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# Urine-Derived Mesenchymal Stromal Cells Alleviate Radiation-Induced TGF-β1 Production through the Inhibition of the NF-κB Signaling Pathway in MRC-5 Cells

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**Authors' Contribution**

**L-nL, SL, FA and L-lC presented the concept and designed the study. L-nL and PG did data acquisition. SL and J-hL analysed and interpreted the data. L-nL and SL drafted the manuscript. FA and L-lC revised the manuscript.**

**Key words**

**Radiation pulmonary fibrosis, Urine-derived mesenchymal stromal cells, NF-κB signaling pathway.**

## ABSTRACT

No effective therapeutic method is available for the treatment of radiation pulmonary fibrosis. Recent studies have suggested that urine can provide an effective and noninvasive source of many differentiating or differentiated progenitor cells and stem cells. The aim of our present study was to assess the therapeutic effects of human urine-derived mesenchymal stromal cells (uMSCs) in a cell model of radiation pulmonary fibrosis. MRC-5 cells were irradiated and cultured in the presence of conditioned medium from uMSCs (MSCCM). MSCCM inhibited MRC-5 cell proliferation and reduced collagen deposition as measured by CCK-8 assay, Sircol assay, qPCR and western blot. MSCCM also reduced the expression of the pro-fibrotic TGF-β1 and increased the expression of anti-fibrotic IFN-γ at both mRNA and protein levels. Furthermore, we found that MSCCM inhibited the activation of NF-κB signaling pathway induced by irradiation as well. Our data suggest that MSCCM could reduce irradiation-induced TGF-β1 production and ameliorate collagen deposition by inhibiting NF-κB signaling pathway. Our present study provides new insights into the treatment effect of uMSCs in radiation pulmonary fibrosis.

## INTRODUCTION

Although significant improvements have been made in radiological technology and reducing damage to normal tissues around tumors, the incidence of radiation pulmonary fibrosis in cancer patients remains high (Yang *et al*., 2017). Moreover, many patients exhibit no evident clinical symptoms until a pulmonary test is performed and indicates reduction in their whole lung function. Currently, the mainstream treatment of acute pneumonitis consists of glucocorticoid and immunosuppressant drugs. However, these therapeutic modalities are not suitable for the treatment of radiation-induced lung injury and no effective treatment is available for radiation pulmonary fibrosis yet (You *et al*., 2014). Consequently, exploring the underlying pathological mechanism of irradiation induced pulmonary fibrosis and seeking effective therapy are urgently needed.

Several lines of evidence have suggested that MSCs can protect tissues from injury via its paracrine effect, the underlying mechanism of which might be that these cells can secrete several protective factors to stimulate endogenous self-repair and angiogenesis, thus attenuating tissue remodeling and cell apoptosis (Stabler *et al*., 2015). One of the most important and useful methods in studying the paracrine effects of MSCs is to evaluate the therapeutic effects of MSC conditioned medium (CM) and analyze its mechanisms *in vitro* and *in vivo*. Direct evidence for the paracrine role of MSCs has been obtained and confirmed in many animal models of lung (Argnoni *et al*., 2012), heart (Timmers *et al*., 2007), and liver (van Poll *et al*., 2008) injury, which have all shown that conditioned medium generated from MSCs significantly reduces tissue damage and stimulates cell regeneration *in vivo*. It has been considered that many growth factors in the CM play important protective roles in lung injury,

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including hepatocyte growth factor (HGF) (Meng *et al*., 2015), epithelial growth factor (EGF) (Katsha *et al*., 2011), keratinocyte growth factor (KGF) (Lee *et al*., 2009), vascular endothelial growth factor (VEGF) (Thebaud *et al*., 2005), angiopoietin-1 (Mei *et al*., 2007; McCarter *et al*., 2007) and adiponectin (Xia *et al*., 2016).

Recently, the mesenchymal stromal cells (MSCs) therapy has been regarded as an effective strategy to prevent radiation pulmonary fibrosis as well (Ionescu *et al*., 2012). Studies indicate that MSCs possess the capability of effectively homing to the injury sites, thus benefitting immunomodulation, epithelial repair, mitochondrial transfer, bactericidal activity and the secretion of protective growth factors and microvesicles (Stabler *et al*., 2015). MSCs isolated from different tissues are of particular interest as a source for pulmonary cell therapy because of their abundance, easy of isolation and characterization, multipotency and pleiotropic therapeutic effects (Lu *et al*., 2019).

In the past, urine was regarded as merely a body waste, but recent studies have demonstrated that urine can provide an effective and noninvasive source of differentiated or differentiating progenitor and stem cells. Moreover, human urine-derived MSCs (uMSCs) have been successfully isolated and cultured (He *et al*., 2016). However, whether uMSCs can ameliorate radiation-induced pulmonary fibrosis is still unclear. The aim of the present study was to explore the therapeutic effects of the paracrine function of uMSCs in a cell model of radiation pulmonary fibrosis. In the present study, we showed that the conditioned medium of uMSCs prevented collagen generation, and reduced the production of pro-fibrotic cytokines by inhibiting the NF-κB signaling pathway. To the best of our knowledge, this is the first translational study to assess and highlight the benefits of human uMSCs therapy for the treatment of radiation pulmonary fibrosis.

## METHODS

*Human pulmonary fibroblast cell line culture*

The human pulmonary fibroblast cell line, MRC-5, was purchased from American Type Culture Collection (Manassas, VA) and maintained in a humidified incubator at 37°C with 5% $CO_2$ in Dulbecco's Modified Eagle's Medium (DMEM) (Hyclone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS) (Invitrogen, USA) and 1% penicillin-streptomycin (Beyotime Biotechnology, Nantong, China). At 70-80% confluency, the complete medium was replaced with medium containing 1% FBS.

*Conditioned medium of urine-derived MSC preparation*

uMSCs were collected from human urine with the consent of the donors. The collection was performed in accordance with the ethical standards of the local ethics committee. uMSCs were successfully isolated from human urine according to the modified methods described in a previous report (He *et al*., 2016). Briefly, urine samples were centrifuged, and cell pellets were washed with phosphate-buffered saline (PBS). Cells were dispersed in 10% FBS-DMEM and passaged into new flasks for further expansion. Most of the urine cells were terminally differentiated and removed when the culture medium was changed. Well-developed colonies of the fibroblast-like cells formed at approximately passage 5-6. The multipotent differentiation capacity of uMSCs was confirmed by their differentiation into osteoblasts and adipocytes using alkaline phosphatase (osteoblasts) and Oil Red O staining (adipocytes), respectively. uMSCs cells were maintained in DMEM containing 10% FBS onto uncoated 25 $cm^2$ culture flasks (Corning, New York, USA) at 37ºC in 5% $CO_2$. When the cell culture reached approximately 70-80% confluence, the conditioned medium of uMSCs (MSCCM) was replaced with medium containing 1% FBS and cultured for an additional 2-3 days. The cells were collected, filtered through a membrane with 0.2 mm pores, aliquoted and stored at -20ºC (Fu *et al*., 2017).

*MRC-5 irradiation and MSCCM treatment*

MRC-5 cells at 70–80% confluency were irradiated with 10-Gy gamma rays using a Gamacell 3000 gamma irradiator (dose rate: 3.75 Gy/min; Nordion International Inc.) After irradiation, the culture medium was abandoned and cultured with MSCCM for another 7 days. Untreated MRC-5 cells were used as control.

*Detection of the paracrine effect of uMSCs*

To confirm the paracrine effect of uMSCs, conditioned medium of uMSCs was collected and the expression of HGF, EGF, KGF, and VEGF in the medium was measured using commercially available ELISA kits according to the manufacturer's protocols. The OD values were determined at the wavelength of 450 nm and the concentrations were read on the linear part of the curve.

*CCK-8 assay*

Cell viability was measured with the CCK-8 assay (cell counting kit-8, Dojindo Molecular Technologies, Tokyo, Japan). MRC-5 cells were seeded at $1\times10^4$ cells per well in 100 µl of complete growth culture media and treated as described above. Finally, CCK-8 solution (10 µl/well) was added into each well. After 2 h of incubation at 37°C, the absorbance of each well was determined at 450 nm using a microplate reader.

*Measurement of collagen content*

MRC-5 cells were treated as described above, then cells were collected and the deposits of collagen were

examined using Sircol soluble collagen assay kit (Biocolor, Belfast, North Ireland).

*Detection of the relationship between NF-κB/p65 and TGF-β1*

Previous studies in hepatofibroblasts indicated that NF-κB/p65 could exacerbate liver fibrosis by regulating the expression of TGF-β1. To determine whether NF-κB/p65 could regulate TGF-β1 in the context of radiation pulmonary fibrosis, MRC-5 cells were pretreated with SN50 (50μg/mL), an inhibitor of NF-κB/p65 and irradiated with gamma rays. The expression of NF-κB/p65 and TGF-β1 was measured by western blot.

*Cytokine expression in MRC-5 cells*

The levels of transforming growth factor-β1 (TGF-β1), interferon-γ (IFN-γ) and NF-κB p65 in lysates of MRC-5 cells were determined using commercially available ELISA kits according to the manufacturer's protocols.

*Real-time fluorescence polymerase chain reaction (q-PCR)*

At the end of the experiment, MRC-5 cells were collected, and total RNA was extracted with Trizol reagent (TaKaRa, Tokyo, Japan). First strand complementary DNA (cDNA) was synthesized using the Reverse transcription Kit (TaKaRa, Tokyo, Japan) according to manufacturer's instructions. For quantitative PCR (q-PCR), 10 μl reactions system that included 5 μl 2×SYBR Green (TaKaRa, Tokyo, Japan), 0.8 μl cDNA templates, and 0.8 μl q-PCR primers were used. The samples were run and analyzed in triplicate with CFX Connect Real-Time System (Bio-Rad, Hercules, USA). The q-PCR conditions were as follows: an initial 3 min denaturation step at 95°C, followed by 40 cycles of 95°C for 5 s, 58°C for 30 s, and 72°C for 30 s. The primer sets used are listed below. Col1A1: Forward 5'-GTGTTGTGCGATGACG-3', Reverse 5'- TCGGTGGGTGACTCTG-3'. Col1A2: Forward 5'-GTGGCAGTGATGGAAGTGTG-3', Reverse 5'-AGGACCAGCGTTACCAACAG-3'. TGF-β1: Forward 5'- CCAGGCUCGTTCTAGATGCUC-3', Reverse 5'- CCGCUTCCTAGGAGACACCG-3'. IFN-γ: Forward 5'-CCCTGCCCCAATCCCTTTAT-3', Reverse 5'-CCCTAAGCCCCCAATTCTCTTT-3'. GAPDH: Forward 5'- AGAAGGCTGGGGCTCATTTG-3', Reverse 5'- AGGGGCCATCCACAGTCTTC-3'. Melting curve analysis showed a single amplification peak for each reaction. Ct values for targets were expressed as relative expression compared to the average of the housekeeping genes GAPDH. The expression of target genes were normalized using the comparative quantification method ($2^{-\Delta\Delta CT}$).

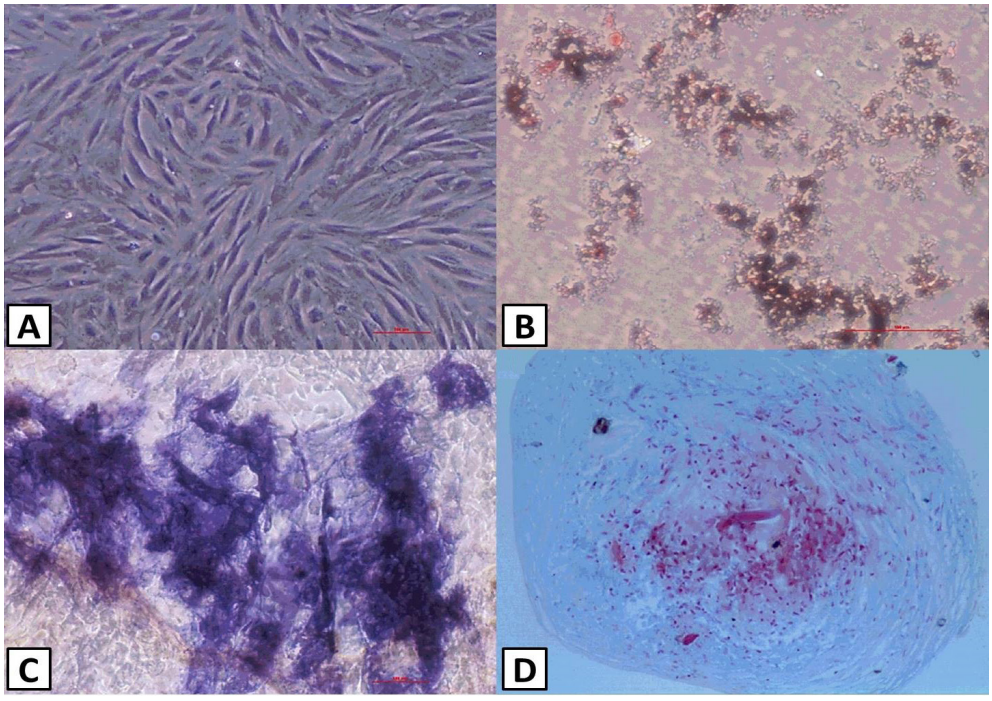


Fig. 1. Identification of uMSCs. uMSCs exhibited a spindle- and fibroblast-like shape (A). B-D shows multipotential differentiation of uMSCs. uMSC differentiation into adipocytes, osteoblasts and chondroblasts, as shown by Oil Red O (B), alkaline phosphatase (C), and alcian blue (D) staining of *in vitro* differentiation cultures, respectively.

*Western blotting assay*

After MRC-5 cells were lysed, the nuclear and cytoplasmic proteins were extracted according to the manufactures' instructions (Beyotime, China). After centrifugation, the supernatants were collected and the protein concentration was detected by bicinchoninic acid protein assay (BCA, Beyotime, China). Proteins from each sample were subjected to 10% SDS-polyacrylamide gels and electrophoretically transferred onto a polyvinylidene fluoride (PVDF) membrane (Millipore). Subsequently, the nonspecific binding sites in the PVDF membranes were blocked with 5% non-fat dried milk dissolved in Tris-buffered saline tween (TBST) for 90 min. The PVDF membranes were incubated with primary antibodies against Col1A1 (Abcam, ab Ab34710), GAPDH (Abcam, ab), Col1A2 (Abcam, ab96723), TGF-β (Proteintech, 21898-1-AP), IFN-γ (CST, 9102), NF-κB/p65 (Bioworld, BS1257), IκB-α (Abcam, Ab85803) overnight at 4°C, followed by incubation with the corresponding secondary antibodies for 60 min. The protein bands were visualized with an enhanced chemiluminescence (ECL) system. GAPDH was used as the internal loading control.

*Statistical analysis*

All experiments were conducted at least in triplicate, and representative data are expressed as the mean ± SD. The comparisons were evaluated by one-way analysis of variance and for those significant, post-hoc multiple comparisons between means were determined with Turkey test. All statistical analyses were performed using SPSS Statistics Software, and values of $p<0.05$ were considered to be statistically significant.

## RESULTS

*Characterization of uMSCs and MSCCM inhibition of MRC-5 cell proliferation*

The uMSCs exhibited typical spindle-like and fibroblast-like shapes (Fig. 1A). The multipotent differentiation capacity of the uMSCs was confirmed by their differentiation into adipocytes, osteoblasts and chondroblasts, as shown by the staining of the vitro differentiated cultures with Oil Red O (Fig. 1B, adipocytes), alkaline phosphatase (Fig. 1C, osteoblasts), and alcian blue (Fig. 1D, chondroblasts).

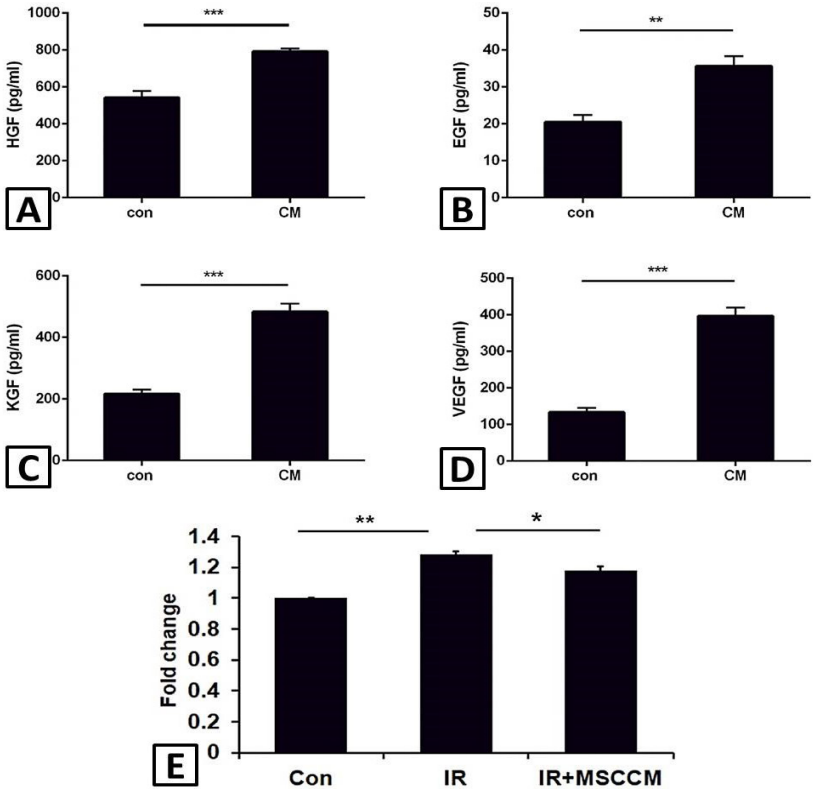


Fig. 2. uMSCs secrete many types of protective cytokines and MSCCM inhibits the proliferation of MRC-5 cells. **A-D,** Expression of the cytokines of HGF, EGF, KGF and VEGF measured in the control media and the conditioned medium of uMSCs. **E,** cell viability analyzed by CCK8 assay. The results were compared among control cells (Con), single-irradiated MRC-5 cells (IR), and irradiation + MSCCM treated MRC-5 cells (IR+MSCCM). Data are expressed as the mean ± SD (n=5). *$p< 0.05$, **$p< 0.01$.

*uMSCs could secrete a lot of protective cytokines and MSCCM inhibits MRC-5 cell proliferation induced by irradiation*

As shown in Figure 2A-D, the expression of cytokines such as HGF, EGF, KGF, and VEGF in the conditioned medium of uMSCs was significantly increased compared with control ($p$<0.05); these date indicated that uMSCs could indeed secrete growth factors by a paracrine effect. As shown in Figure 2E, irradiation could remarkably increase the proliferation ability of MRC-5 cells, however, MSCCM significantly reduced irradiation-induced cell proliferation compared with MRC-5 cells that irradiated with gamma rays only ($p$<0.05).

*MSCCM reduces the collagen generation in MRC-5 cells*

We next examined the impact of irradiation on collagen synthesis in MRC-5 cells. As the Sircol assay showed (Fig. 3A), increased synthesis of collagen induced by irradiation was evidently suppressed by MSCCM ($p$<0.05). We also examined the mRNA and protein levels of Col1A1 and Col1A2, which reflected collagen synthesis in MRC-5 cells. The mRNA and protein levels of Col1A1 and Col1A2 in irradiated MRC-5 cells were highly elevated compared with control ($p$<005). However, the mRNA and protein levels of Col1A1 and Col1A2 in irradiated MRC-5 cells treated with MSCCM were markedly decreased ($p$<0.05) (Fig. 3B-E).

*SN50 could downregulate TGF-β1 expression by inhibiting NF-κB signaling pathway in gamma ray-irradiated MRC-5 cells*

As shown in Figure 4, compared with the control group, irradiated with gamma rays remarkably increased the protein levels of NF-κB/p65 and TGF-β1 ($p$<0.05). However, pretreatment with SN50 dramatically reversed this phenomenon. Data above indicated that TGF-β1 might be regulated by NF-κB/p65, and that NF-κB/p65 might be a key upstream regulatory factor of TGF-β1 in the context of radiation pulmonary fibrosis.

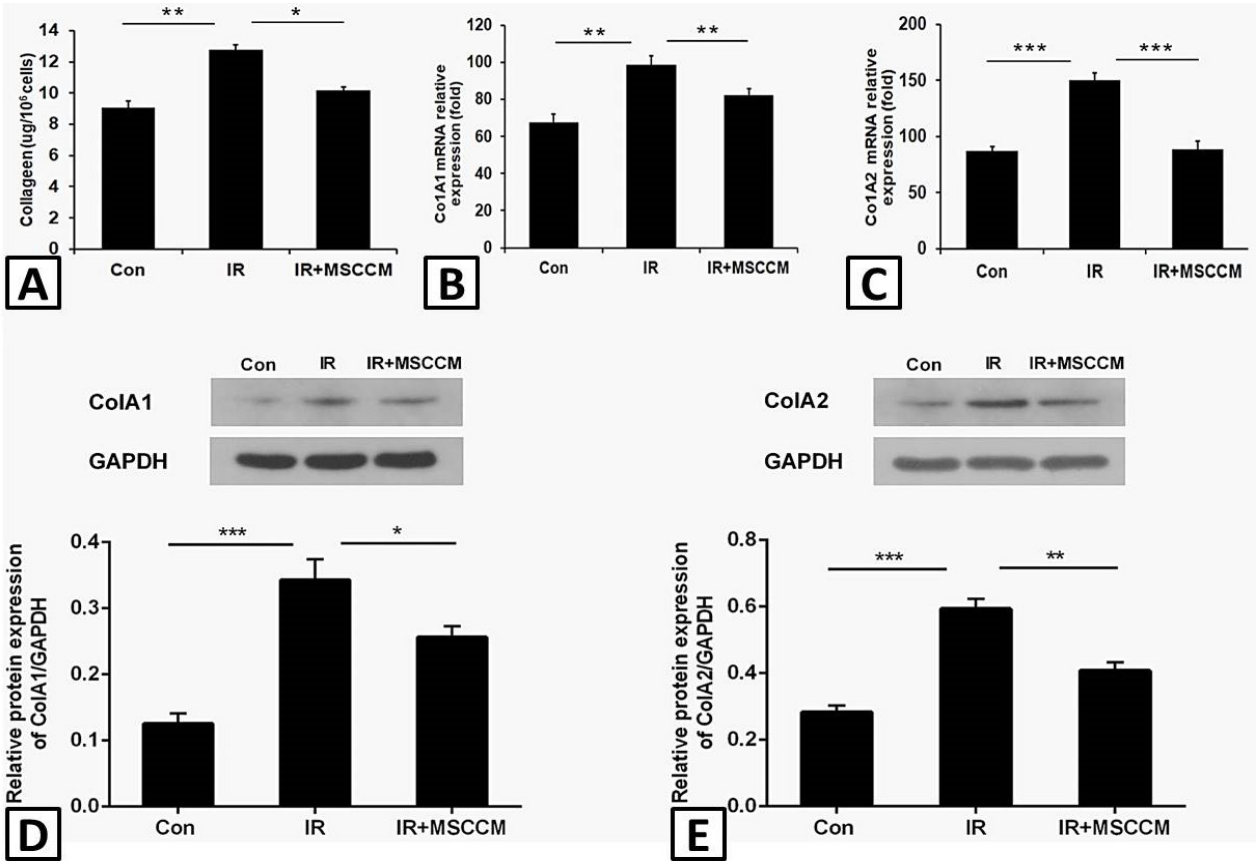


Fig. 3. MSCCM reduces the collagen generation in MRC-5 cells. **A,** the collagen content was examined using Sircol soluble collagen assay in control cells (Con), single-irradiated MRC-5 cells (IR), and irradiation + MSCCM treated MRC-5 cells (IR+MSCCM). **B-C,** mRNA levels of Col1A1 (B) and Col1A2 (C) were detected by q-PCR. **D-E,** protein levels of Col1A1 (D) and Col1A2 (E) were detected by western blot. Data are expressed as the mean ± SD (n=5). *$p$< 0.05, ** $p$<0.01, ***$p$<0.001.

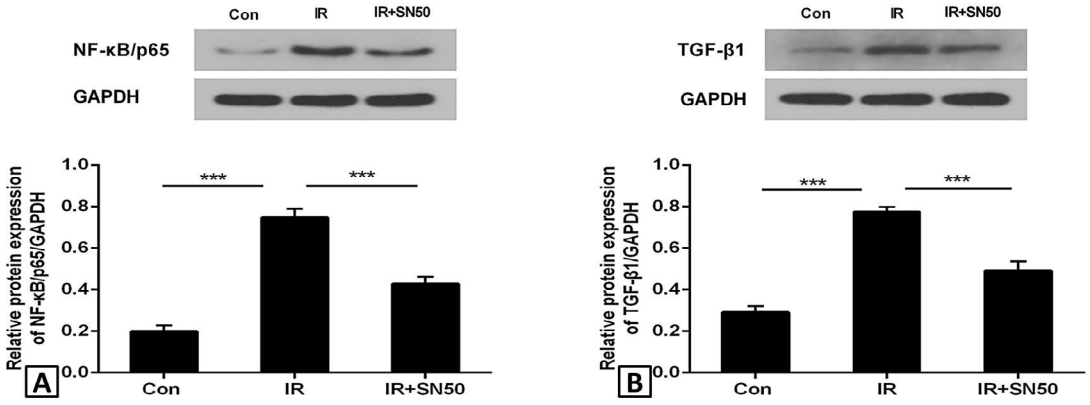


Fig. 4. NF-κB inhibition could reduce irradiation induced upregulation of TGF-β1. **A,** protein level of NF-κB/p65 in different treatment groups detected by western blot. **B,** Protein level of TGF-β1 in different treatment groups detected by western blot. Data are expressed as the mean ± SD (n=5). *$p$< 0.05, **$p$<0.01, ***$p$<0.001.

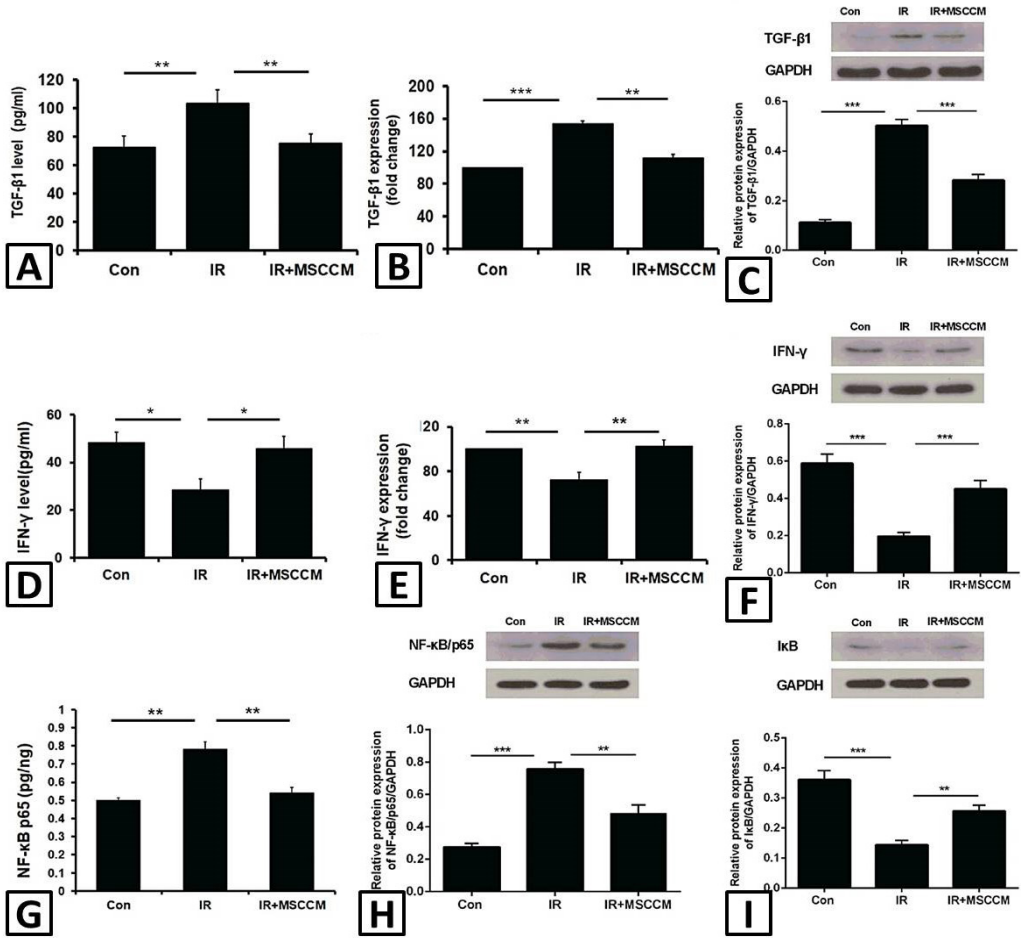


Fig. 5. MSCCM inhibits the expression of TGF-β1 and prevents the NF-κB activation in MRC-5 cells after irradiation. **A, D and G,** TGF-β1, IFN-γ and NF-κB/p65 levels were determined by ELISA in lysate of MRC-5 cells in control cell group (Con), single-irradiated MRC-5 cell group (IR), and irradiation + MSCCM treated MRC-5 cell group (IR+MSCCM). **B and E,** the mRNA levels of TGF-β1 and IFN-γ were examined by q-PCR in different treatment groups. **C, F, H and I,** protein levels of TGF-β1, IFN-γ, NF-κB/p65 and IκB were detected by western blot. Data are expressed as the mean ± SD (n=5). **$p$<0.01, ***$p$<0.001.

*MSCCM down regulates the expression of pro-fibrotic cytokine TGF-β1 in MRC-5 cells*

We measured the levels of key pro-fibrotic cytokine TGF-β1 and the anti-fibrotic cytokine IFN-γ in cell lysate of MRC-5 cells by ELISA. The TGF-β1 level in MSCCM-treated MRC-5 cells was significantly lower than that in MRC-5 cells treated with radiation only ($p<0.05$) (Fig. 5A), however, the IFN-γ level in MSCCM-treated MRC-5 cells was significantly higher than that in MRC-5 cells treated with radiation only ($p<0.05$) (Fig. 5D). We also measured the mRNA and protein levels of TGF-β1 and IFN-γ in MRC-5 cells by q-PCR and western blot, and similar results were obtained (Fig. 5C-F).

*MSCCM prevents the activation of the NF-κB signaling pathway*

To examine whether irradiation would activate the NF-κB signaling pathway and whether MSCCM would prevent the activation of NF-κB signaling pathway in irradiated MRC-5 cells, we investigated the expression of NF-κB/p65 in MRC-5 cells by ELISA and western blot. We also measured the protein level of IκB. As shown in Figure 5G and H, NF-κB/p65, which was detected in the nuclear fraction, was significantly increased after irradiation compared with control ($p<0.05$), however, treatment of irradiated MRC-5 cells with MSCCM could markedly reduce the expression of NF-κB/p65 ($p<0.05$). At the same time, gamma rays irradiation dramatically decreased the expression of IκB ($p<0.05$), whereas treatment with MSCCM counteracted this effects (Fig. 5I).

## DISCUSSION

In the present study, we aimed to investigate the therapeutic potential of uMSCs paracrine effect in a cell model of radiation pulmonary fibrosis. Our data clearly demonstrated that MSCCM could markedly inhibit irradiation-induced cell proliferation and collagen deposition, as well as downregulate the expression of pro-fibrotic TGF-β1 and increase the level of anti-fibrotic cytokine IFN-γ. Furthermore, our data indicated that MSCCM prevented the pro-fibrotic effect induced by irradiation through blocking the NF-κB signaling pathway. Our present study not only defined the potential beneficial effect of the delivery of MSCCM, but also provided supporting evidence of the therapeutic potential of the paracrine effect of uMSCs in a cell model of radiation pulmonary fibrosis.

How do human MSCs exert their beneficial effects? MSCs are known to possess multi-lineage differentiation potential and it could differentiate into multiple types of cells in order to replace the damaged cells in response to adverse stimulus (Xu *et al*., 2018). However, their engraftment or retention time in most organs are relatively short-lived. A previous study has indicated the retention of MSCs in lungs up to 24 h after intravenous infusion (Qazi *et al*., 2011). MSCs' retention time in the heart is even shorter, it has been reported that 4 h after intramyocardial administration, 10 % of injected MSCs were present, and 24 h later, the retention rate was only 1% (Williams and Hare, 2011). Therefore, the direct differentiation mechanism cannot account for the beneficial effect of MSC therapy. Recent studies have revealed that even though its retention time in tissue is short, MSCs possess powerful secretory functions and secrete a various paracrine factors such as cytokines and growth factors, which inhibit apoptosis, and fibrosis, activate immune cells and facilitate angiogenesis (Pashoutan *et al*., 2018). As MSC-conditioned media is a source of growth factors (You *et al*., 2015), we hypothesized that these beneficial effects of MSCCM might be mediated by cytokines secreted by uMSCs in a paracrine manner. In our present study, we found that the conditioned media of uMSCs contain various cytokines such as HGF, EGF, KGF and VEGF, which might protect cells from irradiation injury.

Wang *et al*. (2010) have demonstrated that TGF-β1 level positively correlates with the incidence of radiation lung injury among lung cancer patients. However, some reports indicated that no correlation relationship is observed between changes in the respiratory function parameters and the TGF-β1 level in idiopathic pulmonary fibrosis patients (Alhamad *et al*., 2013; Molina-Molina *et al*., 2006). The gene polymorphisms of TGF-β1 among different ethnic groups have contributed to this different observation (Alhamad *et al*., 2013). A growing body of recent evidence suggest that TGF-β1 could potentially induce epithelial cells to trans-differentiate into myofibroblasts through a process termed "epithelial-mesenchymal transition" (EMT), which depends on the TGF-β/Smad pathway (Kage and Borok, 2012), and leads myofibroblasts to produce large amounts of collagens in the lung. Contrary to TGF-β1, IFN-γ can inhibit fibroblast proliferation, counteract the differentiation of fibroblasts and myofibroblasts and collagen synthesis (Mahmood *et al*., 2013). Our results clearly demonstrated that MSCCM significantly reduced the level of TGF-β1 and increased the expression of IFN-γ. These observations confirmed that downregulation of pro-fibrotic cytokines and upregulation of anti-fibrotic cytokines have been proposed as one of the paracrine actions of MSCs in a previous study (Katsha *et al*., 2011). MSCCM could adjust the balance of pro-fibrotic cytokines and anti-fibrotic cytokines to attenuate irradiation induced radiation pulmonary fibrosis.

NF-κB activation occurs following IκB

phosphorylation, which is induced by the activation of IκB kinase α/β (IKKα/β) (Kim *et al*., 2010). It has been well demonstrated that phosphorylated NF-κB can bind to the target gene in the nucleus and affect the transcription of nuclear genes, thus stimulating fibrosis, which plays an important role in the course of liver fibrosis (Hou *et al*., 2018). Recent studies have revealed an intimate association exists between the NF-κB signaling pathway and the TGF-β1 signaling pathway in the pathological process of fibrosis (Feng *et al*., 2018). Researchers have found that NF-κB/TGF-β1 signaling pathway is of vital importance in pulmonary fibrosis airway remodeling, and it is also a crucial path to induce heart and lung injury (Fu *et al*., 2015; Li *et al*., 2014). The activation of this signaling pathway could stimulate the production of inflammatory cytokines, cause sustained damage to cells, promote fibroblast differentiation and the deposition of collagen fibers. However, no study has explored whether NF-κB/TGF-β1 signaling pathway participates in the pathogenesis of radiation pulmonary fibrosis. In our present experiment, we used an inhibitor of NF-κB to pretreat the irradiated MRC-5 cells and found that NF-κB inhibition could significantly down-regulate the expression of TGF-β1. Subsequently, our data show that MSCCM could also inhibit irradiation induced up-regulation of TGF-β1 and increase fibrous deposition, while the expression of NF-κB/p65 was down-regulated and IκB was up-regulated accordingly. Data above indicated that irradiation could promote the expression of TGF-β1 and facilitate fibrosis by activating NF-κB signaling pathway in MRC-5 cells, while MSCCM could inhibit the activation of NF-κB signaling pathway, thus suppressing the expression of TGF-β1and ameliorating fibrosis.

In conclusion, the present study was the first to demonstrate the protective effect of MSCCM for irradiation induced radiation pulmonary fibrosis. More importantly, this compelling evidence highlighted that uMSCs could attenuate irradiation-induced fibrin deposition in a NF-κB/TGF-β1 dependent manner. It is noteworthy that pulmonary fibrosis is a complex pathological process, and that other signaling pathways such as Wnt/β-catenin and Notch1/eIF3 might be involved as well. However, these assumptions need to be further confirmed. Taken together, the present study comprehensively substantiates the hypothesis of MSCCM-mediated resistance to radiation pulmonary fibrosis induced by irradiation and indicates that uMSCs might be a promising therapeutic agent to prevent radiation pulmonary fibrosis.

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*Ethics approval and consent to participate*

Human tissue samples and animals were not used in the present study. The study was approved by the ethical committee of Hubei University of Medicine.

*Statement of conflict of interest*

The authors declare no conflict of interest.

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