from CHI Scientific, Inc. (Jiangyin, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 2 mM L-glutamine (Sigma, USA) and 10% fetal bovine serum (FBS) (Gibco).

Statistical analysis

The data are presented as the means ± SD (standard deviation), and n indicates the number of animals studied. The statistical analysis was performed using Student's test or a one-

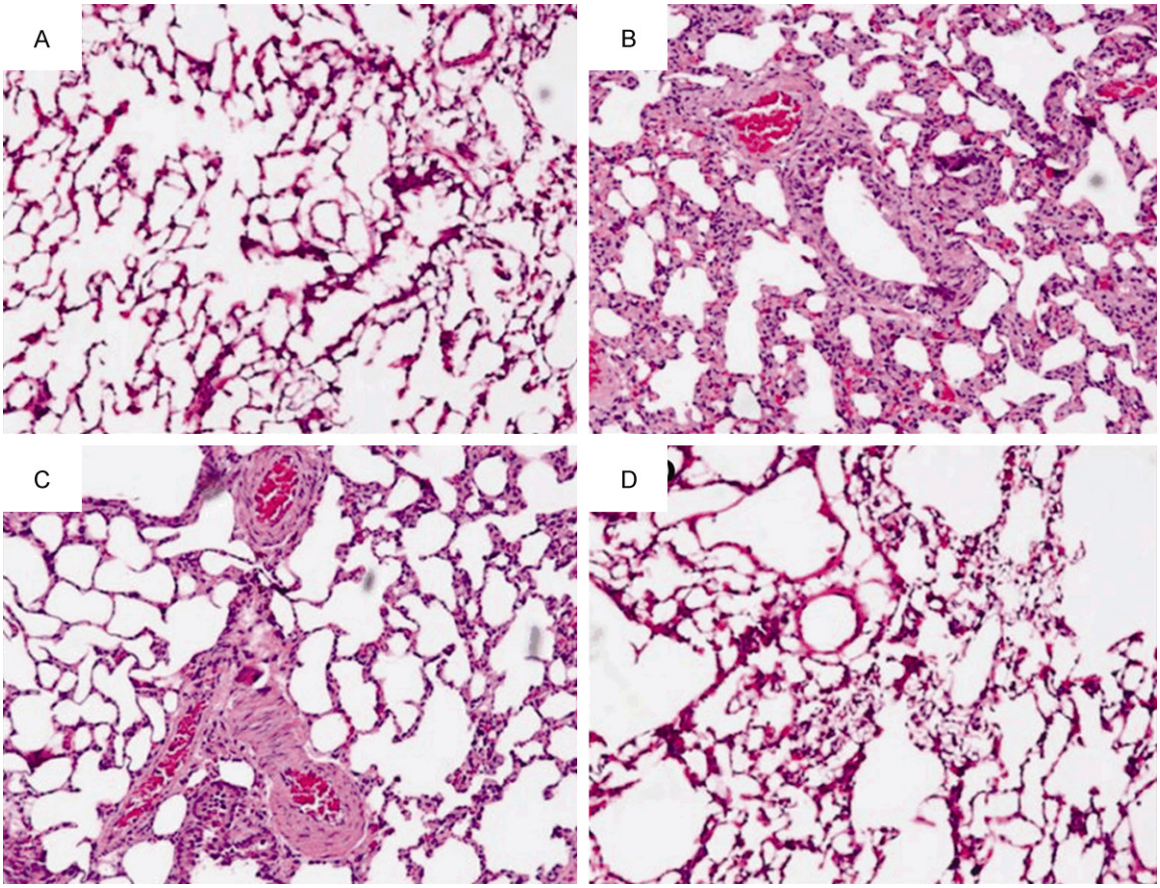

Figure 2. Histology of BMSCs on bleomycin-induced lung in rats. A. Lung tissue sections of control animals showing normal lung morphologies: thin lined interalveolar septa with well-organized alveolar space; B. Lung tissue sections of bleomycin-induced animals showing distorted lung morphologies: collapsed alveolar spaces with inflammatory exudates, wider and thickened interalveolar septa; C. Lung tissue sections of BMSCs treated animals: lower inflammatory infiltrates with lessened alveolar thickening; and D. Lung tissue section of BMSCs alone administered animals showing similar morphology with that of control animals. Representative histological sections were stained by hematoxylin and eosin (×400).

way analysis of variance, followed by the Tukey post hoc test for multiple comparisons. All the statistical analyses used SPSS software (SPSS, Chicago, XXII, USA). $P \leq 0.05$ was considered significant.

Results

Protective effects of BMSCs against bleomycin modulated wet/dry ratio (W/D)

A significant increment in the W/D was observed in the bleomycin-treated animals, compared to saline-treated control animals ($P < 0.05$). BMSCs administration decreased the W/D in animals treated with bleomycin. Simultaneously, the increased W/D of the bleomycin-treated animals were prominently reduced when treated with BMSCs ($P < 0.05$). Moreover, no adverse effect was observed in animals administered BMSCs alone, similar to the profiles of control animals (**Figure 1**).

BMSCs attenuated bleomycin mediated histological changes

The lung tissue sections of bleomycin-treated animals showed markedly histopathological abnormalities, including disturbed alveolar structure, extensive thickening of the interalveolar septa, and dense interstitial infiltration by lymphocytes, neutrophils, and fibroblasts (**Figure 2B**). No such pathological changes were observed in the control group and the group treated with BMSCs alone (**Figure 2A**, **2D**). In contrast, the BMSCs treatments provided pro-

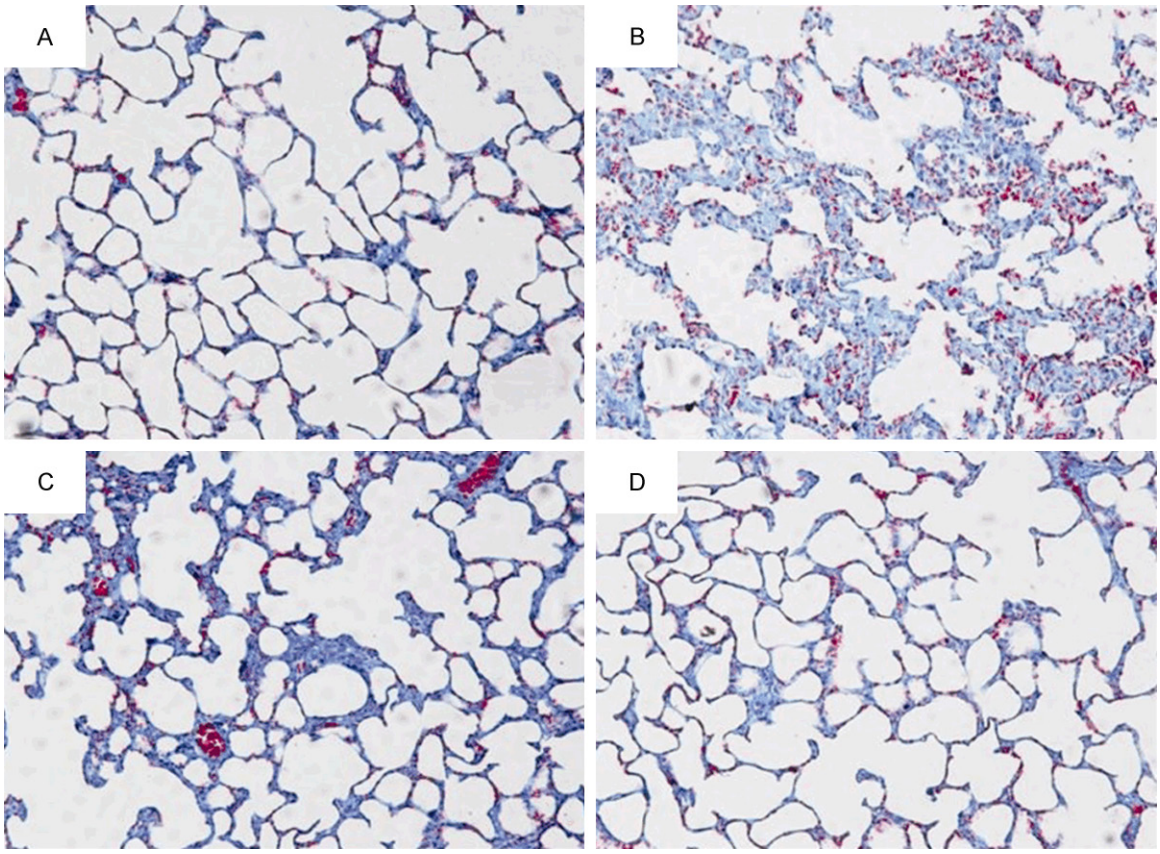

Figure 3. Effects of BMSCs on histopathological changes of bleomycin-induced lung with Masson's trichrome stain (×400). A, D. Lung tissue sections of control and BMSCs-alone administrated animals with normal lung morphologies: scarcely deposited collagen in the lung parenchyma; B. Lung tissue sections of bleomycin-induced animals showing dense collagen accumulations: collagen accumulations between alveoli; and C. Lung sections of BMSCs treated animals showing reduced collagen depositions: reduced alveolar thickening with meager collagen.

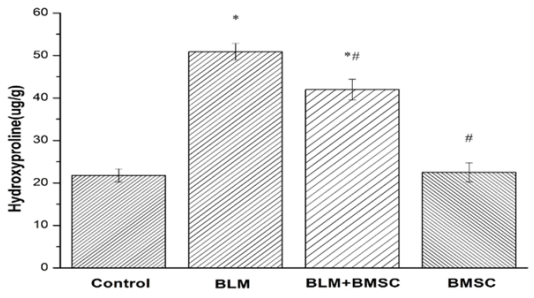

Figure 4. Effects of BMSCs on the hydroxyproline content in the lungs of bleomycin-induced pulmonary fibrosis rats. Values are given as mean ± SD for groups of ten rats each. $^{*}P < 0.05$ vs. the control group; $^{\#}P < 0.05$ vs. the BLM group.

tection against bleomycin-induced lung tissue distortion. Significant amelioration in the cellular infiltrates and thin-lined alveolar septa were observed in the lung tissue sections of the BMSCs-treated group compared to those of the bleomycin-treated animals (**Figure 2C**).

Inhibition effects of BMSCs on collagen depositions and hydroxyproline content

Masson's trichrome staining showed that the bleomycin-treated animals had abnormal collagen deposition and distorted lung morphologies compared with those of control animals (**Figure 3B**). The BMSCs treatment strongly inhibited the extent and intensity of collagen, compared to the bleomycin treatment (**Figure 3C**). No such abnormalities were apparent in the control animals and the animals treated with BMSCs alone (**Figure 3A**, **3D**). We analyzed the hydroxyproline content of lung tissues, which is considered to be a fibrotic marker of deposited collagen. As summarized in **Figure 4**, the pulmonary hydroxyproline levels in the bleomycin group were drastically increased, compared to those in the control animals. The BMSCs treatment reduced the level of hydroxyproline, which was consistent with the changes in Masson's trichrome staining. There was no

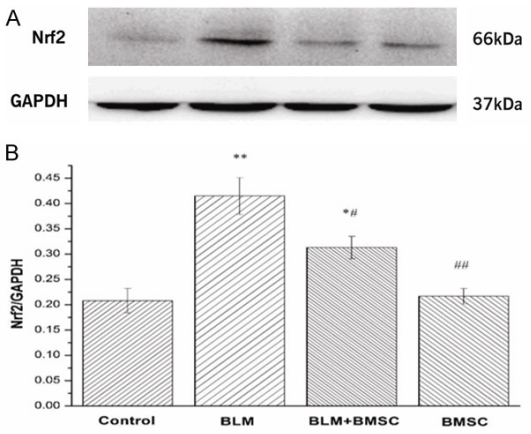

Figure 5. Western blot analysis of Nrf2 levels in the lungs of bleomycin-induced pulmonary fibrosis rats. The decreased levels of Nrf2 protein expression were significantly increased by administration of BMSCs. A. Representative blots are shown and protein size is expressed in kDa; and B. Densitometry showed the intensity ratio of target proteins to GAPDH protein (mean ± SD, n = 10). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. the control group, respectively; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. the BLM group, respectively.

significant difference in the hydroxyproline content of animals administered BMSCs alone compared to that in control animals.

Down-regulation of Nrf2, NQO-1, γ-GCS and HO-1 expression by BMSCs treatment

Several recent studies underscore the importance of Nrf2 in regulating pulmonary fibrosis [11-13]. Nrf2 is a transcription factor which is considered as the "master regulator" of the antioxidant response. It regulates the expression of phase II detoxifying and antioxidant genes. To determine whether BMSCs promote phase II detoxifying and antioxidant genes production via the increase of Nrf2 expression, Nrf2 protein levels were measured using western blot analysis. Compared to the control group and BMSCs group, there was a significant decrease in Nrf2 protein levels in the bleomycin group. The BMSCs treatment significantly promoted the increase of Nrf2 protein in the lung tissue of the bleomycin-treated animals (**Figure 5A**). The results suggested that BMSCs promotes Nrf2 production in rats with bleomycin-induced pulmonary fibrosis. The quantitative analysis of Western blots for Nrf2 expression is depicted in **Figure 5B**.

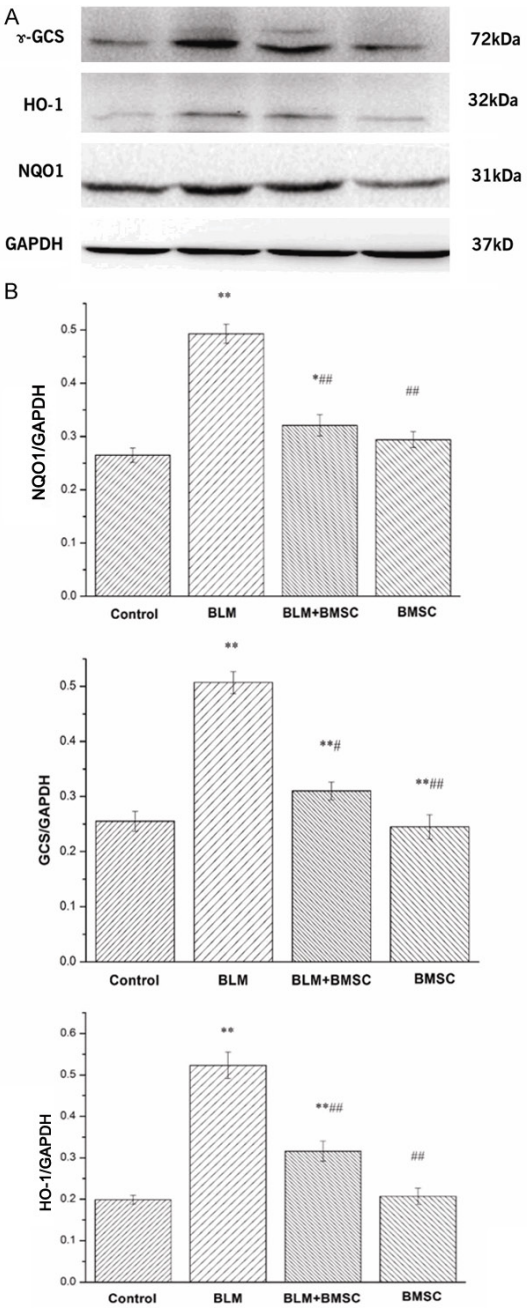

Figure 6. Western blot analysis of NQO1, γ-GCS, and HO-1 levels in the lungs of bleomycin-induced pulmonary fibrosis rats. The decreased levels of NQO1, γ-GCS, and HO-1 protein expression were significantly increased by administration of BMSCs. A. Representative blots are shown and protein size is expressed in kDa; and B. Densitometric quantification data showed the intensity ratio of target proteins to GAPDH (mean ± SD, n = 10). $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. the control group, respectively; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. the BLM group, respectively.

Nrf2 is an essential transcription factor that regulates antioxidant response element (ARE)-mediated expression of phase II antioxidant enzymes, including NAD(P)H: quinine oxidore-

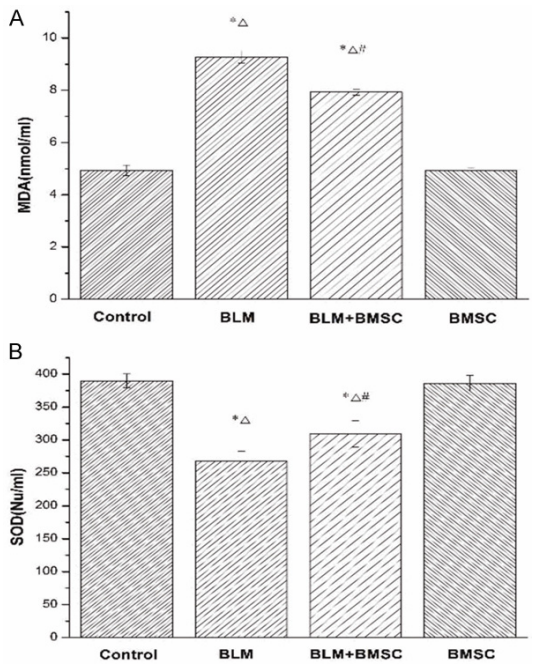

Figure 7. Effects of BMSCs on the (A) malondialdehyde (MDA) and (B) superoxide dismutase (SOD) levels in the lungs of bleomycin-induced pulmonary fibrosis rats. Values are given as mean ± SD for groups of 10 rats each. $^{*}P < 0.05$ vs. the control group; $^{\Delta}P < 0.05$ vs. the BMSC group; $^{\#}P < 0.05$ vs. the BLM group.

ductase 1 (NQO1), Gama-glutamylcysteine synthetase (γ-GCS), and heme oxygenase-1 (HO-1). To analyze the anti-fibrotic efficacy of BMSCs and to confirm the role of phase II antioxidant enzymes, Western blot analysis for NQO1, γ-GCS, and HO-1 were performed. The Western blot analysis of the lung tissue of the bleomycin-treated animals showed decreased expression profiles of NQO1, γ-GCS, and HO-1 compared to the control animals. A significant upregulation of NQO1, γ-GCS, and HO-1 were observed after the BMSCs treatments compared to those of the bleomycin-treated animals (**Figure 6A**). The quantitative analysis of the Western blots for NQO1, γ-GCS, and HO-1 are shown in **Figure 6B**. No significant change was observed between the control animals and the animals treated with BMSCs alone.

Suppressive effects of BMSCs on oxidative stress markers

To evaluate the oxidative injury of lung, the malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations were measured in the experimental group of animals. As expected, the MDA levels were considerably increased in the lung tissue of bleomycin-exposed animals compared to those of the control animals. Conversely, the SOD levels were significantly suppressed in the bleomycin-treated animals, compared to those of the control animals. Treatment with BMSCs reduced the levels of MDA and increased SOD, compared to those of the bleomycin treatment. There was no significant difference between the control animals and the animals treated with BMSCs alone (**Figure 7**).

Discussion

Pulmonary fibrosis is a progressive and fatal lung disease with histopathological characteristics including excessive extracellular matrix (ECM) deposition, patchy chronic interstitial inflammation, fibroblast proliferation, and collapse of alveoli, leading to progressive fibrosis and loss of lung functions [14]. Despite extensive research efforts in experimental and clinical studies, pulmonary fibrosis responds poorly to available therapy [5]. The development of efficient therapeutic interventions to ameliorate the pathogenic events in pulmonary fibrosis seems to gain significance. The present study showed that BMSCs attenuates the histological abnormal changes and reduces the levels of hydroxyproline in pulmonary fibrosis induced by bleomycin in rats, significantly increases the activities of antioxidant enzymes (SOD) and decreases MDA levels in lung tissues. Furthermore, BMSCs significantly promotes the expression and nuclear translocation of Nrf2 that regulated the expression of many antioxidant enzymes.

Bleomycin, a glycopeptide antibiotic, has been used clinically for a variety of cancers [15]. As a side effect of its therapeutic use, bleomycin causes destruction of the lung architecture, leading to pulmonary fibrosis that is characterized by an increase in hydroxyproline levels and collagen deposition in the lungs [16]. Bleomycin-induced lung fibrosis is a widely used to develop animal model of human idiopathic pulmonary fibrosis [17, 18]. The route of intratracheal instillation generally causes an inflammatory response and increased epithelial apoptosis for the first seven days, closely resembling acute lung injury. These effects are followed by three days of transition, in which the inflamma-

tion resolves and followed by three days of transition, in which the inflammation resolves and fibrosis is detected. The fibrotic stage persists until three to four weeks after the bleomycin instillation, and is characterized by the excessive deposition of extracellular matrix, causing areas of fibrosis [19, 20]. The present study showed a substantially increased intensity of collagen in the bleomycin-treated animals, which reflected the detrimental alterations associated with fibrosis. The amplified hydroxyproline levels correlates with the accumulated collagen in the alveolar space. Our data also indicated that the BMSCs-exposed rats exhibited significantly lower W/D ratio (wet/dry). The ameliorating effects of BMSCs on histological changes might be due to their radical scavenging activities, which prevent the accumulation of hydroxyproline in bleomycini-induced lung tissues.

Growing evidence indicates the essential role of oxidative stress in the pathophysiology of many diseases, including lung fibrosis [7, 21]. Reactive oxygen species (ROS) were reported to increase in bleomycin-induced pulmonary fibrosis in animals [22]. In this study, we found that bleomycin induced a marked increase of lipid peroxidation as indicated by the MDA level, and this level was significantly decreased upon BMSCs supplementations. In addition, BMSCs treatments significantly attenuated the bleomycin-mediated oxidative stress. Nrf2 is a transcription factor considered the “master regulator” of the antioxidant response [23]. The upregulation of many antioxidant enzymes or the inhibition of lipid peroxidation in the lung is mediated by Nrf2. Upon exposure to oxidative or electrophilic stress, Nrf2 dissociates from Keap1 and translocates to the nucleus, at where it binds to the ARE and leads to an array of transcriptional regulatory proteins [24, 25], including HO-1, NQO1, SOD, and γ-GCS [26-30]. The deficiency of Nrf2 enhances the susceptibility to experimental acute lung injury and impairs the resolution of lung inflammation in mice. In this study, we found that bleomycin induced a marked increase in lipid peroxidation as indicated by the MDA level. In addition, the Western blot analysis showed that the attenuated expression of Nrf2 in the nuclear transplantation by bleomycin is consistent with the decreased activities of antioxidant enzymes by bleomycin. Our findings suggest that Nrf2 play critical roles during bleomycin-induced pulmonary fibrosis, and therefore, Nrf2 can be applied as biological markers and potential therapeutic target in treatments.

The optimal therapy for pulmonary fibrosis remains controversial. No agent has been rigorously shown to improve the survival or quality of life for patients with pulmonary fibrosis [31]. Lung transplantation is presently the only effective therapy for pulmonary fibrosis [32]. However, lung transplantation has many limitations attributable to organ shortages and complications associated with long-term immunosuppression [33]. Therefore, the development of effective therapies to reduce or reverse pulmonary fibrosis is important for reducing the morbidity and mortality associated with pulmonary fibrosis and the need for lung transplantation. Recent studies have also shown that cells derived from marrow, especially MSCs, may also repopulate the lung and repair the injured lung tissue. Mesenchymal stem cell-based therapy is currently a promising and novel treatment for lung injury [34, 35]. The ability of MSCs to engraft in organs remotely from bone marrow suggests that exogenously administered MSCs contribute to the repair of the injured alveolar epithelium after lung injury. These findings are potentially of significant clinical benefit for the regeneration of injured lung tissue. In this study, we found that BMSCs-exposed rats exhibited significantly lower ratio of W/D and improved histological changes. BMSCs prevented the accumulation of hydroxyproline in bleomycin-induced lung tissues. In addition, BMSCs treatment significantly attenuated the bleomycin-mediated oxidative stress, as indicated by the decreased levels of MDA. The Western blot analysis showed that BMSCs treatment strikingly increased the level of Nrf2 protein as well as phase II enzymes such as NQO, HO-1 and γ-GCS in the lung tissue of the bleomycin-treated animals. These results indicated that BMSCs could attenuate oxidative stress via promote Nrf2 expression in bleomycin-induced pulmonary fibrosis, Nrf2 pathway is an endogenous compensatory adaptation against pulmonary fibrosis induced by bleomycin.

In the present study, we demonstrated the antifibrotic efficacy of BMSCs against bleomycin-induced pulmonary fibrosis. We found BMSCs attenuated bleomycin-induced oxidative stress,

histological alterations and collagen depositions via the activation of NQO1, γ-GCS, and HO-1 expression and the Nrf2 pathway. Our results suggest that BMSCs is a promising treatment in protecting early lung tissue damage induced by bleomycin exposure or might be utilized in combination therapy for pulmonary fibrosis in clinics.

Acknowledgements

This work was supported by the Natural Science Foundation of China (Grant No.81200513).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Kunyuan Guo, Department of Hematology, Zhujiang Hospital, Southern Medical University, 253 Industrial Road, Guangzhou 510282, Guangdong, China. Tel: (+86) 20-62782320; Fax: (+86) 20-85143034; E-mail: kunyuanguo51@163.com

References

[1] Selman M, Thannickal VJ, Pardo A, Zisman DA, Martinez FJ, Lynch JP 3rd. Idiopathic pulmonary fibrosis: pathogenesis and therapeutic approaches. Drugs 2004; 64: 405-430.

[2] Kim DS. Acute exacerbations in patients with idiopathic pulmonary fibrosis. Respir Res 2013; 14: 86.

[3] Raghu G, Chang J. Idiopathic pulmonary fibrosis: current trends in management. Clin Chest Med 2004; 25: 621-636.

[4] Maher TM. Idiopathic pulmonary fibrosis: pathobiology of novel approaches to treatment. Clin Chest Med 2012; 33: 69-83.

[5] Antoniou KM, Margaritopoulos GA, Siafakas NM. Pharmacological treatment of idiopathic pulmonary fibrosis: from the past to the future. Eur Respir Rev 2013; 22: 281-291.

[6] Rahman I, Skwarska E, Henry M, Davis M, O'Connor CM, FitzGerald MX, Greening A, MacNee W. Systemic and pulmonary oxidative stress in idiopathic pulmonary fibrosis. Free Radic Biol Med 1999; 27: 60-68.

[7] Kinnula VL, Fattman CL, Tan RJ, Oury TD. Oxidative stress in pulmonary fibrosis: a possible role for redox modulatory therapy. Am J Respir Crit Care Med 2005; 172: 417-422.

[8] Daniil ZD, Papageorgiou E, Koutsokera A, Kostikas K, Kiropoulos T, Papaioannou AI, Gourgoulianis KI. Serum levels of oxidative stress as a marker of disease severity in idiopathic pulmonary fibrosis. Pulm Pharmacol Ther 2008; 21: 26-31.

[9] Yang J, Song TB, Zhao ZH, Qiu SD, Hu XD, Chang L. Vasoactive intestinal peptide protects against ischemic brain damage induced by focal cerebral ischemia in rats. Brain Res 2011; 1398: 94-101.

[10] Punithavathi D, Venkatesan N, Babu M. Curcumin inhibition of bleomycin-induced pulmonary fibrosis in rats. Br J Pharmacol 2000; 131: 169-172.

[11] Pekovic-Vaughan V, Gibbs J, Yoshitane H, Yang N, Pathiranage D, Guo B, Sagami A, Taguchi K, Bechtold D, Loudon A, Yamamoto M, Chan J, van der Horst GT, Fukada Y, Meng QJ. The circadian clock regulates rhythmic activation of the NRF2/glutathione-mediated antioxidant defense pathway to modulate pulmonary fibrosis. Genes Dev 2014; 28: 548-560.

[12] Zucker SN, Fink EE, Bagati A, Mannava S, Bianchi-Smiraglia A, Bogner PN, Wawrzyniak JA, Foley C, Leonova KI, Grimm MJ, Moparthy K, Ionov Y, Wang J, Liu S, Sexton S, Kandel ES, Bakin AV, Zhang Y, Kaminski N, Segal BH, Nikiforov MA. Nrf2 amplifies oxidative stress via induction of Klf9. Mol Cell 2014; 53: 916-928.

[13] Artaud-Macari E, Goven D, Brayer S, Hamimi A, Besnard V, Marchal-Somme J, Ali ZE, Crestani B, Kerdine-Römer S, Boutten A, Bonay M. Nuclear factor erythroid 2-related factor 2 nuclear translocation induces myofibroblastic dedifferentiation in idiopathic pulmonary fibrosis. Antioxid Redox Signal 2013; 18: 66-79.

[14] White ES, Lazar MH, Thannickal VJ. Pathogenetic mechanisms in usual interstitial pneumonia/idiopathic pulmonary fibrosis. J Pathol 2003; 201: 343-354.

[15] Gothelf A, Mir LM, Gehl J. Electrochemotherapy: results of cancer treatment using enhanced delivery of bleomycin by electroporation. Cancer Treat Rev 2003; 29: 371-387.

[16] Azambuja E, Fleck JF, Batista RG, Menna Barreto SS. Bleomycin lung toxicity: who are the patients with increased risk? Pulm Pharmacol Ther 2005; 18: 363-366.

[17] Moeller A, Ask K, Warburton D, Gauldie J, Kolb M. The bleomycin animal model: a useful tool to investigate treatment options for idiopathic pulmonary fibrosis? Int J Biochem Cell Biol 2008; 40: 362-382.

[18] Wan YY, Tian GY, Guo HS, Kang YM, Yao ZH, Li XL, Liu QH, Lin DJ. Endostatin, an angiogenesis inhibitor, ameliorates bleomycin-induced pulmonary fibrosis in rats. Respir Res 2013; 14: 56.

[19] Chaudhary NI, Schnapp A, Park JE. Pharmacologic differentiation of inflammation and fibrosis in the rat bleomycin model. Am J Respir Crit Care Med 2006; 173: 769-776.

[20] Mouratis MA, Aidinis V. Modeling pulmonary fibrosis with bleomycin. Curr Opin Pulm Med 2011; 17: 355-361.

[21] Cheresh P, Kim SJ, Tulasiram S, Kamp DW. Oxidative stress and pulmonary fibrosis. Biochim Biophys Acta 2013; 1832: 1028-1040.
[22] Fubini B, Hubbard A. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) generation by silica in inflammation and fibrosis. Free Radic Biol Med 2003; 34: 1507-1516.
[23] Mukaigasa K, Nguyen LT, Li L, Nakajima H, Yamamoto M, Kobayashi M. Genetic evidence of an evolutionarily conserved role for Nrf2 in the protection against oxidative stress. Mol Cell Biol 2012; 32: 4455-4461.
[24] Itoh K, Tong KI, Yamamoto M. Molecular mechanism activating Nrf2-Keap1 pathway in regulation of adaptive response to electrophiles. Free Radic Biol Med 2004; 36: 1208-1213.
[25] Kobayashi M, Yamamoto M. Molecular mechanisms activating the Nrf2-Keap1 pathway of antioxidant gene regulation. Antioxid Redox Signal 2005; 7: 385-394.
[26] Bardag-Gorce F, Oliva J, Lin A, Li J, French BA, French SW. Proteasome inhibitor up regulates liver antioxidative enzymes in rat model of alcoholic liver disease. Exp Mol Pathol 2011; 90: 123-130.
[27] Farombi EO, Shrotriya S, Na HK, Kim SH, Surh YJ. Curcumin attenuates dimethylnitrosamine-induced liver injury in rats through Nrf2-mediated induction of heme oxygenase-1. Food Chem Toxicol 2008; 46: 1279-1287.
[28] Mulcahy RT, Wartman MA, Bailey HH, Gipp JJ. Constitutive and beta-naphthoflavone-induced expression of the human gamma-glutamylcysteine synthetase heavy subunit gene is regulated by a distal antioxidant response element/TRE sequence. J Biol Chem 1997; 272: 7445-7454.
[29] Jeong WS, Jun M, Kong AN. Nrf2: a potential molecular target for cancer chemoprevention by natural compounds. Antioxid Redox Signal 2006; 8: 99-106.
[30] Mann GE, Niehueser-Saran J, Watson A, Gao L, Ishii T, de Winter P, Siow RC. Nrf2/ARE regulated antioxidant gene expression in endothelial and smooth muscle cells in oxidative stress: implications for atherosclerosis and preeclampsia. Sheng Li Xue Bao 2007; 59: 117-127.
[31] Adamali HI, Maher TM. Current and novel drug therapies for idiopathic pulmonary fibrosis. Drug Des Devel Ther 2012; 6: 261-272.
[32] Whelan TP. Lung transplantation for interstitial lung disease. Clin Chest Med 2012; 33: 179-189.
[33] McShane PJ, Garrity ER Jr. Minimization of immunosuppression after lung transplantation: current trends. Transpl Int 2009; 22: 90-95.
[34] Cargnoni A, Gibelli L, Tosini A, Signoroni PB, Nassuato C, Arienti D, Lombardi G, Albertini A, Wengler GS, Parolini O. Transplantation of allogeneic and xenogeneic placenta-derived cells reduces bleomycin-induced lung fibrosis. Cell Transplant 2009; 18: 405-422.
[35] Chang YS, Oh W, Choi SJ, Sung DK, Kim SY, Choi EY, Kang S, Jin HJ, Yang YS, Park WS. Human umbilical cord blood-derived mesenchymal stem cells attenuate hyperoxia-induced lung injury in neonatal rats. Cell Transplant 2009; 18: 869-886.