Bone marrow mesenchymal stem cells protect against radiation-induced lung injury by regulating AngII/ACE2/Ang(1-7) axis

Shiying Niu
Shandong Academy of Medical Sciences
Changsheng Cong
Jinan Central Hospital
Zhaopeng Wang
Shandong First Medical University
Meili Sun
Jinan Central Hospital
Yueying Zhang (zhangyueying828@126.com)
Shandong First Medical University https://orcid.org/0000-0002-5006-2483

Research Article

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Abstract

Background Radiation-induced lung injury (RILI) is one of the most common complications of thoracic tumors radiotherapy. Since therapeutic strategies remains limited, the exploration of new approaches to treat RILI is on high demands. The use of bone mesenchymal stem cells (BMSCs) to treat RILI holds great promise. Here, we investigate the therapeutic potential of BMSCs in RILI.

Methods C57BL/6 mice received thoracic irradiation except for the control group.Within 24 hours after irradiation, BMSCs were injected via the tail vein in BMSCs group. At 5 weeks after irradiation, H&E staining and immunohistochemistry were used to observe the pathological changes of lung tissue and the expression of inflammatory factors. Electron microscopy was used to detect changes in cell structure.Immunofluorescence and western blot were uesd to detect the expression of ACE2, ACE and AT1R.. The expression of Ang II and Ang (1-7) was detected by ELISA. The expression of MasR mRNA in lung tissue was detected by qRT-PCR.

Results we found that radiation obviously caused lung tissue injury, especially alveolar type II epithelial cells by H&E and electron microscopy. Immunofluorescence and western blot showed that ACE2 decreased significantly in irradiation group. After BMSCs injection, BMSCs significantly reduced RILI by H&E and immunohistochemistry. Immunofluorescence results showed that BMSCs migrated to injuried lung tissue and differentiated into alveolar type II epithelial cells to up-regulated AngII/ACE2/Ang(1-7) axis.

Conclusions The study demonstrated that BMSCs may be transplanted into damaged lung tissue where they differentiated into AEC II to regulated AngII/ACE2/Ang(1-7) axis to alleviate RILI.

Introduction

Over the last few decades, radiation therapy (RT) has been remained a cornerstone of treatment whether radical or palliative treatment for many malignancies [1]. Although technology improvements in radiotherapy, The occurrence of radiation-induced lung injury (RILI) is still inevitable [2]. It is mainly divided into two stages—radiation pneumonitis (RP) and radiation-induced pulmonary fibrosis (RIPF), which represents irreversible damage [3]. Due to the precise mechanism of RILI is not fully clear, there is still a lack of effective treatment [1]. Bone mesenchymal stem cells (BMSCs), a pluripotent stem cell, have the positive effect for regenerative medicine [4]. It has been reported that BMSCs can migrate to sites of tissue injury and release anti-apoptotic, anti-inflammatory and angiogenic factors to exert a series of effect [5]. Studies have showed that BMSCs may exert great therapeutic potential in several diseases, including acute lung injury (ALI), acute respiratory distress syndrome (ARDS) [1]. Therefore, these studies suggested that BMSCs might be a potential therapy in RILI.

RAS plays a significant role in modulating blood pressure homeostasis, as well as fluid and salt balance[6]. Whereas, there is a growing body of evidence suggested that it was also important in regulating inflammatory responses [7]. ACE2, a homologue of ACE and a negative regulator of the renin–

angiotensin system (RAS), downregulated the protein expression of angiotensin II through catalyzing the conversion of Ang II to angiotensin-(1–7) (Ang-(1–7)), which restrains the vasoconstrictive and inflammatory reaction [8]. Besides, research showed recombinant ACE2 significantly reduced the expression levels of inflammatory cytokines and protected from severe acute lung failure [9]. The RILI may occur due to a series of inflammatory reactions caused by cell damage. Alveolar epithelial cells, which are sensitive to irradiation, were the first to be damaged. Due to cell injury, the expression of ACE2 was decreased, which aggravated RILI. MSCs have potential as regenerative therapeutics due to differentiation potential, which are able to repair damage in a number of tissues.Previous studies have reported that up-regulation of the ACE2/Ang-(1–7)/Mas axis protected against sepsis-induced acute lung injury[10, 11, 12, 13]. However, detailed mechanism of this effect and the downstream molecular have not yet been fully elucidated.

Therefore, our study mainly discussed the mechanism of BMSCs protects against RILI. We showed that BMSCs could differentiate into alveolar II epithelial cells to regulate ACE2/Ang(1–7)/Mas axis to protect from RILI.

Methods

Isolation and culture of BMSCs

The BMSCs are a cell line obtained from rats thighbone. First, we took out the femur of rats with intact femoral head, washed the femur with 75% alcohol and double antibody, then removed the epiphyseal end and exposed the bone marrow cavity, finally the culture medium was used to wash the cells. BMSCs cells were cultured with OriCell Wistar Rat Bone Marrow Mesenchymal Stem Cell Medium supplemented with 10% OriCell Superior-Qualified Fetal Bovine Serum, Penicillin-Streptomycin and L-Glutamine (Cyagen Biosciences, China) at 37℃ in a humidified atmosphere of 5% CO2. Non-adherent cells were removed by changing the culture medium after incubation for 24 hours. The medium was replaced every 2-3 days. All the experiments in this study were carried out using 4th generation cells.

Identification of BMSCs

To identify whether they are BMSCs, Immunofluorescence was used to detect expression of CD34 and CD44 of BMSCs. The sections containing BMSCs were fixed with 4% paraformaldehyde for 30 min and blocked by 5% goat serum at 37℃ for 30 min. BMSCs were incubated with antibody CD34 and antibody CD44 at 4℃ overnight. Then BMSCs were incubation with Alexa Fluor 594 conjugated goat anti-rabbit IgG antibodies (1:50, #ZF-0516, ZSGB-BIO, Beijing, China) for visualization. The nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). The fluorescent signal was showed using 3D scanner (3DHISTECH, Hungary).

To test the multidifferentiation potential of BMSCs toward the osteogenic and adipogenic, the BMSCs were cultured in adipogenic and osteogenic differentiation medium (Cyagen Biosciences, China). After 4

weeks of differentiation induction, adipose cells were stained with Oil Red O and osteogenic cells were were stained with Alizarin Red. The stained cells were observated by a microscope.

Animals and Experimental models

45 male C57BL/6 mice with an average body weight of 20 g at 8 weeks of age purchased from Jinan pengyue experimental animal co., Ltd were utilized for all experiments. All animal procedures were approved by the Ethics Committee of The First Affiliated Hospital of Shandong First Medical University. Totally 45 Mice were evenly divided into three groups including control group, model group and BMSCs group with 15 mice in each group. Thorax irradiation was delivered to all except the mice from control group. The mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.04ml/10g) and exposed to chest irradiation at a dose rate of 2 Gy / min, with a total dose of 20 Gy. Within 24 hours after irradiation, the mice in BMSCs group were injected with BrdU labeled BMSCs (1×10^6 cells/0.1ml) via tail vein. All the mice were sacrificed 4 weeks after irradiation. The lung tissues were procured for histological and molecular biological analysis.

Animal sample collection

Four weeks after irradiation, the mice were anesthetized by intraperitoneal injection of 10% chloral hydrate (0.04ml/10g). Firstly, the eyeballs of the mice were removed and the blood was collected. The supernatant was collected by centrifugation and frozen at - 20 ℃. The left lung was cryopreserved at - 80 ℃ to prepare for the molecular experiment. The right lung was fixed in 10% formaldehyde for pathological experiment.

Histologic examination

H&E staining was performed to evaluate the pathological changes of lung tissue. Briefly, the lung tissues were fixed in 10% formaldehyde, paraffin-embedded and then sectioned into 4 μm slices (Leica, Germany). Then the nucleus and cytoplasm were stained with hematoxylin and eosin respectively, the neutral gum was used for sealing. All sections were observed under light microscope. Immunohistochemistry staining was used to detect expression of inflammatory factors in lung tissues. The paraffin-embedded lung tissue sections were dewaxed to water, repaired by EDTA for 15 min at 100℃, then incubated with 5% BSA (Solarbio, Beijing, China) for 30 min at 37℃. The slides were incubated with Interleukin 6 (IL-6) (1:100, #bs-0782R, Bioss, Beijing, China) antibody and Tumor necrosis factor α (TNF-α) (1;100, #bs-10802R, Bioss, Beijing, China) at 4 ℃ overnight. Subsequently, the slides were incubated with secondary antibody (1:200, #bs-0295D-HRP, Bioss, Beijing, China) for 60 min at 37℃. The slides were visualized by 3,3-diaminobenzidin (DAB). Finally, the slices were stained with hematoxylin, dehydrated with gradient alcohol, transparent with xylene and sealed with neutral gum. The positive signal was determined by a 3D scanner (3DHISTECH, Hungary).

Electron microscope sample preparation

The lung tissue fixed with 3% glutaraldehyde was rinsed with PBS for 5-6 times, 15min each time, and then fixed with 1% osmic acid in dark for 90min. PBS was rinsed twice, 15min each time, rinsed with ultrapure water for 3-4 times, 15min each time, and then dehydrated with gradient ethanol for 20min each stage. Pure acetone was used for 3 times, 20min each time. In order to gradually infiltrate the embedding agent into the tissue cells in the later stage, the infiltration treatment is carried out before embedding, mainly using acetone and resin 1:1 mixed and infiltrated for 1h, then acetone and resin 1:2 mixed and infiltrated for 4h or overnight, and pure resin infiltrated twice for 4H each time. After infiltration, put the tissue sample into both ends of the embedding plate, add pure resin, put it into the polymerization instrument, and perform the procedure of 37 ℃ 24h-45 ℃ 24h-60 ℃ 48h to form the resin block of the embedded sample. After that, trim the resin block, make ultra-thin sections with a thickness of 70nm, and fish the pieces to three aromatic membrane carrier nets; Dye with 2% uranyl acetate solution for 15min and lead citrate solution for 8min, wash with water, bake the sheet under infrared lamp for 10min, dry, observe with Hitachi ht-7800 transmission electron microscope at 80kV, and take photos at different magnification.

Cells and tissues Immunofluorescence

The BrdU-Labeled BMSCs and lung tissue sections were fixed with 4% neutral formaldehyde solution for 15 min, then denatured with 2m hydrochloric acid for 15 minutes, neutralized with sodium borate and washed with distilled water, followed with 0.1% Triton X-100 for 10 min and incubated with 5% BSA (Solarbio, Beijing, China) for 30 min at 37°C. The cells were then incubated with primary antibodies: mouse anti-BrdU antibody (1:100, #ZM-0013, ZSGB-BIO, Beijing, China) and rabbit anti-AQP5 (1:100; #bs-10927R, Bioss, Beijing, China) and rabbit anti-Pro-spc (1:200; #ab90716, Abcam, China) at 4 °C overnight. Subsequently, these cells were incubated with the corresponding goat anti-mouse IgG (H + L) secondary antibody, Alexa Fluor 488 ((1:50, #ZF-0512, ZSGB-BIO, Beijing, China) and goat anti-rabbit IgG(H + L) secondary antibody, Alexa Fluor 594 ((1:50, #ZF-0516, ZSGB-BIO, Beijing, China) for 60 min at 37°C. The cell nuclei were counterstained by DAPI. The cells stained with green fluorescence were counted as the BrdU-positive cell. The cells stained with red fluorescence were counted as the AQP5 and Pro-spc positive cell. BrdU positive and AQP5 positive or Pro-spc positive were positive signal. Immunofluorescence images were detected by a 3D scanner (3DHISTECH, Hungary).

Enzyme-linked immunosorbent assay (ELISA)

We used a commercially available mouse ELISA kits, according to the manufacturer's instructions, to detect the levels of AngII (CK-E21284, MLBIO, Shanghai, China) and Ang-(1-7) (CK-E20733, MLBIO, Shanghai, China) in lung tissues. The absorbance was read at 450 nm with a microplate reader.

qRT-PCR

Total RNA was extracted from freshly isolated lung tissues using Trizol reagent (Vazyme biotech co., Nanjing, China) according to the manufacturer's instructions. Then RNA was reverse-transcribed into cDNA with cDNA Synthesis Kit (Vazyme Biotech Co., Nanjing, China). Quantitative real-time PCR was

performed with SYBR qPCR Mix (Vazyme Biotech Co., Nanjing, China). The mRNA levels of MasR in lung tissues were examined by qRT-PCR. Data were calculated using the $2^{-\triangle\triangle t}$ method. MasR forward primer 5'-ACAACACGGGCCTCCTATCTG-3' and reverse primer 5'-GAAGGGCACAGACGAATGCT-3', β-actin forward primer 5'-GGCTGTATTCCCCTCCATCG-3' and reverse primer 5'-CCAGTTGGTAACAATGCCATGT-3'.

Western blots

Western blots was used to analyse the related proteins of ACE/ACE2/ Ang(1-7) and MAPK parhway in lung tissue. The lung tissues were lysed in RIPA lysis buffer (Beyotime, China) with protease inhibitors for 30 min at 4°C, followed by centrifugation at 12,000 rpm for 15 min at 4 °C. The supernatant will be collected and added to the loading buffer for denaturation. Protein samples were separated by 8-15% SDS-PAGE, then transferred to a PVDF membrane and blocked in 5% skim milk at room temperature for 90 min. The membranes were incubated with primary antibody including ACE (1:1000; #bs-0439R, Bioss, Beijing, China), ACE2 (1:1000, #21115-1-Ig, Proteintech, China), AT1R (1:1000; #bs-0630R, Bioss, Beijing, China), overnight at 4 °C and the corresponding secondary antibody at room temperature for 1h. Finally, proteins were visualized with ECL(Milipore, USA) and analyzed with Image J.

Statistical analysis

All data are shown as mean±SEM. Data was analyzed using SPSS 25 software. The measurements for this study were performed with one-way analysis of variance (ANOVA) for multiple groups. $P<0.05$ was considered statistically significant.

Results

The expression of ACE2 decreased in RILI

In order to verify the effect of irradiation on the expression of ACE2, we detected the changes of lung tissue structure and the expression of ACE2 in the RILI model. First we examined the pathological changes of lung tissue by H&E staining. The results demonstrated that compared with the control group, the capillaries were dilated and congested, inflammatory cells were infiltrated, and part of the alveolar septum was thickened in the irradiation group (Fig. 1A); Next,we further examined the changes of cells in alveolar structure by electron microscope. The results showed that alveolar epithelial cells and endothelial cells were vacuolized in varying degrees in the irradiation group, especially alveolar type II epithelial cells were seriously damaged. Reduced lamellar bodies, shrunken mitochondria and less mitochondrial cristae were observed in IR group compared to the control group (Fig. 1B). In order to verify the changes of ACE2, we detected the expression of ACE2 by immunofluorescence and western blot. The results showed that the expression of ACE2 in the irradiation group was significantly lower than that in the control group (Fig. 1C,1D). These results showed that irradiation reduced the expression of ACE2.

BMSCs were identified and did not express ACE2

BMSCs are cell lines isolated from the bone marrow cavity of rat femur. During the process of cell expansion, fibroblastic-shaped cells could be observed under microscope. Considering the survival rate and proliferation ability of BMSCs, the fourth generation of BMSCs will be used for phenotypic analysis and differentiation experiments. Next, to verify the osteogenic and adipogenic differentiation ability of BMSCs, we used osteogenic and adipogenic differentiation medium to culture BMSCs. Under osteogenic inducement, BMSCs synthesized matrix which were stained red by Alizarin red, which indicates formation of calcium. BMSCs displayed red droplets within the cytoplasm by oil red staining after adipogenic induction, indicating oil droplets deposition (Fig. 2A). Additionally, we identified the surface marker molecules of BMSCs by immunofluorescence. Immunofluorescence analysis showed that BMSCs were positive expression of CD44, but negative expression of CD34 (Fig. 2B). These results verifyed that BMSCs were successfully derivated. Since subsequent experiments will study the expression of ACE2, we used immunofluorescence and western blot to detect the expression of ACE2 in BMSCs. We used overpressed-ACE2 mouse lung tissues as positive control, the results showed that BMSCs did not express ACE2 (Fig. 2C,2D).

BMSCs homed to the lung tissues and ameliorated RILI

In order to verify the success of BrdU labeling, we used immunofluorescence technology to verify BrdU Labeled BMSCs. The results showed that BrdU Labeled BMSCs produced bright green fluorescence, which proved that BrdU-Labeled BMSCs successfully. Subsequently, BrdU-labeled BMSCs were injected into mice through tail vein with irradiation. Immunofluorescence technique was used to monitor BMSCs BrdU-labeling. BrdU-labeled BMSCs with irradiation group produced stronger green fluorescence signal after 4 weeks (Fig. 3A). To evaluate the therapeutic effects of BMSCs on RILI, we observed pathological changes in the lung tissues. Compared with the Control group, irradiation led to severe pathological changes in the lung tissues, including structural destruction, vasodilation and congestion, and infiltration of inflammatory cells. Pathological changes in the BMSCs group significantly alleviated. Similarly, we determined the protein expression levels of TNF-α and IL-6 in lung tissues, compared with the control groups, expression levels of TNF-α and IL-6 were dramatically increased under irradiated stimulation, suggesting radiation causes a profound inflammatory response, in which markedly reduces in brdu-labeled BMSCs with irradiation group (Fig. 3C). These results showed that BMSCs can home to the lung tissues and ameliorated RILI.

BMSCs regulated AngII/ACE2/Ang(1-7) axis

To verify whether BMSCs can increase the expression of ACE2 and regulate AngII/ACE2/Ang(1–7) axis after treatment, western blotting and Immunofluorescence were used to detect related proteins expression of AngII/ACE2/Ang(1–7) axis. Compared with the control group, the protein levels of AngII, ACE and AT1R were significantly upregulated (Fig. 4B,4C), but the protein levels of ACE2 and Ang(1–7) and the mRNA levels of MasR were remarkably downregulated in the irradiation group, which were reversed by BMSCs treatment (Fig. 4A,4B,4D,4E). Compared with the irradiation group, the protein levels of AngII, ACE and AT1R were significantly downregulated but the protein levels of ACE2 and Ang(1–7) and the mRNA levels

of MasR were remarkably upregulated in the brdu-labeled BMSCs with irradiation group. As expected, these results suggested that BMSCs could regulate AngII/ACE2/Ang(1–7) axis to ameliorated RILI.

BMSCs differentiated into alveolar II epithelial cells

To further verify whether BMSCs increase the expression of ACE2 by differentiating into alveolar epithelial cells.Immunofluorescence was used to assess the differentiation of BMSCs. Compared with the control group, the protein levels of AQP5 (a marker of alveolar type I cells) and of Pro-SPC (markers of alveolar type II cells) were significantly downregulated in the irradiation group. Compared with the irradiation group, the protein expression levels of AQP5, Pro-SPC were significantly upregulated in brdu-labeled BMSCs with irradiation group (Fig. 5). The results explained that brdu-labeled BMSCs homed to the injured lung tissues and differentiated into alveolar cells, mainly alveolar type II cells. In order to further verify the origin of ACE2, we used four-color multiplex fluorescent immunohistochemical staining kit to detect the co-localization of BrdU, Pro-SPC and ACE2. The results showed that compared with the control group, BrdU, Pro-SPC and ACE2 positive cells in BMSCs group were significantly increased (Fig. 5), which indicated that BMSCs could increase the expression of ACE2 by differentiating into alveolar type II epithelial cells. Therefore, BMSCs can not only repair some damaged epithelial cells but also produce ACE2 by differentiating into alveolar II epithelial cells to alleviate radiation-induced lung injury (Fig. 6).

Discussion

Nowadays, radiotherapy has become one of the most important treatment methods for thoracic tumors[17]. Although advanced radiation techniques can decrease radiation-related toxicity and increase the survival rate, it is still inevitable for occurrence of RILI, which limits the maximum dose for thoracic radiotherapy and reduces tumor control efficiency. RILI occurs in about 5–20% of patients with thoracic tumor clinically [15]. It is mainly divided into two stages: early radiation pneumonia and late radiation pulmonary fibrosis. Currently, molecular events the development of RILI is not fully elucidated [18]. Furthermore, there are no effective trearments for improving the clinical outcome of RILI [19]. BMSCs, a new treatment, have mediated well many beneficial therapeutic effects in various diseases [16, 20]. Hence, we speculated whether the BMSCs can alleviate RILI. In this study, we demonstrated a possible mechanism of BMSCs in the treatment of RILI.

In this study, we firstly established a RILI model to detect the pathological changes of lung tissue and the expression of ACE2. The results showed that endothelial cells and alveolar epithelial cells in the irradiated group were damaged to varying degrees, especially alveolar type II epithelial cells, and the expression of ACE2 decreased. Therefore, we speculate that ACE2 may play a protective role in RILI and its decrease may be related to cell injury. Next, we successfully separated BMSCs from rat thighbone and invetigated phenotype and differentiation potential of BMSCs. To verify whether BMSCs can alleviate the pathological changes of RILI, H&E and immunohistochemistry techology were used to detect the pathological changes of lung tissue and the changes of inflammatory factors. The results showed that the irradiated lung tissues induced severe lung injury, including blood capillary congestion and dilatation,

destruction of alveolar histological structure and inflammatory cells infiltration. It is reported that RILI was associated with expressed highly proinflammatory mediators, including IL-6 and TNF-α [21]. Our analysis showed the obviously elevated levels of IL-6 and TNF-α under irradiation. This result is consistent with the previous results [22]. However, After infusion of BMSCs, the injured lung tissues showed more remarkable relief with the relative reduction of alveolar tissue damage and lower degree of inflammation. Furthermore, BMSCs also reduced the expression levels of systemic TNF-α and IL-6. These results suggested that the protective effects of BMSCs were associated with anti-inflammatory, which consistented with previous studies [23].

The renin–angiotensin system (RAS) plays a significant role in modulating blood pressure homeostasis, as well as fluid and salt balance [26]. Whereas, there is a growing body of evidence suggested that it was also important in regulating inflammatory responses. These effects are considered to be regulated mainly via the AngII/ACE2/Ang(1–7) axis [27]. Ang II plays as a critical regulatory molecule in the RAS system and promotes inflammatory process by increasing the release of inflammatory cytokines combined with the AT1R [28]. ACE2, a homologue of ACE and a negative regulator of RAS, is expressed in numerous tissues especially the lung alveolar epithelial cells, kidney, heart and plays a key role in regulating inflammatory responses[14]. Recent studies have shown that ACE2 significantly inhibits inflammatory responses [29, 30]. However, nothing is known about AngII/ACE2/Ang(1–7) function in RILI. Our research found that the protein levels of AngII, ACE and AT1R were markedly upregulation in lung tissues under irradiation compared with control group, but irradiation resulted in significant downregulation of ACE2, Ang(1–7) and MasR protein. However, After infusion of BMSCs, the effects reversed these results which protein expression levels of AT1R, Ang II and ACE were significant decrease, whereas protein expression levels of ACE2, Ang(1–7) and MasR was dramatically increased. Besides, We verified that BMSCs do not express ACE2 by immunohistochemistry and western blotting. In addition, previous research had reported the possibility of differentiation of BMSCs into alveolar epithelial cells [24, 25]. The differentiation ability of BMSCs can help repair the integrity of alveolar epithelium cells, reducing RILI. Therefore, we speculate that BMSCs can increase the expression of ACE2 by differentiating into alveolar epithelial cells. The results showed that compared with irradiation group, the expression of Pro-spc (a marker of alveolar type II cells) and AQP5 (markers of alveolar type I cells) was markedly upregulated injected with BMSCs exposed to irradiation. Indeed, BMSCs mainly differentiated into alveolar type II cells. These results suggested that the BMSCs may home injured lung tissues and differentiate into alveolar cell especially alveolar type II cells to prevent RILI. In order to further verify the origin of ACE2, we used four-color multiplex fluorescent immunohistochemical staining kit to detect the co-localization of BrdU, Pro-SPC and ACE2. The results showed that compared with the control group, BrdU, Pro-SPC and ACE2 positive cells in BMSCs group were significantly increased, which indicated that BMSCs could increase the expression of ACE2 by differentiating into alveolar type II epithelial cells. These results supported the hypothesis that the AEC II generation following injection of MSCs may responsible for the alleviation of RILI, at least in part due regulating AngII/ACE2/Ang(1–7) axis.

Conclusions

In conclusion, we have demonstrated treatment with BMSCs can alleviate RILI. Our study have revealed that the protective mechanism of BMSCs is associated with upregulating the ACE2/Ang (1–7) axis which will provide experimental basis for better treatment of RILI in the future.

Abbreviations

RILI: Radiation-induced lung injury;

MSCs: Mesenchymal stem cells;

RT: Radiation therapy

RP: Radiation pneumonitis;

RILF: Radiation-induced lung fibrosis;

ALI: Acute lung injury;

ARDS: Acute respiratory distress syndrome;

RAS: renin–angiotensin system;

Ang-(1-7): angiotensin-(1-7);

MAPKs: Mitogen-activated protein kinases;

IL-6: Interleukin6;

TNF-α: Tumor necrosis factor α;

DAB: 3,3-diaminobenzidin;

Declarations

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Competing interests

The authors declare that there is no conflict of interests.

Authors’contributions

YYZ and SML designed and conducted the study and performed animal study. NSY,

WZP and CCS performed the experiments. NSY analyzed the data and wrote the paper. All authors read and approved the final manuscript.

Ethical approval and consent to participate

This study was approved by the Ethics Committee of The First Affiliated Hospital of Shandong First Medical University. Our institution's committee on human research gave approval for this study, and all participants gave informed consent.

All animal experiments were approved by the Institutional Animal Care and Use Committee of The First Affiliated Hospital of Shandong First Medical University. Treatment of experimental animals followed the internationally recognized 3R principle, that is, replacement, reduction, and refinement of experimental animals and the welfare ethics of experimental animals in accordance with the requirements of the guidelines for ethical review of experimental animal welfare (GB/T 35892-2018) of China.

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Availability of data and materials

Not applicable.

Consent for publication

Not applicable.

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Figures

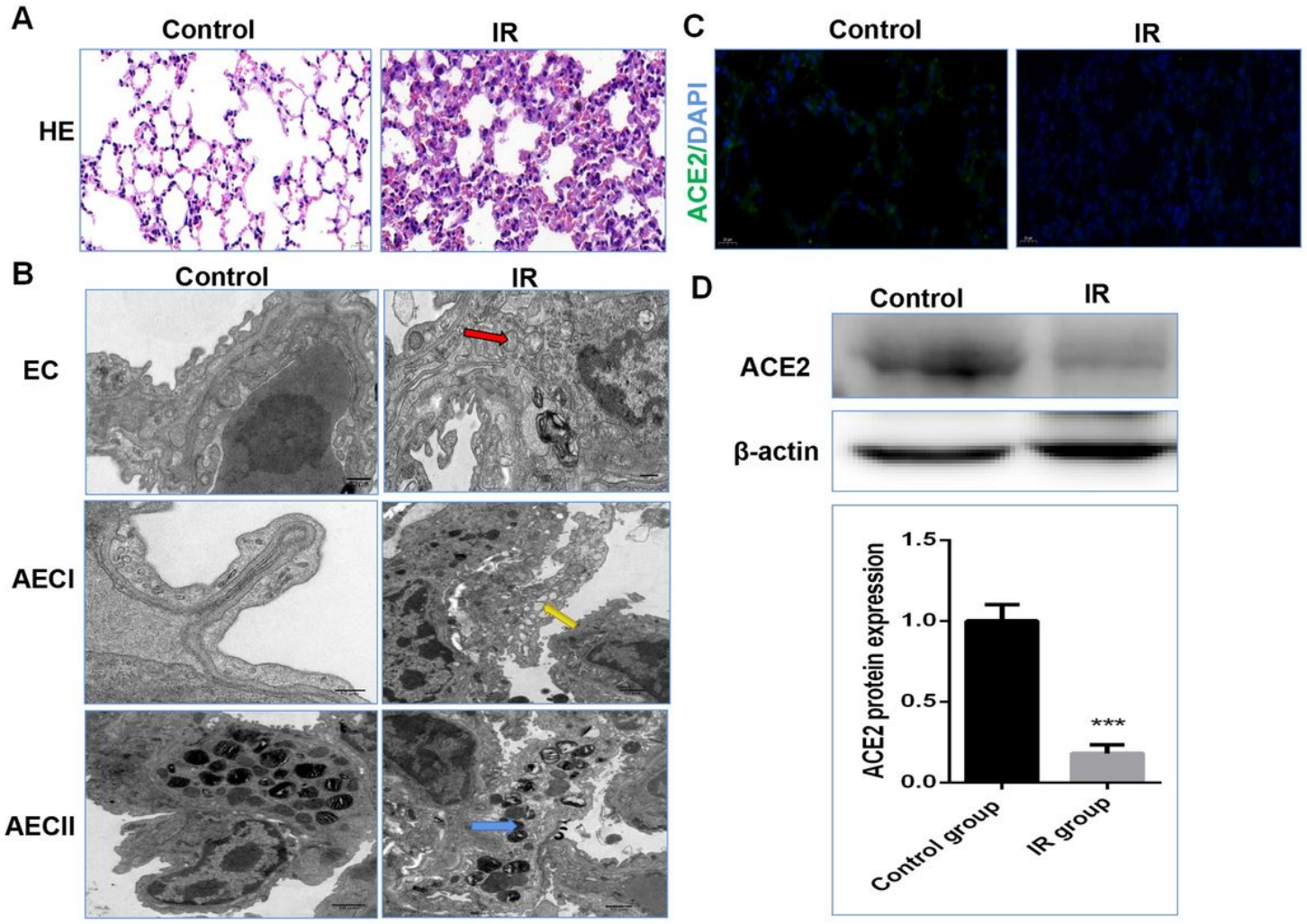

Figure 1

Pathological changes and expression of ACE2 in lung tissue in RILI. **A** H&E staining was used to detect the pathological changes of lung tissue.Scale bars, 20μm. **B** The damage of various cells in lung tissue was detected by electron microscope. Scale bars, 100 μm. **C** Irradiation decreased the expression of the ACE2 in the lung tissue by immunofluorescence staining and western blot (**D**). Scale bars, 20 μm. ***P < 0.001 versus the control group. EC:Endothelial cells. AECI:Alveolar type I epithelial cells. AECII:Alveolar type II epithelial cells.

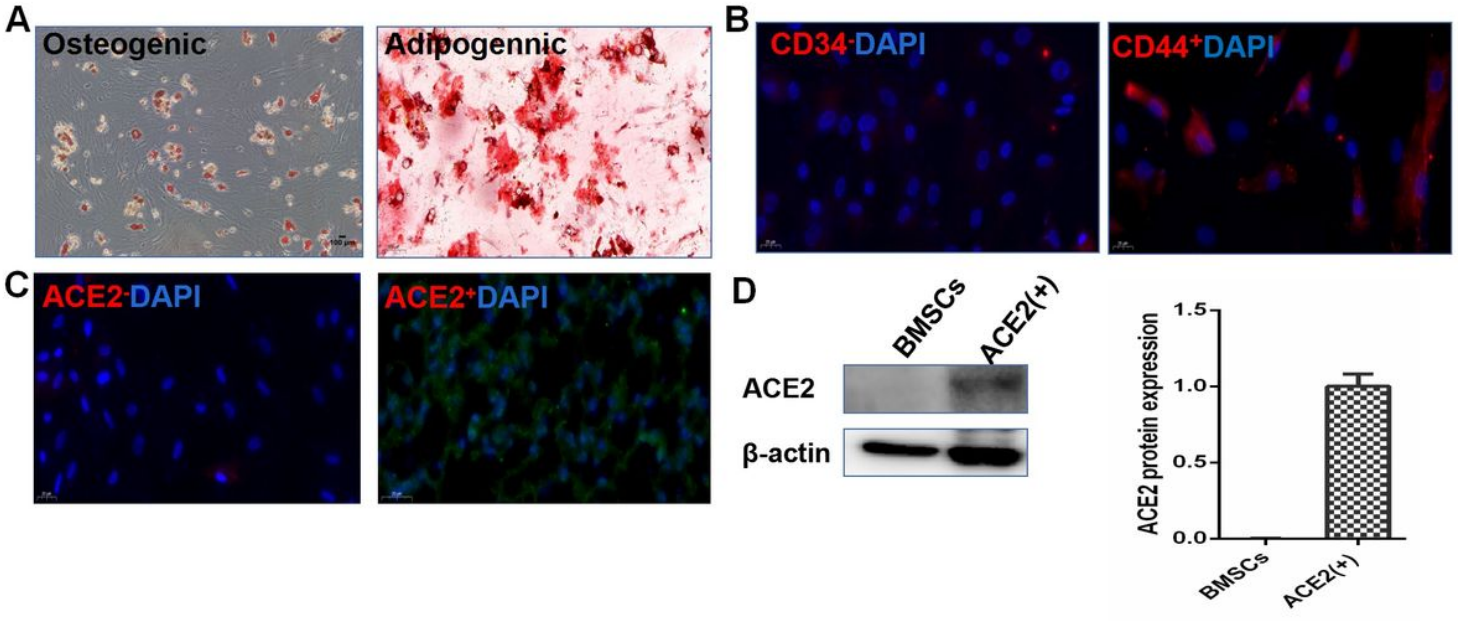

Figure 2

Identification of BMSCs. **A** Identification of osteogenic and adipogenic cells by Alizarin red staining and oil red O staining. Scale bars, 100 μm. **B** Immunofluorescence was used to detect surface molecular expression of the BMSCs, which were negative in CD34 expression, but positive in CD44 expression. Scale bars, 20 μm. **C** BMSCs did not express ACE2 by immunofluorescence staining and western blot (**D**). Scale bars, 20 μm. BMSCs: Bone mesenchymal stem cells.

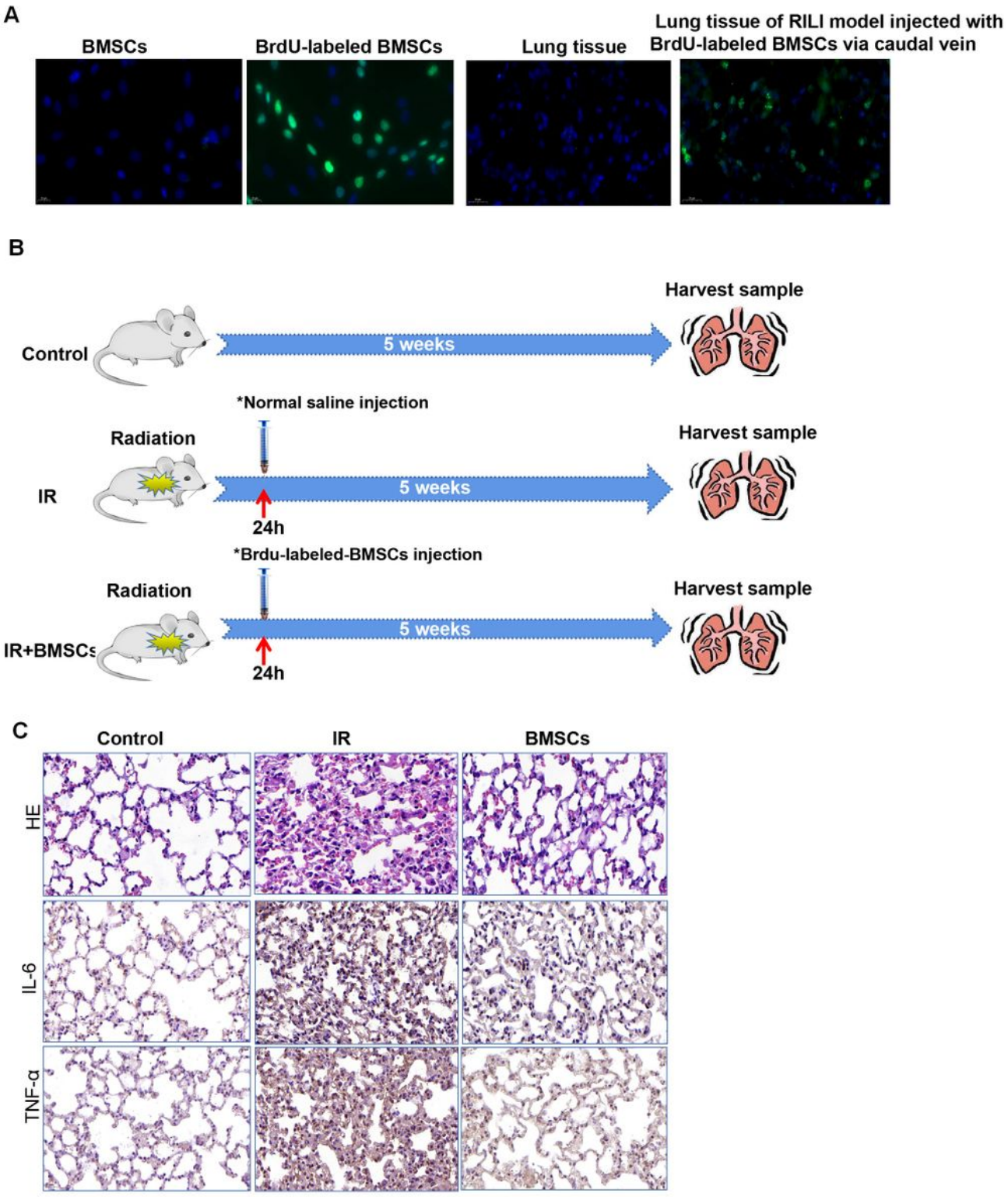

Figure 3

BMSCs homed to the lung tissues and histopathological changes in lung tissues. **A** Identification of BrdU-Labeled BMSCs in vitro and vivo, which showed strong green fluorescent staining in BrdU expression. Scale bars, 20μm. **B** Flowchart of the in vivo experiment. **C** Histopathological changes in lung tissues and the expression changes of IL-6 and TNF-α from each experimental group by H&E staining and immunohistochemical staining. Scale bars, 20μm. IL-6: Interleukin6; TNF-α: Tumor necrosis factor α.

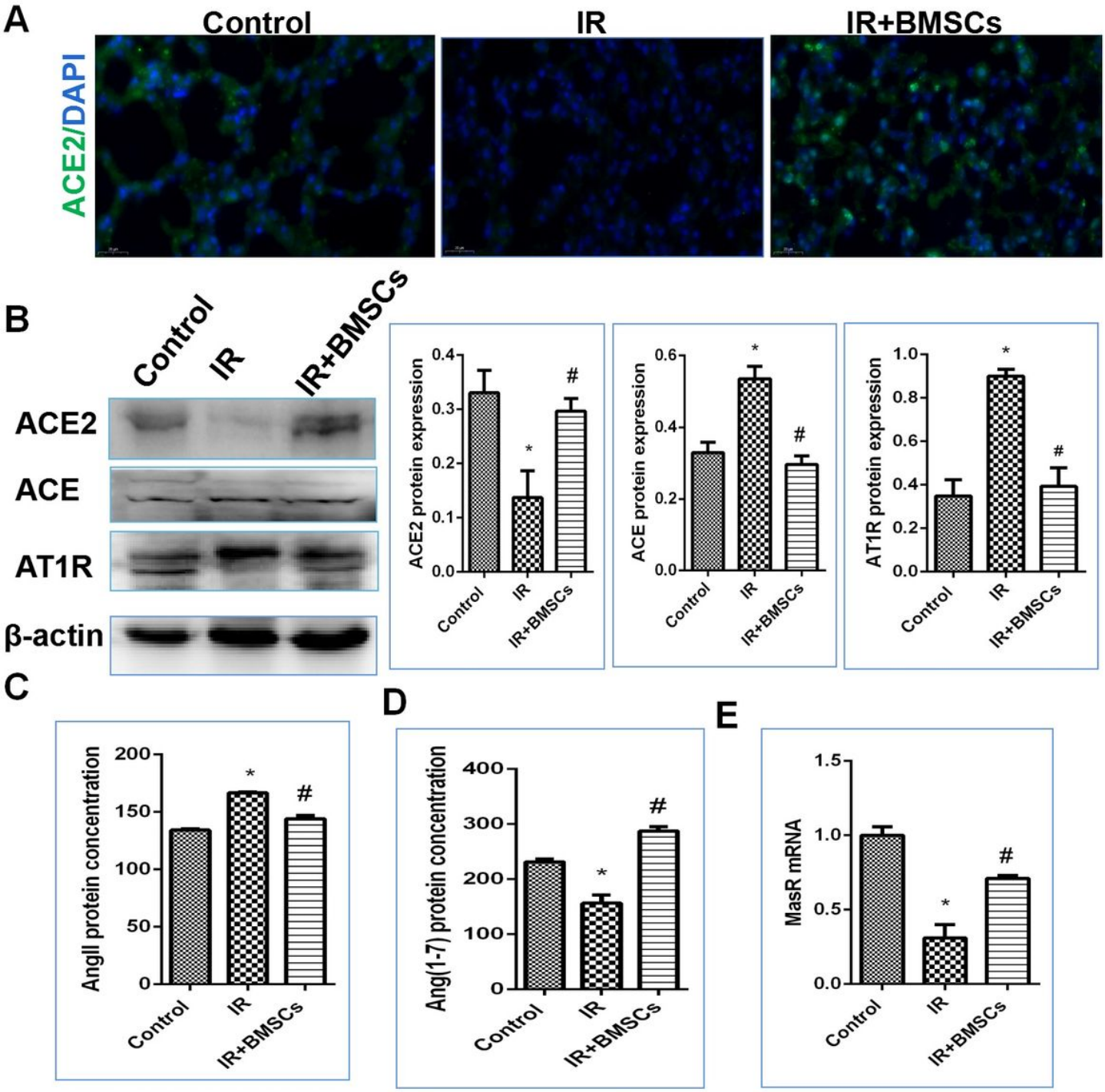

Figure 4

BMSCs regulate AngII/ACE2/Ang(1-7) axis. **A** The protein expression levels of ACE2 in the lung tissues were measured by immunofluorescence. Scale bars, 20μm.**B** The protein expression levels of ACE2, ACE and AT1R in the lung tissues were measured by western blot assay and quantification of ACE2, ACE and AT1R protein expression. **C** The protein expression levels of AngII in the lung tissues were measured by ELISA. **D** The protein expression levels of Ang(1-7) in the lung tissues were measured by ELISA. **E** The

mRNA expression levels of MasR in the lung tissues were measured by qRT-PCR. *Compared with the contol group, P<0.05. #Compared with the IR group, P<0.05.

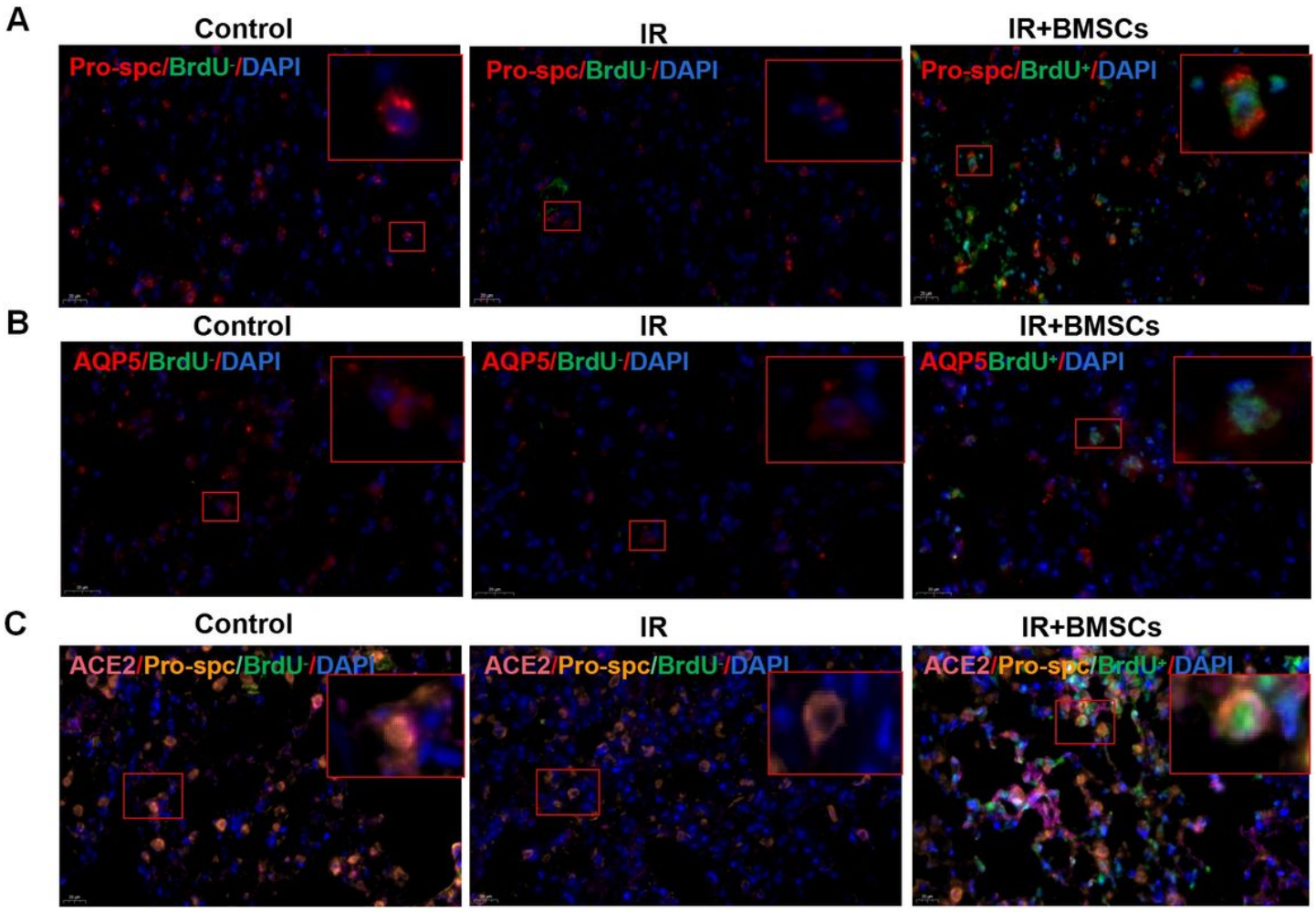

Figure 5

BMSCs differentiated into alveolar II epithelial cells to upregulate experssion of ACE2 in RILI. **A** The co-expression levels of BrdU and Pro-spc in the lung tissues were measured by immunofluorescence. Scale bars, 20 μm. **B** The co-expression levels of BrdU and AQP5 in the lung tissues were measured by immunofluorescence. Scale bars, 20 μm.**C** The co-expression levels of BrdU, ACE2 and Pro-spc in the lung tissues were measured by immunofluorescence. Scale bars, 20 μm.

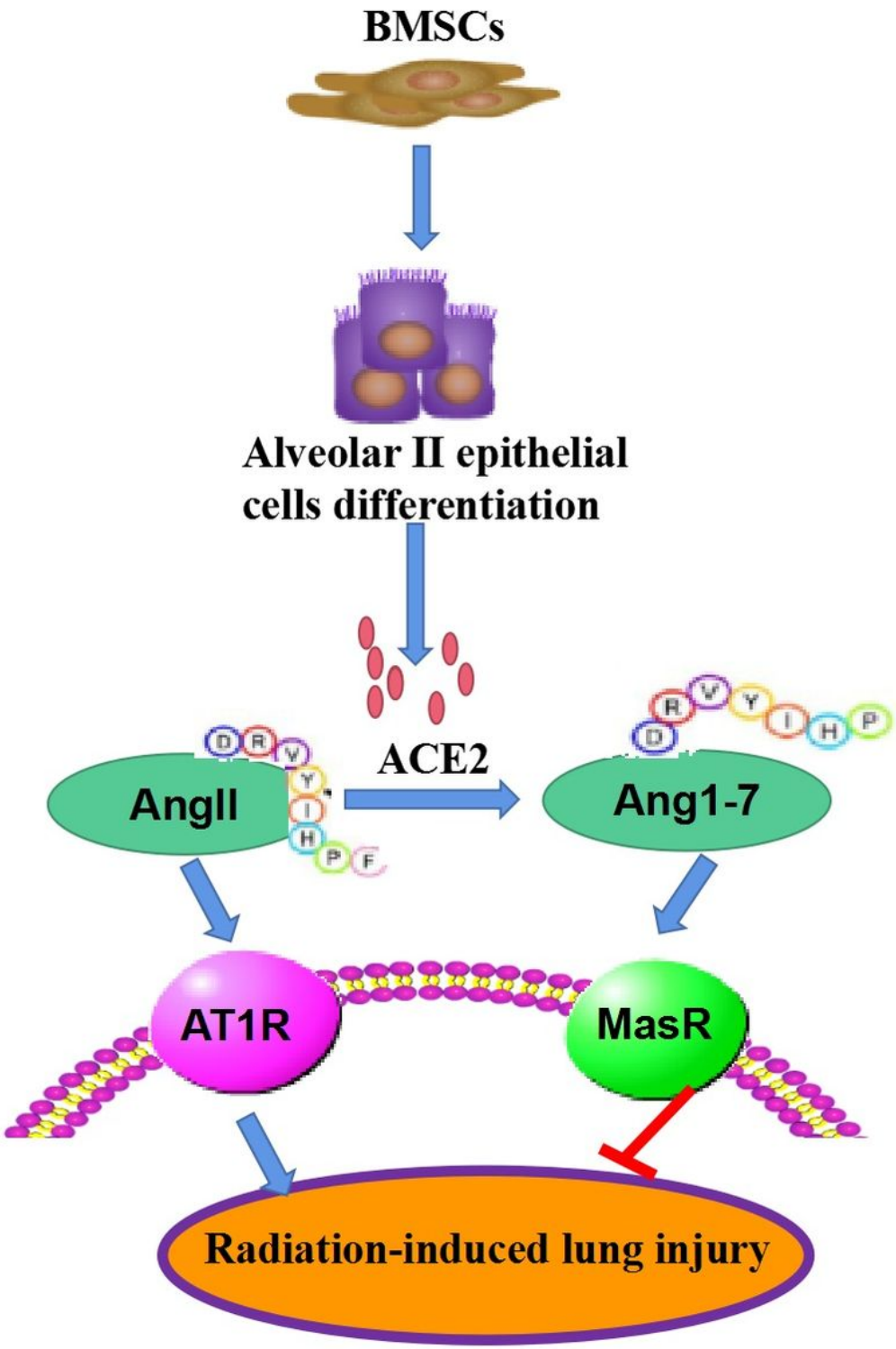

Figure 6

Illustration of possible mechanisms that BMSCs can not only repair some damaged epithelial cells but also produce ACE2 by differentiating into alveolar II epithelial cells to alleviate radiation-induced lung injury.