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# Anti-fibrotic effects of bone morphogenetic protein-7-modified bone marrow mesenchymal stem cells on silica-induced pulmonary fibrosis

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ABSTRACT

Silicosis is an occupational lung disease caused by exposure to small particles of crystalline silica, which ultimately results in diffuse pulmonary fibrosis. Evidence indicates an anti-fibrotic role of bone morphogenetic protein-7 (BMP-7) and bone marrow mesenchymal stem cells (BMSCs) in lung diseases. Therefore, strategies incorporating genetic engineering and stem cell biology might have a tremendous potential to treat critical injuries and diseases. Therefore, we modified BMSCs to overexpress the BMP-7 gene (BMP-7-BMSCs) by lentivirus transduction, and then evaluated whether fibrotic processes were inhibited by these cells in vivo. Wistar rats were divided into four groups: control, silica, BMSCs, and BMP-7-BMSCs. The control group received saline, the silica group received silica and saline, the BMSCs group received silica and BMSCs, and the BMP-7-BMSCs group received silica and BMP-7-BMSCs. Rats were sacrificed on days 15 or 30 after silica instillation. Hematoxylin and eosin, and Masson's trichrome staining were performed for histological examination. The severity of fibrosis was evaluated by the levels of hydroxyproline, fibronectin (FN), and transforming growth factor (TGF)-β1. Restoration of the alveolar epithelium was detected by the epithelial marker surfactant protein (SP)-C and aquaporin (AQP)-5. Histopathological results showed that BMP-7-BMSCs could remarkably block the progression of silica-induced fibrosis. Hydroxyproline, FN, and TGF-β1 contents in the BMP-7-BMSCs-treated group were significantly lower than those in the BMSCs group ($P < 0.05$). Furthermore, the expression of SP-C and AQP-5 in the BMP-7-BMSCs-treated group was significantly higher than those in the BMSCs group ($P < 0.05$). In conclusion, the pulmonary fibrosis induced by silica in rats was significantly reduced by treatment with BMP-7-BMSCs and BMSCs. The anti-fibrotic effect of BMSCs can be strengthened by BMP-7. Treatment with BMP-7-BMSCs might be a potential therapeutic intervention for silicosis.

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## 1. Introduction

Silicosis is caused by the inhalation of respirable crystalline silica dust, which can lead to pulmonary fibrosis, lung structure distortion, and respiratory failure. It is one of the major occupational diseases worldwide (Greenberg et al., 2007; Steenland and Ward, 2014). The pulmonary injury develops progressively, even after escaping from occupational exposure. No proven curative treatment exists and the only effective therapy is lung transplantation (Leung et al., 2012). However, high expenses, donor supply, and surgical invasiveness continue to be challenges. Consequently, it is necessary to develop an effective alternative to cure this life-threatening disease.

In light of these facts, mesenchymal stem cell (MSC)-based cell therapy is regarded as an innovative experimental treatment. MSCs, a population of multipotent stem cells, are original from various tissues and organs, including bone marrow, adipose, cord blood, and placenta. Multipotentiality is one of the properties for these cells that differentiate under particular conditions into not only adipocytes, chondrocytes and osteoblasts, but also vascular smooth muscle cells and lung epithelial cells. Based on their capability to differentiate into specific cell types, bone marrow-derived MSCs (BMSCs) might promote tissue regeneration (Peng et al., 2015; Zhang et al., 2014). In addition to their regenerative capability, BMSCs exhibit immunomodulatory and anti-fibrotic activities that can be significant in the response to injury. The anti-fibrotic effects of cultured BMSCs have been demonstrated in several injured tissues such as the heart (Galie and Stegemann, 2014), kidney (Wang et al., 2012), liver (Fiore et al., 2015), and lung (Cruz and

*Abbreviations:* BMP-7, bone morphogenetic protein-7; BMSCs, bone marrow mesenchymal stem cells; BMP-7-BMSCs, BMSCs overexpress the BMP-7 gene by lentivirus transduction; FN, fibronectin; TGF-β1, transforming growth factor beta 1; SP-C, epithelial marker surfactant protein-C; AQP-5, aquaporin-5; MSC, mesenchymal stem cell; BAL, bronchoalveolar lavage; Bcl-2, B-cell lymphoma-2; HO-1, heme oxygenase-1; GFP, green fluorescent protein.

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Rocco, 2015). In animal models of pulmonary fibrosis, transplanted BMSCs home to sites of injury (Xu et al., 2015), ameliorate the histological alterations (Ni et al., 2015), inhibit production of proinflammatory mediators (Xue et al., 2013), and decrease collagen deposition (Lan et al., 2015). In addition, BMSCs attenuate lung injury and pulmonary fibrosis by secreting various types of factors with anti-apoptotic, anti-inflammatory, and anti-fibrotic functions. Because BMSCs are easily harvested, isolated, and purified, they have been considered to be a promising and novel treatment.

Bone morphogenetic protein-7 (BMP-7), a member of the transforming growth factor (TGF)-β superfamily, is regarded as a critical protein that negatively regulates TGF-β in tissue fibrosis. Our previous study revealed that BMP-7 significantly reduces the silica-induced hydroxyproline content and the expression of other fibrosis markers in the lung tissue of rats (Yang et al., 2013), suggesting that BMP-7 may exert positive effects to inhibit lung fibrosis processes. Indeed, several studies have shown that BMP-7 has beneficial effects on renal (Manson et al., 2014), liver (Wang et al., 2014), and cardiac fibroses (Urbina and Singla, 2014). These findings indicate that BMP-7 is a potential therapeutic agent for the treatment of silicosis.

Because BMSCs and BMP-7 exert protective effects against pulmonary fibrosis, we integrated them and evaluated their potential for inhibiting lung fibrosis processes. In our study, we verified enhancement of the protective effect of BMSCs by BMP-7 in a silicosis model in vivo.

## 2. Materials and methods

### 2.1. Animals and ethics statement

A total of 10 male and 64 female Wistar rats weighing 190–240 g were purchased from Vital River Laboratory Animal Technology Co. Ltd. (Beijing, China). They were kept in a temperature-controlled room (24 ± 1 °C) with a 12:12 h light/dark cycle and provided free access to food and water. This study was approved by the Laboratory Animal Care and Use Committee at Capital Medical University (Approval number: 2012- X-25), which abided by the National Institute of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize suffering.

### 2.2. BMSCs isolation, culture, and characterization

Primary BMSCs were isolated from the femurs and tibias of male Wistar rats and cultured in alpha-modified minimum essential medium (Hyclone, Logan, UT, USA) containing 14% fetal bovine serum (Gemini Bio-Products, West Sacramento, CA, USA), 100 IU/mL penicillin, and 100 μg/mL streptomycin (KeyGEN, Nanjing, China) at 37 °C in a humidified atmosphere with 5% $CO_2$. Non-adherent cells were removed by a medium change after 72 h. When colonies of fibroblast-like appearance showed up, BMSCs were conducted at passages 3–5 in all experiments.

Surface marker expression was evaluated by flow cytometry. BMSCs were positive for CD90 and CD44, but negative for CD45 and CD11b, which is consistent with BMSCs characteristics (Dominici et al., 2006). An antibody against CD90 was obtained from Becton Dickinson (Mississauga, ON, Canada), and antibodies against CD11b, CD44, and CD45 were obtained from eBioscience (San Diego, CA, USA). All antibodies were conjugated with phycoerythrin.

To induce BMSCs to differentiate into adipocytes and osteocytes in vitro, specific differentiation media were applied at 37 °C in a humidified atmosphere containing 5% $CO_2$. Three weeks later, fat droplets and calcium were detected by staining with Oil Red O and Alizarin Red, respectively.

### 2.3. Lentiviral vector construction and transduction

Replication-defective lentiviruses with green fluorescent protein (GFP) only or the BMP-7 gene were used for the control (Lenti-GFP) and target gene (Lenti-BMP-7) groups. Production of the lentiviral vector encoding BMP-7-GFP (LV-BMP-7-GFP) has been previously described (Liang et al., 2015). The lentivirus expressing BMP-7 was propagated and harvested using a virus packaging system (Telebio, Shanghai, China). The optimal multiplicity of infection for lentiviral

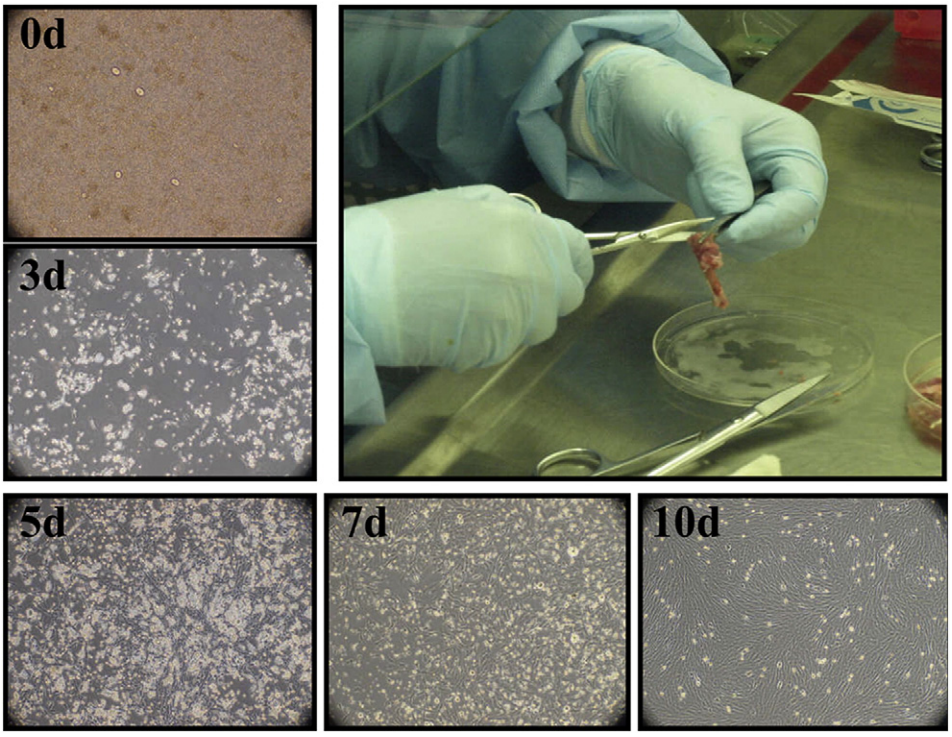


**Fig. 1.** Isolation of BMSCs in vitro. BMSCs were isolated from the femurs and tibiae of rats. They were adherent and displayed a 'spindle-like' shape during growth (0–10 days [d]). Images were obtained with a phase-contrast microscope at a magnification of ×100.

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