[Frontiers in Bioscience 13, 3415-3422, May 1, 2008]

Mesenchymal stem cells transplantation protects against rat pulmonary emphysema

Guohua Zhen, Hongmei Liu, Naibing Gu, Huilan Zhang, Yongjian Xu, Zhenxiang Zhang

Division of Respiratory Diseases, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430030, China; Key Laboratory of Respiratory Diseases, Ministry of Health, China

TABLE OF CONTENTS

1. ABSTRACT

Pulmonary emphysema is characterized by loss of alveolar structure. Bone marrow mesenchymal stem cells (MSCs) have been shown to differentiate into alveolar epithelial cells. However, the effect of MSCs transplantation on pulmonary emphysema is unknown. To address this question, cultured bone marrow MSCs from male donor rats were infused into female recipients treated with irradiation and instillation of papain. We found that the emphysematous changes in rats received MSCs transplantation were ameliorated when compared with the rats without MSCs transplantation. Y chromosome fluorescent *in situ* hybridization (FISH) and immunohistochemical staining for SP-C, confirmed that MSCs engrafted in recipient lungs and differentiated into type II alveolar epithelial cells. Additionally, MSCs transplantation reduced the extent of irradiation and papain-induced alveolar cell apoptosis, likely due to the up-regulation of the expression of *Bcl-2* and *Bax* gene. We conclude that MSCs transplantation protects against the irradiation and papain-induced pulmonary emphysema. The mechanisms of protection may involve the engraftment of MSCs in the lungs, differentiation of MSCs into type II alveolar epithelial cells and suppression of alveolar cell apoptosis.

2. INTRODUCTION

Chronic obstructive pulmonary disease (COPD) is a significant global health problem. The key pathologic change of COPD is pulmonary emphysema which is characterized by the loss of alveolar wall and enlargement of alveolar space. Pulmonary emphysema is considered as an irreversible pathological process. So far, there is no effective therapy for pulmonary emphysema.

Bone marrow stem cells play an important role in tissue repair. Bone marrow stem cells are divided into at least two populations, hematopoietic stem cells (HSCs) and MSCs. HSCs are nonadherent cells negative for lineage-specific markers (1). MSCs are plastic adherent cells which are also referred to as marrow stromal cells or multipotent mesenchymal stem cells (2). Bone marrow MSCs are capable of differentiating into a variety of cell types, including endothelial, epithelial, neuronal cells and adipocytes (3, 4). In the lungs, bone marrow MSCs has been shown to differentiate into type I alveolar epithelial cells (5), type II alveolar epithelial cells (6, 7), and bronchial epithelial cells (8, 9). This makes bone marrow MSCs as a potential cellular source for clinical applications in the regeneration of lung. However, the effect of bone marrow MSCs transplantation on pulmonary emphysema

remains unclear. Recently, bone marrow MSCs transplantation has been reported to ameliorate bleomycin-induced lung fibrosis (7, 9), and restore the cystic fibrosis lung epithelium (6). These studies raise the possibility that bone marrow MSCs transplantation may be developed to an effective intervention of pulmonary emphysema.

To determine the effect of bone marrow MSCs transplantation on the pathogenesis of pulmonary emphysema and its possible mechanisms, we instilled papain intratracheally to produce rat model of pulmonary emphysema, and in one experimental group, rats were exposed to Co60 irradiation before the intratracheal instillation of papain. After the treatment with papain and / or irradiation, female rats were infused with male rat bone marrow MSCs through tail vein. 28 d after MSCs transplantation, we compared the morphology of the lungs of rats which received irradiation and instillation of papain with the lungs of rats which received bone marrow MSCs transplantation after irradiation and papain treatment. We also determined the engraftment and differentiation of bone marrow MSCs in the lungs by Y chromosome FISH and immunohistochemistry. Moreover, we examined the effect of bone marrow MSCs transplantation on alveolar cell apoptosis in the lungs treated with irradiation and papain.

3. MATERIALS AND METHODS

3.1. Isolation and expansion of rat bone marrow MSCs

Bone marrow cells were collected by flushing the femurs and tibias from 3-month-old male Lewis rats (Vital River, China) with Dulbecco's modified Eagle's medium (DMEM; Hyclone, USA) containing 1% penicillin-streptomycin. The DMEM media containing bone marrow cells was added on the Percoll gradient (Pharmacia, Sweden) and centrifuged at 2,000 rpm for 30 min. The mononuclear cells in the medium portion was harvested and added to DMEM containing 10% fetal bovine serum (FBS; Gibco, USA). The cells were plated at a density of 10^6 cells/cm^2 in DMEM containing 10% FBS and cultured at 37℃ in 5% CO_2 incubator. After 24 h, non-adherent cells were removed by changing the medium. Then the medium was changed every 3-4 d. The passage number of cultured MSCs refers to the number of times that the cells had been trypsinized. MSCs of passage 2 and passage 3 were used in the experiments.

3.2. Papain-induced pulmonary emphysema and bone marrow MSCs transplantation

Female recipient rats were anesthetized and exposed to Co60 irradiation (7.5 Gy). Immediately after that, rats were treated with intratracheal instillation of papin (Sigma, USA; 8% in PBS) or PBS alone (0.05ml / 100g body weight). Then cultured bone marrow MSCs (4×10^6 in 0.4 ml PBS) were infused through tail vein. At day 28 after MSCs infusion, rats were sacrificed.

3.3. Morphologic analysis of the lungs

Lungs were fixed for 5 min by instillation of 4% paraformaldehyde-PBS (Sigma, USA) through a tracheal catheter at a transpulmonary pressure of 15 cmH_2O. Then the harvested lungs were fixed in 4% paraformaldehyde-PBS overnight. Fixed lungs were embedded in paraffin and sectioned by standard methods. Sections were processed for staining with hematoxylin and eosin. The extent of emphysematous changes was assessed by measuring the mean linear intercept (MLI) using the method of Thurlbeck with modification (10). Briefly, MLI was obtained by dividing the total length of all lines in the frames counted by the total number of intercepts encountered in the counted lines. A minimum of 10 fields at 100 × magnification, 200 intercepts for two sections in each rat were measured. Each counting was performed by three independent observers who were unaware of the subjects' profiles.

3.4. Y chromosome fluorescent *in situ* hybridization (FISH)

PCR product of *Sry* gene was used to prepare the Y chromosome probe. The primers used to amplify the rat *Sry* gene were 5'-AAT TCA GAG ATC AGC AAG CAG -3' and 5'-TGC AGC TCT ACT CCA GTC TTG-3'. Genomic DNA isolated from male rat lungs was used for amplification. The PCR conditions were incubation at 94°C for 5 min; 30 cycles of incubation at 95°C for 45 s, 55.6°C for 45 s, and 72°C for 1 min; followed by a final incubation at 72°C for 8 min. The PCR products were resolved by 1.2% agarose gel electrophoresis and sequenced to confirm that it was identical to the published sequence. Biotin DecaLabel DNA Labeling Kit (Fermentas, Lithuania) was used to label the Y chromosome probe. The probe was denatured at 95°C for 5 min and immediately put on ice. Female recipient lung paraffin sections were deparaffinized and rehydrated. Then they were digested with protease K and washed with 4 × SSC at room temperature. The sections were incubated at 75℃ for 5 min and immediately dehydrated through gradient ethanol series (70%, 90%, and 100%; 2 min each). Samples were air dried at room temperature and then overlaid with denatured probe. The sections were covered with a cover slip and incubated at 37°C overnight. Sections were washed twice in 2 × SSC for 10 min at 70°C and then twice in 0.1 × SSC for 10 min at 70°C. Slides were incubated in 4 × SSC containing 0.1% Tween-20 for 3 min at room temperature, then a 1:400 dilution of avidin-FITC (Santa Cruz Biotechnology, USA) in 4 × SSC containing 1% BSA, and 0.1% Tween-20 was added. Slides were incubated at 37°C for 60 min in a humidified chamber before washing with three changes of 4 × SSC containing 0.1% Tween-20 for 3 min at room temperature. Sections were counterstained with DAPI before visualization by fluorescence microscopy. The percentage of Y chromosome-positive cells was calculated by dividing the number of Y chromosome-positive cells by the number of DAPI-positive cells in twenty fields at 1,000 × magnification randomly sampled in two sections for each rat in different groups.

3.5. Terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling (TUNEL)

TUNEL was performed on the lung sections with commercially available kit (Roche, USA), following the manufacturer's instructions. Briefly, after deparaffinization and rehydration, sections were digested with proteinase K at a concentration of 20 mg/ml for 15

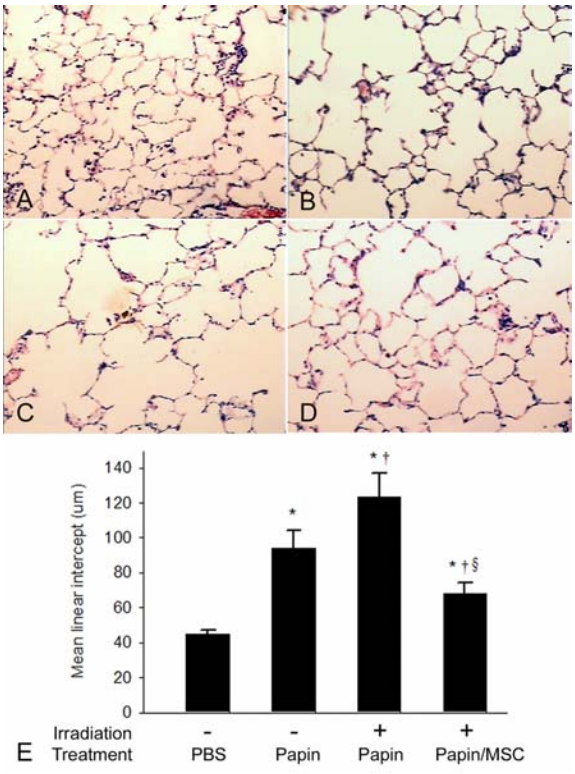

Figure 1. Bone marrow MSCs transplantation protects against the irradiation and papain-induced pulmonary emphysema. Representative lung sections stained with hematoxylin and eosin were chosen for each group. Compared with the lung section from control group treated with intratracheal instillation of PBS (A), lung section from the group treated with intratracheal instillation of papin exhibited emphysematous changes (B). The emphysematous changes were exacerbated in the group exposed to irradiation before instillation of papain (C). Ameliorated emphysematous changes were observed in the group received infusion of MSCs after irradiation and papain treatment (D). (E) The value of MLI in the group received MSCs transplantation was significantly less than that of the group treated with papain or the combination of irradiation with papain. Data are means (± SEM) for six rats in each group. * $p < 0.01$ compared with control group. † $p < 0.01$ compared with the group treated with papain. § $p < 0.01$ compared with the group treated with irradiation and papain. The original magnification was × 100.

minutes. Endogenous peroxidase activity was quenched with 2% H_2O_2 for 5 minutes. The slides were immersed in terminal deoxynucleotidyl transferase (TdT) buffer. TdT, 1 mM Mn^{2+}, and biotinylated dNTP in TdT buffer were then added to cover the sections and incubated in a humid atmosphere at 37°C for 60 minutes. The slides were washed with PBS and incubated with streptavidin–horseradish peroxidase for 10 minutes. After rinsing with PBS, the slides were immersed in diaminobenzidine (DAB) solution. The slides were counterstained with hematoxylin. The percentage of TUNEL-positive cells was calculated by dividing the number of TUNEL-positive cells by the total cell number in twenty fields at 400 × magnification randomly sampled in two sections for each rat.

3.6. Immunohistochemistry for surfactant protein C (SP-C), Bcl-2 and Bax

Immunohistochemistry was performed on paraffin-embedded, formalin-fixed rat lungs. Briefly, after paraffin removal in xylene, the sections were rehydrated and submitted to microwave treatment (800 W/15 min) in 10 mM citric acid monohydrate solution. After quenching of endogenous peroxidase with 3% H_2O_2 for 20 minutes, the sections were exposed to anti-SP-C antibody (1:500; Abcam, USA), anti-Bcl-2 antibody (1:100 dilution; Zhongshan, China) and anti-Bax antibody (1:100 dilution; Zhongshan, China) for 30 minutes. After incubation with the primary antibody, immunodetection was performed using biotinylated anti-rabbit or anti-mouse IgG. Finally, peroxidase-conjugated streptavidin and DAB (Vector Laboratories, USA) were added. For SP-C immunofluorescent staining, anti-rabbit TRITC secondary antibody (Santa Cruz Biotechnology, USA) was used. Negative controls for nonspecific binding included normal rabbit or mouse serum. The percentage of Bcl-2 or Bax-positive cells was calculated by dividing the number of Bcl-2 or Bax-positive cells by the total cell number in twenty fields at 400 × magnification randomly sampled in two sections for each rat in different groups.

3.7. Statistical analysis

Data are presented as mean ± SEM. Student's *t* test was used for comparisons between groups. Statistical difference was accepted at $p < 0.05$.

4 RESULTS

4.1. Rat bone marrow MSCs transplantation protected against irradiation and papain-induced rat pulmonary emphysema

To examine the effect of bone marrow MSCs transplantation on the pathogenesis of pulmonary emphysema, we instilled papain intratracheally to induce pulmonary emphysema, and in one experimental group, rats were exposed to Co60 irradiation before the intratracheal instillation of papain. After the treatment with papain and / or irradiation, female rats were infused with male rat bone marrow MSCs through tail vein. Animals were sacrificed 28 d after MSCs transplantation. We observed enlarged air spaces, the feature of pulmonary emphysema, in the lungs of rats treated with papain, and the emphysematous changes were exacerbated in the lungs of rats which were exposed to irradiation before papain treatment when compared with that in the group treated with papain only. However, the pulmonary emphysema was ameliorated in the lungs of rats which received male bone marrow MSCs transplantation after irradiation and papain treatment when compared with that in the groups treated with papain only or treated with irradiation and papain (Figure 1A – 1D). Morphologic assay showed that mean linear intercept (MLI), a parameter to quantitate the degree of alveolar enlargement, was significantly greater in the group treated with papain only (94.28 ± 10.11 micron) and

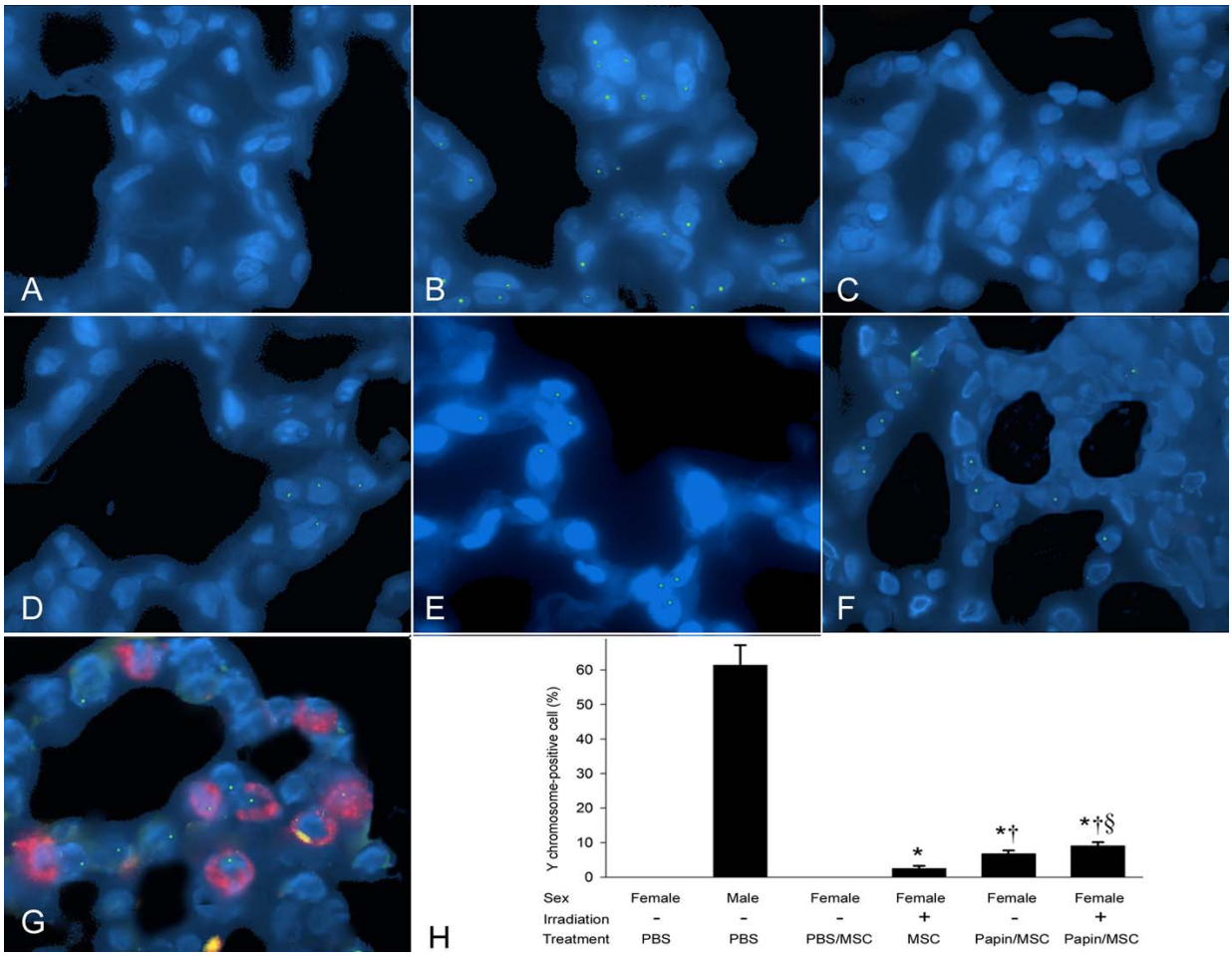

Figure 2. Bone marrow MSCs from male donor rats engraft in the lungs of female recipient rats and differentiate into type II alveolar epithelial cells. Engrafted male MSCs were detected by fluorescent *in situ* hybridization for Y chromosome (in green color). DAPI was used for counterstaining (in blue color). Y chromosome-positive cells were observed in the lungs of normal male rats (B) but not in normal female rats (A). No Y chromosome-positive cells were observed in the lungs from the group received MSCs transplantation after intrachacheal instillation of PBS (C). However, Y chromosome-positive cells were observed in the lungs from the group received MSCs transplantation after irradiation (D), the group received MSCs transplantation after papain treatment (E) and the group received MSCs transplantation after both of irradiation and papain treatment (F). (G) After Y chromosome FISH and SP-C immunohistochemistry were performed on the same lung section. It was observed that some of the Y chromosome-positive cells were also positive for SP-C staining (in red color). (H) The percentage of Y chromosome-positive cells was calculated by dividing the number of Y chromosome-positive cells by the number of DAPI-positive cells in twenty fields at 1,000 × magnification randomly sampled in two sections for each rat. Data are means (± SEM) for six rats in each group. * $p < 0.01$ compared with the group received MSCs transplantation after PBS treatment. † $p < 0.01$ compared with the group received MSCs transplantation after irradiation. § $p < 0.05$ compared with the group received MSCs transplantation after papain treatment. The original magnification was ×1,000.

the group treated with irradiation and papain (123.14 ± 13.84 micron) when compared with the control group (44.97 ± 2.36 micron). However, the MLI was significantly reduced in the group received MSCs transplantation (68.20 ± 5.82 micron) when compared with the group treated with papain only or the group treated with irradiation and papain (Figure 1E). Our results indicate that irradiation exacerbates the papain-induced pulmonary emphysema, and transplantation of male bone marrow MSCs protects against the rat pulmonary emphysema induced by irradiation and intratracheal instillation of papain.

4.2. Male bone marrow MSCs engrafted in the lungs of female recipient rats and differentiated into type II alveolar epithelial cells

To investigate the mechanism by which MSCs transplantation protects against the pulmonary emphysema induced by irradiation and papain treatment, we first examined whether the male MSCs engrafted in the lungs of female recipient rats. By Y chromosome FISH, we found that there were Y chromosome-positive cells in the lungs of normal male control rats but no Y chromosome-positive cell in the lungs of normal female control rats (Figure 2A

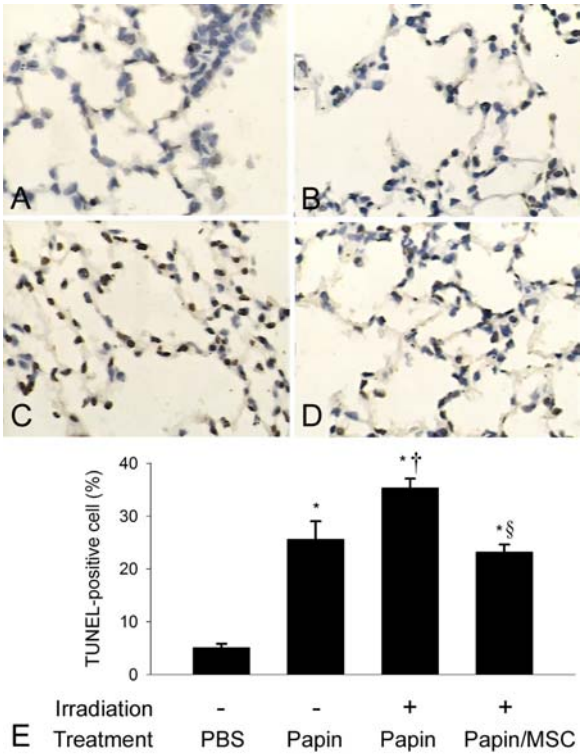

Figure 3. The effects of irradiation, papain treatment and bone marrow MSCs transplantation on rat lung alveolar cell apoptosis. TUNEL assay was performed to detect the apoptotic alveolar cells (in deep brown color). Hematoxylin was used for counterstaining. Very few apoptotic cells were observed in the lungs of rats received intratracheal instillation of PBS (A). More apoptotic alveolar cells were observed in the lungs of rats treated with intratracheal instillation of papain (B), or in the lungs of rat treated with irradiation and papain (C). There were fewer apoptotic alveolar cells in the lungs of rats received MSCs transplantation after irradiation and papain treatment when compared with the group treated with irradiation and papain (D). (E) The percentage of TUNEL-positive cells was calculated by dividing the number of TUNEL-positive cells by the total cell number in twenty fields at 400 × magnification randomly sampled in two sections for each rat. Data are means (± SEM) for six rats in each group. * $p < 0.01$ compared with control group. † $p < 0.01$ compared with the group treated with papain. § $p < 0.01$ compared with the group treated with irradiation and papain. The original magnification was ×400.

2B). No Y chromosome-positive cells were observed in the lungs of female rats which received male bone marrow MSCs transplantation after intratracheal instillation of PBS (Figure 2C). However, we found that there were Y chromosome-positive cells in the lungs of female rats which received MSCs transplantation after irradiation alone (Figure 2D) or papain treatment alone (Figure 2E). Moreover, we found that there were Y chromosome-positive cells in the lungs of female rats which received MSCs transplantation after irradiation and papain treatment (Figure 2F). These findings indicate that the male MSCs engrafted in the lungs of the female recipient rats which were injured by irradiation alone, papain alone or by the combination of irradiation with papain. Furthermore, we found that there were more Y chromosome-positive cells engrafted in the lungs of female rats which received MSCs transplantation after treatment with both papain and irradiation (9.03 ± 1.13%) when compared with those received MSCs transplantation after irradiation alone (2.53 ± 0.71%) or papain alone (6.82 ± 0.93%) (Figure 2H). Therefore, preexisting injury such as irradiation or papain treatment is associated with the engraftment of bone marrow MSCs in the lungs and the extent of bone marrow MSCs engraftment is associated with the types of injuries to the lungs.

Next we asked whether the engrafted male MSCs could differentiate into type II alveolar epithelial cells. To answer this question, we did Y chromosome FISH and SP-C staining in the same lung sections from the female recipient rats which received male bone marrow MSCs transplantation after irradiation and papain treatment. We found that part of the Y chromosome-positive cells were also positive for SP-C staining (4.33 ± 0.16 %) (Figure 2G). Therefore, the engrafted MSCs can differentiate into type II alveolar epithelial cells in the lungs of recipient rat treated with irradiation and papain.

4.3. MSCs transplantation prevented alveolar cell apoptosis in the lungs of rats treated with irradiation and papain

Besides the bone marrow MSCs' engraftment and differentiation into type II alveolar epithelial cells in the recipient lungs, we sought to find other mechanisms that may contribute to the prevention of pulmonary emphysema by MSCs transplantation. It has been reported that apoptosis is involved in the development of emphysema (11-13). To determine whether apoptosis is also involved in the pulmonary emphysema induced by irradiation and papain, and the effects of MSCs transplantation on cell apoptosis, we performed the TUNEL assay on the lungs sections. We observed that there were more apoptotic alveolar cells in the lungs of rats treated with papain (25.58 ± 3.42%) when compared with the control *(*5.03 ± 0.77%). There were even more apoptotic alveolar cells in the lungs of rats treated with both irradiation and papain (35.33 ± 1.75%) when compared with the group treated with papain only. However, in the group which received MSCs transplantation after irradiation and papain treatment there were less apoptotic alveolar cells (23.16 ± 1.47%) when compared with the group treated with irradiation and papain (Figure 3). Our results indicate that alveolar cell apoptosis is involved in the pulmonary emphysema induced by irradiation and papain. MSCs transplantation prevents the alveolar cell apoptosis induced by irradiation and papain. This could be another mechanism by which bone marrow MSCs transplantation protects against pulmonary emphysema.

We also examined the expression of the apoptosis associated gene, *Bcl-2* and *Bax,* in the alveolar cells. *Bcl-2* is an antiapoptosis gene and *Bax* is an apoptosis gene (14, 15). By immunohistochemichal staining, we

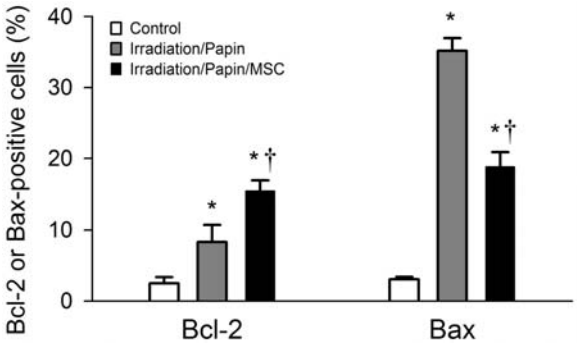

Figure 4. Bone marrow MSCs transplantation increases the expression of Bcl-2 and decreases the expression of Bax. Immunohistochemistry was performed to detect the expression of Bcl-2 and Bax. The percentage of Bcl-2 or Bax-positive cells was calculated by dividing the number of Bcl-2 or Bax-positive cells by the total cell number in twenty fields at 400 × magnification randomly sampled in two sections for each rat. Data are means (± SEM) for six rats in each group. * $p < 0.01$ compared with control group. † $p < 0.01$ compared with the group treated with irradiation and papain.

found that the number of Bcl-2 and Bax-positive cells was significantly greater in the group treated with both irradiation and papain (8.33 ± 2.41%, 35.16 ± 1.82%) when compared with those of the control group (2.50 ± 0.84, 3.08 ± 0.34). In the lungs from rats which received bone marrow MSCs transplantation after papain and irradiation treatment, the number of Bcl-2-positive cells (15.50 ± 1.51%) was significantly greater than that in the group without MSCs transplantation after papain and irradiation treatment. In contrast, the number of Bax-positive cells in the group received MSCs transplantation after irradiation and papain treatment (18.85 ± 2.04%) was significantly less than that in the group without MSCs transplantation (Figure 4). These findings indicate that irradiation and papain treatment increases the expression of *Bcl-2* and *Bax* in the lungs, and that MSCs transplantation further increases the expression of *Bcl-2* but reduces the expression of *Bax* in the lungs of rats treated with irradiation and papain.

5. DISCUSSION

In this study, for the first time, we demonstrate that transplantation of bone marrow MSCs protects against irradiation and papain-induced rat pulmonary emphysema. In the investigation of the mechanisms by which transplantation of exogenous MSCs prevents the development of pulmonary emphysema, we show that MSCs engraft in the recipient lungs and differentiate into type II alveolar epithelial cells. We also show that MSCs transplantation prevents cell apoptosis induced by irradiation and papain at least partly through the regulation of *Bcl-2* and *Bax* gene expression.

First of all, we show that administration of exogenous male bone marrow MSCs has protective effect against the female rat pulmonary emphysema induced by irradiation and papain. In support of this, we found that 28 d after the infusion of male rat MSCs into female recipients which were treated with irradiation and papain, the pulmonary emphysema was significantly ameliorated when compared with the rats which did not receive MSCs transplantation. Ishizawa and colleagues reported that all-trans retinoic acid and granulocyte colony-stimulating factor improved the elastase-induced pulmonary emphysema by inducing endogenous bone marrow cells mobilize into the lungs (16). Different to their study, we systemically administered exogenous bone marrow MSCs into recipient rats. In addition to our findings that MSCs transplantation protects against pulmonary emphysema, MSCs transplantation has also been shown to have functional implications in mouse models of lung fibrosis and cystic fibrosis. Reports in the literature indicate that transplantation of MSCs protects against bleomycin-induced lung fibrosis, and that the engrafted MSCs differentiate into alveolar epithelial cells (7, 9). Roberto and colleagues showed that after the transplantation of MSCs obtained from wild-type mice into cystic fibrosis transmembrane conductance regulator protein (CFTR) knockout mice, MSCs can be recruited to airway epithelium and induced to express CFTR in the recipient CFTR knockout mice (6). These studies and ours suggest that bone marrow MSCs are promising cellular source for clinical application of lung regeneration.

In our experiments to investigate the mechanisms by which MSCs transplantation protects against the irradiation and papain-induced emphysema, we demonstrate that MSCs from male donors engraft in the lungs of female recipients treated with irradiation or papain or the combination of irradiation with papain. The reason for the engraftment of exogenous MSCs in the irradiation and / or papain-treated lungs remains unclear. One possibility is that irradiation and papain damage the lungs including the stem cell population and stem cell niches, and chemotactic signals released by injured lungs attract bone marrow MSCs into the lung (17-19). The type and degree of injury to the lungs are thought to be critical initiating factors for the recruitment of MSCs into lungs. There were two forms of injuries in our study, irradiation and papain treatment. Irradiation alone does not induce emphysematous changes (data not shown). However, we demonstrate that irradiation alone leads to the engraftment of MSCs into the lungs. This could be attributed to the direct damage of irradiation to the lungs, and the suppression of the recipient bone marrow which inhibits the recruitment of endogenous bone marrow stem cells into the injured lung (20). Papain is a proteolytic enzyme which has broad proteolytic activity. Its enzymatic attack on lung proteins results in dilation of air spaces (21). We also show that papain treatment alone results in pulmonary emphysema, and that irradiation exacerbates the emphysematous changes induced by instillation of papain. The combination of irradiation with papain reinforces the damage to the lungs, which could explain the exacerbated emphysematous changes in the lungs treated with irradiation and papain, and the more engraftment of MSCs in the lungs of rats revceived MSCs transplantation after irradiation and papain when compared with the lungs treated with papain only.

Furthermore, we demonstrate that the engrafted MSCs can differentiate into type II alveolar epithelial cells. By performing Y chromosome FISH and SP-C immunohistochemistry on the same lung sections from rat which received MSCs transplantation after irradiation and papain treatment, we found that some of the Y chromosome-positive cells also expressed SP-C. Our finding is consistent with other studies which also demonstrate that in the recipient mice lung the engrafted MSCs differentiate into type II alveolar epithelial cells (6, 7). However, it is controversial regarding into which type of lung cells the MSCs can differentiate. Kotton and colleagues reported that the engrafted MSCs cells only differentiate into type I alveolar epithelial cells in the bleomycin-injured mice lungs (5). Anjos-Afonso and colleagues showed that engrafted MSCs obtained the phenotype of respiratory bronchial epithelial cells (8). Rojas and colleagues showed that MSCs differentiate into type I alveolar epithelial cells, type II alveolar epithelial cells, fibroblast and myofibroblast (9). This controversy could be attributed to different culture conditions of MSCs, different types of injuries and different methods used to identify the phenotype of the engrafted MSCs in these studies.

Recently, apoptosis has been shown to play an important role in the pathogenesis of pulmonary emphysema (11-13). Kasahara and colleagues reported that inhibition of VEGF receptors resulted in apoptosis and pulmonary emphysema (12). Consistent with this report, we demonstrate that irradiation and / or papain treatment induce alveolar cell apoptosis in rat lungs. Interestingly, our study indicates that MSCs transplantation ameliorates the alveolar cell apoptosis in the lungs of the rats which received MSCs transplantation after irradiation and papain treatment. This could be another mechanism by which MSCs transplantation protects against the irradiation and papain-induced pulmonary emphysema. However, the reason why MSCs transplantation ameliorates the irradiation and papain-induced apoptosis is not clear. One possibility is that MSCs transplantation changes the expression of apoptosis or antiapoptosis genes. As we demonstrate in this study, the expression of apoptosis gene *Bax* is decreased and the expression of anti-apoptosis gene *Bcl-2* is increased in the lungs of rats received MSCs transplantation after irradiation and papain treatment.

In conclusion, bone marrow MSCs transplantation protects against the pulmonary emphysema induced by irradiation and papain treatment. This could be attributed to the MSCs' engraftment in the recipient lung and differentiation into type II alveolar epithelial cells in the lungs treated with irradiation and papain, and that MSCs transplantation also ameliorates the alveolar cell apoptosis induced by irradiation and papain. So far, there is no effective therapy for pulmonary emphysema. Our study suggests that bone marrow MSCs transplantation could be a potential therapy for pulmonary emphysema.

6. ACKNOWLEDGEMENTS

This work was supported by grants from the National Natural Science Foundation of China (No. 30400193, 30500224 and 30770941)

7. REFERENCES

1. Herzog, E. L., L. Chai & D. S. Krause: Plasticity of marrow-derived stem cells. *Blood*, 102, 3483-93 (2003)
2. Horwitz, E. M., K. Le Blanc, M. Dominici, I. Mueller, I. Slaper-Cortenbach, F. C. Marini, R. J. Deans, D. S. Krause & A. Keating: Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy*, 7, 393-5 (2005)
3. Prockop, D. J.: Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science*, 276, 71-4 (1997)
4. Jiang, Y., B. N. Jahagirdar, R. L. Reinhardt, R. E. Schwartz, C. D. Keene, X. R. Ortiz-Gonzalez, M. Reyes, T. Lenvik, T. Lund, M. Blackstad, J. Du, S. Aldrich, A. Lisberg, W. C. Low, D. A. Largaespada & C. M. Verfaillie: Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature*, 418, 41-9 (2002)
5. Kotton, D. N., B. Y. Ma, W. V. Cardoso, E. A. Sanderson, R. S. Summer, M. C. Williams & A. Fine: Bone marrow-derived cells as progenitors of lung alveolar epithelium. *Development*, 128, 5181-8 (2001)
6. Loi, R., T. Beckett, K. K. Goncz, B. T. Suratt & D. J. Weiss: Limited restoration of cystic fibrosis lung epithelium *in vivo* with adult bone marrow-derived cells. *Am J Respir Crit Care Med*, 173, 171-9 (2006)
7. Ortiz, L. A., F. Gambelli, C. McBride, D. Gaupp, M. Baddoo, N. Kaminski & D. G. Phinney: Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. *Proc Natl Acad Sci U S A*, 100, 8407-11 (2003)
8. Anjos-Afonso, F., E. K. Siapati & D. Bonnet: *In vivo* contribution of murine mesenchymal stem cells into multiple cell-types under minimal damage conditions. *J Cell Sci*, 117, 5655-64 (2004)
9. Rojas, M., J. Xu, C. R. Woods, A. L. Mora, W. Spears, J. Roman & K. L. Brigham: Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol*, 33, 145-52 (2005)
10. Thurlbeck, W. M.: Measurement of pulmonary emphysema. *Am Rev Respir Dis*, 95, 752-64 (1967)
11. Demedts, I. K., T. Demoor, K. R. Bracke, G. F. Joos & G. G. Brusselle: Role of apoptosis in the pathogenesis of COPD and pulmonary emphysema. *Respir Res*, 7, 53 (2006)
12. Kasahara, Y., R. M. Tuder, L. Taraseviciene-Stewart, T. D. Le Cras, S. Abman, P. K. Hirth, J. Waltenberger & N. F. Voelkel: Inhibition of VEGF receptors causes lung cell apoptosis and emphysema. *J Clin Invest*, 106, 1311-9 (2000)
13. Tuder, R. M., L. Zhen, C. Y. Cho, L. Taraseviciene-Stewart, Y. Kasahara, D. Salvemini, N. F. Voelkel & S. C. Flores: Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. *Am J Respir Cell Mol Biol*, 29, 88-97 (2003)
14. N'Guessan, P. D., M. Vigelahn, S. Bachmann, S. Zabel, B. Opitz, B. Schmeck, S. Hippenstiel, J. Zweigner, K. Riesbeck, B. B. Singer, N. Suttorp & H. Slevogt: The UspA1 protein of Moraxella catarrhalis induces CEACAM-1-dependent apoptosis in alveolar epithelial cells. *J Infect Dis*, 195, 1651-60 (2007)

15. Wu, C. H., H. H. Lin, F. P. Yan, C. H. Wu & C. J. Wang: Immunohistochemical detection of apoptotic proteins, p53/Bax and JNK/FasL cascade, in the lung of rats exposed to cigarette smoke. *Arch Toxicol*, 80, 328-36 (2006)
16. Ishizawa, K., H. Kubo, M. Yamada, S. Kobayashi, M. Numasaki, S. Ueda, T. Suzuki & H. Sasaki: Bone marrow-derived cells contribute to lung regeneration after elastase-induced pulmonary emphysema. *FEBS Lett*, 556, 249-52 (2004)
17. Gomperts, B. N., J. A. Belperio, P. N. Rao, S. H. Randell, M. C. Fishbein, M. D. Burdick & R. M. Strieter: Circulating progenitor epithelial cells traffic via CXCR4/CXCL12 in response to airway injury. *J Immunol*, 176, 1916-27 (2006)
18. Hattori, K., B. Heissig, K. Tashiro, T. Honjo, M. Tateno, J. H. Shieh, N. R. Hackett, M. S. Quitoriano, R. G. Crystal, S. Rafii & M. A. Moore: Plasma elevation of stromal cell-derived factor-1 induces mobilization of mature and immature hematopoietic progenitor and stem cells. *Blood*, 97, 3354-60 (2001)
19. Phillips, R. J., M. D. Burdick, K. Hong, M. A. Lutz, L. A. Murray, Y. Y. Xue, J. A. Belperio, M. P. Keane & R. M. Strieter: Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest*, 114, 438-46 (2004)
20. Yamada, M., H. Kubo, S. Kobayashi, K. Ishizawa, M. Numasaki, S. Ueda, T. Suzuki & H. Sasaki: Bone marrow-derived progenitor cells are important for lung repair after lipopolysaccharide-induced lung injury. *J Immunol*, 172, 1266-72 (2004)
21. Johanson, W. G., Jr. & A. K. Pierce: Effects of elastase, collagenase, and papain on structure and function of rat lungs *in vitro*. *J Clin Invest*, 51, 288-93 (1972)

Abbreviations: MSCs: mesenchymal stem cells; FISH: fluorescent *in situ* hybridization; COPD: chronic obstructive pulmonary disease; HSCs: hematopoietic stem cells; DMEM: Dulbecco's modified Eagle's medium; MLI: mean linear intercept; TUNEL: terminal deoxynucleotidyl transferase–mediated dUTP nick end-labeling; DAB: diaminobenzidine; SP-C: surfactant protein C; CFTR: cystic fibrosis transmembrane conductance regulator protein.

Key Words: Stem cells, Emphysema, Papain, Irradiation

Send correspondence to: Dr. Guohua Zhen, Department of Internal Medicine, Tongji Hospital, Huazhong University of Science and Technology, Wuhan, China, Tel: 86-13517277794, E-mail: guohuazhen@yahoo.com.cn