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Bone marrow mesenchymal stem cells attenuate silica-induced pulmonary fibrosis via paracrine mechanisms

Xiaoli Li[a,b,1], Yan Wang[a,b,1], Guoliang An[a,b], Di Liang[c], Zhonghui Zhu[a,b], Ximeng Lian[a,b], Piye Niu[a,b], Caixia Guo[a,b], Lin Tian[a,b,*]

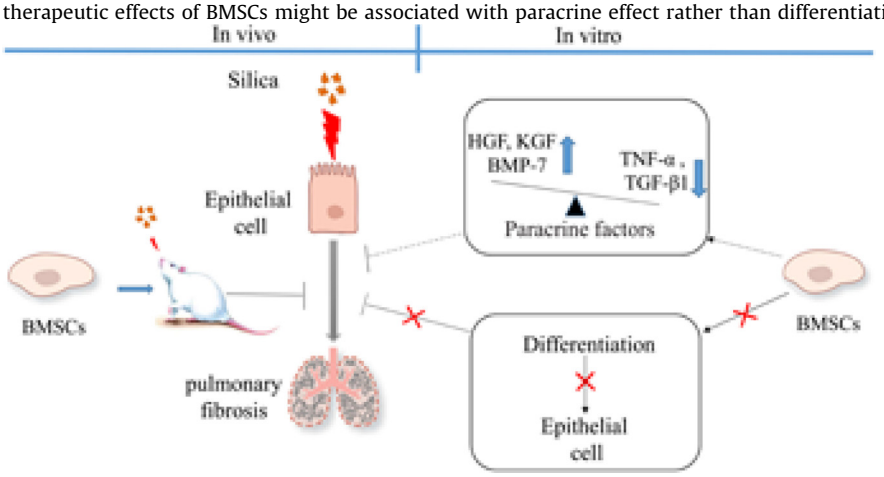
[a] School of Public Health, Capital Medical University, Beijing 100069, China
[b] Beijing Key Laboratory of Environmental Toxicology, Capital Medical University, Beijing 100069, China
[c] Beijing Children's Hospital, Capital Medical University, Beijing, China

HIGHLIGHTS

- BMSCs attenuat silica-induced fibrosis and reduce the injury of alveolar epithelial in vivo and in vitro.
- BMSCs do not differentiate into alveolar epithelial cells.
- The effects of BMSCs might be associated with paracrine rather than differentiation.

GRAPHICAL ABSTRACT

BMSCs attenuated silica-induced pulmonary fibrosis and reduced the injury of alveolar epithelial. The therapeutic effects of BMSCs might be associated with paracrine effect rather than differentiation.



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ABSTRACT

The purpose of this study was to investigate the anti-fibrotic effect and possible mechanism of bone marrow mesenchymal stem cells (BMSCs) in silica-induced lung injury and fibrosis in vivo and in vitro. In vivo, rats were exposed to 50 mg/ml silica intratracheally. The rats were sacrificed on day 15 or day 30 after intravenous injection of BMSCs. Histopathological examination demonstrated that BMSCs decreased the blue areas of collagen fibers and the number of nodules. Alveolar epithelium was damaged by silica, but it was restored by BMSCs. In vitro, BMSCs co-cultured with RLE-6TN cells in 6-Transwell plates were evaluated to determine the possible mechanism. The results demonstrated that BMSCs downregulated the expression of collagen I and III. BMSCs reversed morphological abnormalities and reduced the proliferation of RLE-6TN cells. These data showed that BMSCs did not give rise to alveolar epithelial cells directly, while the levels of hepatocyte growth factor, keratinocyte growth factor and bone

Abbreviations: BMSCs, bone marrow mesenchymal stem cells; AECs, alveolar epithelial cells; ATI, alveolar epithelial type I; ATII, alveolar epithelial type II; FN, fibronectin; SP-C, surfactant protein-C; AQP-5, aquaporin-5; HGF, hepatocyte growth factor; KGF, keratinocyte growth factor; BMP-7, bone morphogenetic protein-7; TGF-β1, transforming growth factor-β1; TNF-α, tumor necrosis factor-α.

* Corresponding author at: Department of Occupational Health and Environmental Health, School of Public Health, Capital Medical University, Beijing 100069, China.
E-mail address: tian_lin@163.com (L. Tian).

[1] These authors contributed equally to this work.

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Differentiation
Paracrine

morphogenetic protein −7 increased and expression of tumor necrosis factor-α and transforming growth factor-β1 decreased in the 6TN + Silica + BMSCs group compared with the 6TN + Silica group. Our results revealed that BMSCs exerted anti-fibrotic effects on silica-induced pulmonary fibrosis, which might be associated with paracrine mechanisms rather than differentiation.

1. Introduction

Silicosis is an inflammatory and diffuse fibrotic lung disease caused by exposure to crystalline silica (Bhandary et al., 2015). It is a common occupational disease among workers who inhale silica particles at low levels but for long periods (Healy et al., 2014). Pathological features are represented by silicotic nodule formation and diffuse pulmonary fibrosis (Steenland and Ward, 2014). There are no effective treatments available for silicosis other than supportive care and allogeneic lung transplantation (Leung et al., 2012). Therefore, new therapies are desired to improve the prognosis of this serious disease.

Alveolar cells play a crucial role in lung tissue repair (Chu et al., 2013). Epithelial cell injury accelerates the fibrotic process. Therefore, any method promoting the proliferation or replenishment of destroyed alveolar epithelial cells could ameliorate pulmonary fibrosis. The alveolar surface of the mammalian lung is covered by an epithelium consisting of two main cell types, alveolar epithelial type I (ATI) and alveolar epithelial type II (ATII) cells (Spitalieri et al., 2012). ATI cells cover most of the internal surface area of the lung (95%) and are important for gas exchange and the regulation of alveolar fluid balance (McElroy and Kasper, 2004). ATII cells are situated between ATI cells and produce pulmonary surfactant (Barkauskas and Noble, 2014). ATI cells are injured during the early period when the lung is constantly exposed to crystalline silica. Then, ATII cells proliferate and differentiate into new ATI cells (Fehrenbach, 2001). However, the proliferative capability of ATII cells is limited. Lung tissue is destroyed when the recovering ability of ATII cells is exhausted, which can also lead to lung structure distortion and respiratory dysfunction. Subsequently, aberrant lung repair and fibroblast activation promote the pulmonary fibrosis process (Zoz et al., 2011).

Numerous studies have reported beneficial effects of mesenchymal stem cells (MSCs), including direct tissue regeneration and modulatory functions in damaged tissues. MSCs are multipotent adult cells that can be isolated from various tissues. They have the ability to differentiate into various kinds of functional parenchymal cells, including chondroblasts, adipocytes, and osteoblasts (Bianco et al., 2013). Importantly, MSCs possess a strong immunosuppressive ability and low immunoreactivity, which makes them safe for transplantation as autografts or allografts to treat multiple diseases (Abumaree et al., 2012). In addition, the therapeutic potential of MSCs is based on their immunomodulatory, proangiogenic, cytoprotective, and anti-fibrotic properties in tissue repair and regeneration (Griffin et al., 2013). It has been reported that the management of various diseases with MSCs involves two probable mechanisms: cell differentiation and paracrine effects. Systemically transplanted exogenous MSCs migrate to damaged lung tissue, differentiate into alveolar epithelial cells, and alleviate lung injury (Xue et al., 2013). MSCs have been shown to differentiate into ATII cells during bleomycin (BLM)-induced fibrosis, suggesting that the primary mechanism of MSCs may be associated with the differentiation underlying their therapeutic action (Huang et al., 2015). However, the low engraftment rate of MSCs in the injured areas appears to be inadequate to explain their therapeutic benefit. Furthermore, exogenously administered MSCs have a poor survival rate and fail to permanently engraftment in t infarcted areas. In recent years, much attention has been focused on MSCs because of their paracrine effects that promote the repair of damaged tissues. MSCs secrete several soluble cytokines and growth factors that modulate the regeneration of tissues in lung injury models (Conese et al., 2013).

Although the tissue repair function of MSCs has been demonstrated in diverse lung injury models, it is unclear whether MSCs can inhibit lung fibrosis induced by silica. It is also unknown whether paracrine-mediated or regenerative therapeutic effect is involved in ameliorating silica induced pulmonary fibrosis. Bone marrow mesenchymal stem cells (BMSCs) are the most frequently studied MSCs (Heise et al., 2016). RLE-6TN cells, which were derived from rat ATII cells, were used for silica-induced fibrosis models in our study. We aimed to verify the anti-fibrotic effects and probable mechanism of action of BMSCs.

2. Materials and methods

2.1. Silica

Crystalline silica particles were purchased from Sigma (St. Louis, MO, USA). The silica samples were suspended in saline at a final concentration of 50 mg/ml for in vivo experiments and 2 mg/ml for in vitro experiments. The silica particle suspension was sonicated and dispersed vigorously by a vortex shaker prior to instillation in rats (Zhu et al., 2013).

2.2. Preparation of primary rat BMSCs cultures

Specific pathogen-free male Wistar rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Bone marrow harvested from their femur and tibia was cultured in alpha-modified minimum essential medium (α-MEM; 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 with 5% CO_2. After 72 h, non-adherent cells were removed by a medium change. Differentiation of BMSCs was performed over 21 days in differentiation media for adipocytes or osteocytes in vitro. Passage 3–5 BMSCs were used for all experiments.

2.3. Animals and experimental design

Ten adult male and sixty female Wistar rats (specific pathogen-free class, weighing 200–240 g) were obtained from Vital River Laboratory Animal Technology Co. Ltd. Animals were maintained in specific pathogen-free conditions (24 ± 1 °C, 12:12 h light/dark cycles) and allowed free to water and chow. All animal procedures were approved by the Laboratory Animal Care and Use Committee at Capital Medical University and carried out in strict accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

The sixty female Wistar rats were divided into three groups (n = 20 each), control, silica and BMSCs groups. Rats were intratracheally administered with 1 ml silica suspension (50 mg/ml/rat) except for the control group that received 1 ml saline after anesthesia induced by diethyl ether. The rats in control, silica, and BMSCs groups were intravenously injected with 1 ml saline, 1 ml