


MINI REVIEW

Mesenchymal stem cells: A double-edged sword in radiation-induced lung injury

Yi Yao¹ , Zhongliang Zheng² & Qibin Song¹¹ Cancer Center, Renmin Hospital of Wuhan University, Wuhan, China² State Key Laboratory of Virology, College of Life Sciences, Wuhan University, Wuhan, China**Keywords**

Gene modification; lung injury; mesenchymal stem cells; radiation; transplantation.

Correspondence

Yi Yao, Cancer Center, Renmin Hospital of Wuhan University, 238# Jiefang Road, Wuchang, Wuhan, 430060 China.
Tel: +86 27 8804 1911 ext 85570
Fax: +86 27 8807 5927
Email: yivanrobin@sina.com

Received: 20 September 2017;

Accepted: 10 November 2017.

doi: 10.1111/1759-7714.12573

Thoracic Cancer **9** (2018) 208–217**Abstract**

Radiation therapy is an important treatment modality for multiple thoracic malignancies. However, radiation-induced lung injury (RILI), which is the term generally used to describe damage to the lungs caused by exposure to ionizing radiation, remains a critical issue affecting both tumor control and patient quality of life. Despite tremendous effort, there is no current consensus regarding the optimal treatment approach for RILI. Because of a number of functional advantages, including self-proliferation, multi-differentiation, injury foci chemotaxis, anti-inflammation, and immunomodulation, mesenchymal stem cells (MSCs) have been a focus of research for many years. Accumulating evidence indicates the therapeutic potential of transplantation of MSCs derived from adipose tissue, umbilical cord blood, and bone marrow for inflammatory diseases, including RILI. However, reports have also shown that MSCs, including fibrocytes, lung hematopoietic progenitor cells, and ABCG²⁺ MSCs, actually enhance the progression of lung injuries. These contradictory results suggest that MSCs may have dual effects and that caution should be taken when using MSCs to treat RILI. In this review, we present and discuss recent evidence of the double-edged function of MSCs and provide comments on the prospects of these findings.

Introduction

Radiotherapy is an essential tool for the management of thoracic tumors. However, radiation-induced lung injury (RILI), the term generally used to describe damage to the lungs caused by exposure to ionizing radiation, is the main obstacle limiting dosage. Manifestations of RILI include early-stage radiation pneumonitis (RP) and subsequent stage radiation pulmonary fibrosis (RPF).

Radiation pneumonitis typically occurs within three months after radiation therapy, and is pathologically characterized across diverse grades by numerous infiltrating inflammatory cells in the edema interval of broken alveolar and in the bronchus.¹ RPF, considered a result of chronic lung damage, occurs at 6–24 months post-radiotherapy, and includes irreversible corruption of the alveoli, activation of myofibroblasts, abnormal deposition of extracellular collagen matrix, and deregulated remodeling of lung tissue.¹ Clinical data have demonstrated that the incidence of RILI ranges from 20.3% to 36.9%.² However, only a few

successful therapeutic strategies have been proposed, despite extensive research.

Mesenchymal stem cells (MSCs) have been well characterized and can be isolated from various tissues, such as bone marrow,³ adipose,⁴ umbilical cord,⁵ placenta,⁶ and circulatory vessel walls.⁷ Because of a number of functional advantages, including self-proliferation, multi-differentiation, injury foci chemotaxis, anti-inflammation, and immunomodulation, this cell population has become a focus of research.⁸ MSC-based therapies have been successfully used to repair tissue and several organs, including the lungs.⁹

Based on accumulating evidence, MSC transplantation has been proposed as a potential therapeutic approach to address inflammatory diseases, such as RILI. Controversially, reports have also shown that MSCs actually enhance the progression of lung injuries characterized by inflammation and fibrosis. These contradictory results suggest that MSCs may have dual effects and that caution should be taken when using MSCs to treat RILI.

In this review, we present and discuss recent evidence of the double-edged function of MSCs and provide comments on the prospects of these findings.

Evidence supporting the application of mesenchymal stem cells (MSCs) for the treatment of radiation-induced lung injury (RILI)

The use of MSCs in RILI models

Reports of the therapeutic application of MSCs in RILI animal models have recently been published. Dong *et al.* confirmed that systemic infusion of human adipose tissue-derived MSCs (AMSCs) ameliorated lung fibrosis in rats that received semi-thoracic irradiation (15 Gy) by upregulating HGF and PGE₂, while downregulating TNF- α and TGF- β 1.¹⁰ Furthermore, several similar reports have demonstrated upregulation of IL-10 and downregulation of IL-1, IL-6, and TNF- α in murine serum, and downregulation of TGF- β 1, α -smooth muscle actin (SMA), and type I collagen in irradiated murine lung tissues.^{3,11–13} AMSCs have been shown to protect lung cells from apoptosis by regulating the expression of pro-apoptotic and anti-apoptotic mediators, including Bcl-2, Bax, and caspase-3.^{3,11–13}

Wang *et al.* applied human umbilical cord blood-derived MSCs (hUMSCs) to an acute RP animal model.¹² The results indicated that hUMSCs have definite therapeutic effects on acute RP in rats.

Zhen *et al.* administered bone marrow-derived mesenchymal stem cells (BMSCs) from male donor rats into female recipients in a model of pulmonary emphysema induced by irradiation and papain instillation.¹⁴ Emphysematous changes were ameliorated in rats that received BMSC infusions compared to rats that did not, revealing a protective mechanism of BMSC engraftment in the lungs, their differentiation into type II alveolar epithelial cells, and suppression of alveolar cell apoptosis. Similarly, Klein *et al.* treated whole thorax irradiated mice with allogeneic BMSCs or aorta-derived MSCs.¹⁵ Irradiation induced endothelial cell damage, senescence of lung epithelial cells, and upregulation of invasion and inflammation-promoting factors, while the MSCs antagonized this damage to resident cells as well as the resulting secretome changes and abrogated the metastasis-promoting effects of thorax irradiation.

MSCs for acute lung injury (ALI)

Chemical agents, such as bleomycin, hydrochloric acid, and amiodarone, have been employed to establish acute lung injury (ALI) in animal models.^{16,17} Using these animal models, allogeneic BMSCs have been shown to elicit anti-

inflammatory and pro-healing properties. In fact, BMSCs were found to reduce circulating and in situ inflammatory cytokines, such as TGF- β , PDGF-A, PDGF-B, and IGF-1,¹⁸ and to protect against myelosuppression induced by bleomycin.^{3,13} MSCs are also capable of inhibiting the proliferation of effector T lymphocytes and effectively induce T-lymphocyte anergy by changing the secretome of T-lymphocyte subsets.^{19,20} Furthermore, BMSCs were observed to home to sites of tissue injury, where they differentiated into specific lung cell phenotypes.¹³ Recently, the beneficial effect of infused multi-potent adult stem/progenitor cells has been explained by expression of the inflammatory modulating protein TNF- α -stimulated gene/protein-6, which predominantly modulates the early inflammatory phase.²¹

Lipopolysaccharide (LPS) extracted from pathogenic microorganisms, including *Escherichia coli* and *Pseudomonas aeruginosa* (*P. aeruginosa*), is often used as an endotoxin to induce ALI in animal models. BMSCs have been shown to home to and repair both intratracheal LPS-induced intrapulmonary ALI and intravenous LPS/zymosan-induced extra-pulmonary ALI,²² while AMSCs have been found to exert therapeutic effects against *P. aeruginosa* pneumonia.⁴ It can be speculated that the protective mechanism involves inhibition of PGE₂ production and improvements in phagocytosis and the bactericidal properties of macrophages.⁴ BMSCs have also been used successfully in an ovine model of severe acute respiratory distress syndrome (ARDS) caused by *P. aeruginosa* pneumonia. These cells not only improved oxygenation, but also decreased pulmonary edema.¹³

Cobalt gamma-ray radiation has been reported to inhibit the differentiation potential of BMSCs without significantly affecting their paracrine activity, cell proliferation, viability, or homing potential.²³ Zhu *et al.* treated rats with smoke inhalation lung injuries using BMSCs and found that lung vascular endothelial injury and increased permeability were alleviated, predominantly as a result of enhanced angiogenesis, regulated by the notch signaling pathway.²³

To assess the safety of allogeneic BMSC administration in ARDS patients, a phase I clinical trial (NCT01775774) was performed using nine cases. No pre-specified infusion-associated events or treatment-related adverse events were reported, demonstrating good tolerability of allogeneic BMSCs in moderate-to-severe ARDS patients.²⁴ Similar results were obtained in a second clinical trial (NCT01902082) in which ARDS patients were treated with allogeneic AMSCs or a placebo. However, in this study, AMSCs failed to exhibit any significantly beneficial clinical effects other than a reduction in the serum levels of surfactant protein-D.²⁵ In contrast, Chang *et al.* reported the successful clinical application of hUMSCs to treat ARDS in a single patient.²⁶

Gene-modified MSCs for the treatment of ALI

Because MSCs are capable of homing to sites of injury, they are often employed as cellular vehicles for gene delivery and have been developed to treat various diseases, including ALI.²⁷

Angiotensin II, which can be degraded by ACE2, plays an important role in the process of endothelial dysfunction in ALI, and ACE2 deficiency enhances lung injuries in mice.²⁸ When infused into ACE2^{-ly} mice, BMSCs^{ACE2+} (transfected with the ACE2 gene) improved lung histopathology but had additional anti-inflammatory effects, reduced pulmonary vascular permeability, improved endothelial barrier integrity, and normalized lung endothelial nitric oxide synthase expression.^{5,28–34} Furthermore, BMSC^{ACE2+} downregulated pulmonary expression of ICAM-1, VCAM-1, TNF- α , and IL-6, and was more effective in treating bleomycin-induced ALI in a murine model than ACE2 or hUMSC alone.^{5,28,30–34} Interestingly, some factors were decreased in BMSC^{ACE2+}-injected mice, including malondialdehyde, oxidized glutathione, TNF- α , IFN- γ , TGF- β , IL-1, IL-2, IL-6, collagen type 1, MMPs, TIMPs, and hydroxyproline, while other factors were increased, including superoxide dismutase, glutathione, ACE2, and IL-10.^{30–34}

TGF- β , which is recognized as both a fibrogenic and inflammatory cytokine, plays critical roles in various pathophysiological processes and is an independent predictor of RILI.³⁵ Following transfection with the TGF- β type II receptor gene, BMSC^{TGFBR+} migrated into injured lungs and obviously alleviated lung injuries in mice challenged with thoracic irradiation.^{31–34} These results were further confirmed by concentration of factor assays, such as malondialdehyde, hydroxyproline, CTGF, and α -SMA.³¹ Furthermore, BMSC^{TGFBR+} adopted the characteristics of alveolar type II cells at the injury site.^{31–34}

FGF-2 is a multifunctional growth factor found in different tissues and cell types.³⁶ BMSC^{FGF2+} expressing exogenous FGF2 following lentivirus-mediated transduction was used in an LPS-induced murine ALI model. Compared to groups treated with BMSCs alone, LPS-induced lung injury was alleviated in the group treated with BMSCs^{FGF2+}. Furthermore, the histopathological index of lung injury was improved and levels of inflammatory cytokines were reduced.^{32–34}

HGF plays mitogenic, morphogenic, and anti-apoptotic roles in a variety of cells, including most epithelial and endothelial cells.²⁷ HGF enhances lung regeneration and inhibits lung fibrosis.^{27,33} Wang *et al.* reported the significant therapeutic effect of BMSC^{HGF+} in an RILI murine model mediated by reducing the secretion of pro-inflammatory cytokines, including TNF- α , IFN- γ , IL-6,

and ICAM-1, and pro-fibrosis factors, including TGF- β , Col-1 α 1, and Col-3 α 1, while increasing the expression of anti-inflammatory cytokines, including IL-10. BMSC^{HGF+} was found to promote the proliferation of lung epithelial cells, thus protecting against apoptosis and stimulating a significant increase in the expression of endogenous HGF and its receptor, c-Met.^{33,34}

CXCR4, also known as fusin or CD184, is involved in MSC mobilization, but is only expressed on the surface of a small proportion of MSCs. The lack of CXCR4 expression on MSCs may underlie their low homing efficiency toward injured tissues.^{37,38} Using a LPS-induced ALI murine model, Yang *et al.* showed that BMSC^{CXCR4+} increased the efficiency of BMSC mobilization to injury sites. Therefore, BMSCs^{CXCR4+} modified to overexpress CXCR4 improved the therapeutic potential of these cells for the treatment of ALI by increasing self-renewal, homing, and epithelial differentiation.^{37,39}

Del-1 is another critical factor involved in cell migration and infiltration. This molecule inhibits the function of the major leukocyte adhesion receptor LFA-1, which prevents leukocyte adhesion to the endothelium.³⁴ Zhao *et al.* showed that treatment of LPS-induced ALI mice with BMSCs^{Del1+} (allogeneic Del-1-overexpressing BMSCs) significantly decreased the severity of endotoxin-induced lung injury and inflammatory cytokine levels.³⁴

“Cell-free” treatments for ALI

In addition to MSCs themselves, MSC-derived extracellular vesicles have also been extensively investigated for their paracrine effect.⁴⁰ Numerous biological functions of MSCs have been demonstrated as a result of the paracrine effects of extracellular vesicles.^{40–42} According to their size, the vesicles are classified as exosomes (40–120 nm) and microvesicles (MVs; 200–1000 nm).

Microvesicles are circular fragments of membrane released from the endosomal compartment as exosomes or shed from the surface membrane.⁴³ Because the central mechanism of cell-to-cell communication involves the packaging of bioactive factors in MVs, it was thought that the therapeutic potential of MSCs was largely mediated by MVs released from intracellular endosomes.^{43,44} When administered intratracheally in endotoxin-induced ALI mice, MVs improved pulmonary edema and lung protein permeability, reduced neutrophil influx, and decreased MIP-2 levels in bronchoalveolar lavage fluid, demonstrating a reduction in inflammation. However, the therapeutic effects were partially eliminated if MVs were released from low expression KGF BMSCs, suggesting that KGF plays an important role in the underlying mechanism.⁴² As highlighted by Sdrimas *et al.*, despite accumulating

evidence of the use of MSC MVs in lung disease, very little is known about the underlying mechanism.⁴³

In recent years, evidence of the therapeutic effect of MSC conditioned medium (CM) on lung injuries has been reported.^{31,45} BMSC CM has been shown to attenuate lung fibrosis of bleomycin-challenged rats in terms of lung inflammation, fibrotic scores, collagen deposition, and cell apoptosis.⁴⁵ Thus, the paracrine capability of MSCs and their anti-inflammatory and anti-fibrotic mechanisms are now recognized.^{27,46}

Evidence supporting the detrimental effects of MSCs in RILI

Pro-fibrotic cells derived from MSCs

Fibrocytes

Fibrocytes are circulating BMSCs with unique growth characteristics and surface phenotype. This cell type can differentiate into fibroblasts and myofibroblasts following entry into the tissues.⁴⁷ Myofibroblasts in the tumor stroma, known as cancer-associated fibroblasts, participate in the support of tumor growth, angiogenesis, metastasis, and therapy resistance.⁴⁸ Clinical biopsies have shown that fibrocytes exist in the lungs of most idiopathic pulmonary fibrosis (IPF) patients (8/9). Furthermore, the risk of pulmonary fibrosis (PF) increases with the number of fibrocytes in the lungs, although the underlying mechanism is unclear.^{49–53} Fibrocytes are believed to be involved in the pathogenesis of several fibrotic disorders affecting the lungs, liver, kidney, and other organs. Furthermore, they have been implicated as potential biomarkers that are easily detected and quantified from peripheral blood samples.⁵⁴

Pulmonary fibrosis was originally thought to be mediated solely by resident lung fibroblasts.¹ However, increasing data indicates that intrapulmonary recruitment of fibrocytes is directly correlated with increased collagen deposition in the lungs. Phillips *et al.* identified a population of human CD45⁺/Col 1⁺/CXCR4⁺ circulating fibrocytes that migrated to sites of lung injury in response to CXCL12 signaling in models of bleomycin-induced PF.^{49–53,55} Mehrad *et al.* reported that fibrocyte recruitment is mediated by the CXCL12/CXCR4 axis in vitro, and revealed high CXCL12 levels in the plasma and injured lungs of patients with fibrotic interstitial lung disease.⁵⁶ There were significantly more circulating peripheral blood fibrocytes in patients with fibrotic interstitial lung disease than in healthy controls. Furthermore, CXCR4, the predominant chemokine receptor, has been identified on human fibrocytes, and has been found to mediate the influx of these cells into the lung during PF. Regulation of CXCR4 is reported to be mediated by hypoxia and growth

factors, such as PDGF, via the PI3-kinase and mTOR signaling pathways.^{50,57}

Lung hematopoietic progenitor cells

Green fluorescent protein (GFP) bone marrow-chimera mice were employed in studies by Nakashima *et al.* to elucidate the roles of bone marrow-derived cells in bleomycin-induced PF.⁵¹ The results showed an increase in high-GFP expressing cells (GFP^{hi}) in the fibrotic lungs, with phenotypic characteristics of CD11c⁺ dendritic cells and macrophages. CM from these cells was chemotactic for fibroblasts from fibrotic lungs in vitro, and adoptive transfer of GFP^{hi} exacerbated disease in a bleomycin-induced mouse model of PF. It was also observed that GFP^{hi} differentiated from lung hematopoietic progenitor cells (LHPCs) (c-Kit⁺/Sca1⁻/Lin⁻ and GFP⁺), with numbers increasing rapidly in response to bleomycin treatment. These findings indicated that LHPCs represent a novel therapeutic approach for chronic fibrotic lung diseases.

ABCG2⁺ MSCs

Adult lung tissue contains a population of perivascular ABCG2⁺ MSCs, which are proven precursors of myofibroblasts distinct from NG2 pericytes. Marriott *et al.* found that resident lung MSCs were increased in human PF samples.⁵² ABCG2⁺ MSCs were found to increase in number and localize to the interstitial areas during fibrotic microvessel remodeling in bleomycin-challenged mice. Furthermore, these cells responded to bleomycin treatment by expressing pro-fibrotic genes. Thus, ABCG2⁺ lung MSCs are implicated as a novel cell population that contributes to detrimental myofibroblast-mediated remodeling during PF.

Summary and prospects

Despite the considerable number of studies that have been performed, the clinical management of RILI using a stem cell approach requires further investigation. Based on the reports of studies focusing on the effects of MSCs in RILI, ALI, ARDS, and chronic obstructive pulmonary disease models (as shown in Table 1), we can conclude that:

- 1 There have been no serious adverse events reported in any pre-clinical or clinical study of MSC reported to date.
- 2 MSCs shown to elicit a therapeutic potential in RILI were predominantly from allogeneic donor tissues, such as AMSCs, BMSCs, and UMSCs.
- 3 The majority of MSCs had a deleterious effect by promoting RILI and other endogenous forms of the lung, such as fibrocytes, LHPCs, and BMSCs^{ABCG2⁺}. These cell populations produce detrimental pro-inflammatory and

Table 1 Different types of stem cells used in lung injury models produce various effects

Study	SCs	Origins of SCs	Biomarkers	Gene modification	Animal models	Treatment phases	Effect of SC on lung injury
Mao et al. 2015 ⁴	ASC	Allogeneic	CD34(+), CD45(+), CD90(+), CD105(+)	—	<i>P. aeruginosa</i> pneumonia injury	1 hour after lung injury	Therapeutic
Min et al. 2015 ⁵	hUMSC	Heterogenic	CD29(+), CD44(+), CD105(+); CD34(-), CD45(-), CD86(-)	ACE2	Bleomycin-induced lung injury	—	Therapeutic
Zhao et al. 2014 ⁶	PMSC	Heterogenic	CD29(+), CD44(+), CD73(+), CD90(+), CD105(+), CD14(-), CD19(-), CD34(-), CD45(-), HLA-DR(-)	—	Bronchiolitis obliterans injury	3 days after lung injury	Therapeutic
Dong et al. 2015 ¹⁰	ASC	Heterogenic	CD73(+), CD90(+), CD105(+); CD11b(-), CD19(-), CD34(-), CD45(-), HLA-DR(-)	—	Radiation-induced pulmonary fibrosis	2 hours after lung injury	Therapeutic
Jiang et al. 2015 ¹¹	ASC	Allogeneic	CD29(+), CD44(+); CD11b(-), CD45(-)	—	Acute radiation-induced lung injury	2 hours after lung injury	Therapeutic
Wang et al. 2014 ¹²	hUMSC	Heterogenic	CD29(+), CD34(+), CD44(+), CD73(+), CD90(+), CD105(+); CD45(-)	—	Radiation pneumonitis	Prevention group: 24 hours before lung injury; Treatment group: 24 hours after lung injury	Therapeutic
Asmussen et al. 2014 ¹³	hUMSC	Heterogenic	—	—	Bacterial pneumonia (ARDS)	1 hour after lung injury	Therapeutic
Zhen et al. 2008 ¹⁴	BMSC	Allogeneic	—	—	Papain-induced pulmonary emphysema	1 day after lung injury	Therapeutic
Zickri et al. 2014 ¹⁶	hUMSC	Heterogenic	CD45(+), CD105(+), CD133(+)	—	Amiodarone-induced lung injury	4 weeks after lung injury	Therapeutic
Zhao et al. 2008 ¹⁸	BMSC	Allogeneic	—	—	Bleomycin-induced lung injury	12 hours after lung injury	Therapeutic
Liu et al. 2015 ²²	Mouse BMSC	Allogeneic	—	—	Acute lung injury	4 hours after lung injury	Therapeutic
Zhu et al. 2015 ²³	Rat BMSC	Allogeneic	CD45(+), VEGF(+), bFGF(+), CD44(-), CD90(-)	—	Smoke-induced lung injury	—	Therapeutic
He et al. 2014 ²⁹	Mouse BMSC	Allogeneic	ACE2(+), β -actin(+)	ACE2	LPS-induced lung injury	4 hours after lung injury	Therapeutic
Liu et al. 2014 ³⁰	hUMSC	Heterogenic	CD29(+), CD44(+), CD105(+); CD34(-), CD45(-), CD86(-)	ACE2	Acute lung ischemia-reperfusion injury	45 minutes, 6 hours and 24 hours after lung injury	Therapeutic
Xue et al. 2013 ³¹	Mouse BMSC	Allogeneic	Sca-1(+), CD29(+), CD44(+), sT β R(+); CD11b(-), CD31(-), CD45(-), CD117(-)	sT β R	Radiation-induced lung injury	Zero days or 14 days after radiation	Therapeutic

Table 1 Continued

Study	SCs	Origins of SCs	Biomarkers	Gene modification	Animal models	Treatment phases	Effect of SC on lung injury
Zhao et al. 2015 ³²	Mouse BMSC	Allogeneic	CD73(+), CD90(+), CD105(+), CD106(+), FGF2(+), CD11b(-), CD14(-), CD34(-), CD45(-)	FGF2	LPS-induced lung injury	1 hour after lung injury	Therapeutic
Wang et al. 2013 ³³	hUMSC	Heterogenic	CD29(+), CD73(+), CD166(+), HGF(+), HLAABC(+); CD31(-), CD34(-), CD45(-), HLA-DR(-)	HGF	Radiation-induced lung injury	6 hours after lung injury	Therapeutic
Zhao et al. 2014 ³⁴	Mouse BMSC	Allogeneic	CD73(+), CD90(+), CD105(+), CD106(+); CD11b(-), CD14(-), CD34(-), CD45(-)	DEL-1	LPS-induced lung injury	1 hour after lung injury	Therapeutic
Yang et al. 2015 ³⁷	BMSC	Allogeneic	CD29(+), CD34(+), CD45(+), CD90(+), VCAM-1(+), ICAM-1(+)	CXCR4	LPS-induced lung injury	1 hour after lung injury	Therapeutic
Hayes et al. 2015 ³⁸	hUMSC	Heterogenic	—	—	Ventilator-induced lung injury	15–30 minutes after lung injury	Therapeutic
Liu et al. 2014 ³⁹	hUMSC	Heterogenic	CD49c(+), CD49d(+), CD49e(+), CD49f(+), CD73(+), CD90(+), CD105(+); CD14(-), CD19(-), CD34(-), CD45(-), HLA-DR(-)	—	Hyperoxia-induced neonatal lung injury	On postnatal day 5	Therapeutic
Wilson et al. 2015 ²⁴	Human BMSC	Allogeneic	—	—	Moderate-to-severe ARDS patients	120 hours after lung injury	Therapeutic
Zheng et al. 2014 ²⁵	Human ASCs	Allogeneic	CD73(+), CD90(+), CD105(+); CD34(-), CD45(-), HLA-DR(-)	—	ARDS patients	1–48 hours of enrollment	Therapeutic
Chang et al. 2014 ²⁶	hUMSC	Allogeneic	CD29(+), CD44(+), CD73(+), CD105(+), CD166(+); CD14(-), CD34(-), CD45(-), HLA-DR(-)	—	ARDS patient	On the 114th HD	Therapeutic
Andersson-Sjoland et al. 2008 ⁴⁹	Fibrocytes	Autologous	CXCR4(+), proly I-4-hydroxylase(+), CD34(+), SMA(+), CD45RO(+)	—	IPF patient	—	Deleterious
Strieter et al. 2009 ⁵⁰	Fibrocytes	Autologous	CD45(+), collagen I(+), CXCR4(+)	—	Bleomycin-induced pulmonary fibrosis	—	Deleterious
Nakashima et al. 2013 ⁵¹	LHPC	Allogeneic	CD11c(+), CD45(-), MHC-II(+), F4/80(+), CXCR4(+); CD11b(-), CD34(-), Sca1(-), c-Kit(-), Ly6c(-), Col 1(-)	—	Bleomycin-induced pulmonary fibrosis	2 days after lung injury	Therapeutic

Table 1 Continued

Study	SCs	Origins of SCs	Biomarkers	Gene modification	Animal models	Treatment phases	Effect of SC on lung injury
Marriot <i>et al.</i> 2014 ⁵²	Mouse MSC	Autologous	ABCG2(+), CD44(+), CD73(+), CD105(+), CD106(+), Scd(+); c-kit(-), CD14(-), CD45(-)	—	Bleomycin-induced lung injury	Inflammatory phase post-injury	Deleterious
Antoniou <i>et al.</i> 2010 ⁶⁰	Mouse BMSC	Autologous	CD29(+), CD44(+), CD73(+), CD90(+), CD105(+), CD146(+); CD34(-), CD45(-)	—	IPF patient	—	Deleterious
Xu <i>et al.</i> 2007 ⁶¹	Mouse BMSC	Autologous	CD44(+), CD105(+), CXCR4(+); CD11b(-), CD45(-)	—	Bleomycin-induced lung injury	1, 3, 7, and 14 days after lung injury	Deleterious
Sun <i>et al.</i> 2014 ⁶²	BMSC	Allogeneic	CD29(+), CD44(+), CD73(+), CD90(+); CD11b(-), CD34(-), CD45(-), CD79(-), CD133(-)	—	HCl-induced acute lung injury	24 hours after lung injury	Therapeutic

ARDS, acute respiratory distress syndrome; ASC, adipose tissue-derived mesenchymal stem cells; BMSC, bone marrow-derived mesenchymal stem cells; HD, hospital day; hUMSC, human mesenchymal stem cells; HUMSC, human umbilical cord mesenchymal stem cells; IPF, idiopathic pulmonary fibrosis; LHP, lung hematopoietic progenitor cells; LPS, lipopolysaccharide; PMSC, placenta-derived human mesenchymal stem cells; sTβR, TGF-β type II receptor.

pro-fibrotic effects under the regulation of CXCL12/CXCR4 and Wnt/β-catenin signaling.

4 The data described in this report is preliminary, obtained from non-RILI animal models, such as bleomycin-induced, LPS-induced, and HCl-induced lung injuries.

However, several points remain unresolved, and these are discussed as follows (Table 1).

Issues of cell selection

Dong *et al.* proposed that AMSCs are beneficial for RILI, while Sun *et al.* demonstrated that BMSCs are not; however, whether AMSCs are more effective for this purpose than BMSCs remains to be confirmed.^{10,62} Wang *et al.* demonstrated that infusion of hUMSCs improved RILI.¹² Shu *et al.* reported that administration of BMSCs promoted lung fibrosis by increasing the number of myofibroblasts.⁵³ These conflicting findings may be explained by differences between the studies. Regarding MSC preparation methods, Dong *et al.* used CD73, CD90, and CD105 as cell-specific markers, while Wang *et al.* used CD29 and CD44 in addition to CD73, CD90, and CD105.^{10,12} Sun *et al.* used CD29, CD44, CD73, and CD90 and Marriott *et al.* used ABCG2.^{52,62} In contrast, Shu *et al.* used GFP-positive expression as the only marker of BMSCs.⁵³ These different MSC selection methods would result in distinct effects on lung injuries; thus, we hypothesize that a more beneficial MSC population exists within the mixed population of MSCs infused, and that the different proportions of this beneficial MSC population in the total population of MSCs may lead to diverse effects on RILI and other lung injuries. However, the identification and characterization of the beneficial MSC population remains a challenge.

Issues of phase selection

The pathophysiological process of RILI is composed of several stages/phases. Foskett *et al.* described the toxic effects exerted by bleomycin as “damage/necrosis of the alveolar epithelium, tissue edema, inflammatory cell infiltration, and pulmonary fibrosis.”²¹ Royce *et al.* concluded that inflammatory cell infiltration, cytokine release, epithelial damage, airway/lung remodeling, and fibrosis are all central features of inflammatory lung disorders.⁶³ However, Graves *et al.* considered that the process of RILI has three phases: injury, inflammation, and repair.¹ Based on the findings of this review, we believe that MSCs exhibit therapeutic effects primarily in the inflammation phase.^{3,10–13} While some MSCs seem to exert detrimental effects during the repair phase in RILI,^{49–53,55} we conclude that administration of MSCs in different phases is likely to result in

distinct therapeutic outcomes; however, more research is necessary.

Issues of gene modification in MSCs

Some reports claim that modifying certain genes in the administered MSCs can improve outcomes in patients with RILI or other lung injuries,^{5,28–34} while others have shown contradictory data.^{37,39} However, all conclude that modification of some genes significantly alters the activity and bio-behavior of the administered MSCs. Identification of the genes that should be changed in MSCs to achieve optimal results is essential. The potential effects of multiple gene modifications and the optimal method of gene modification are also critical issues that warrant further investigation.

Overall, investigation into the effect of MSC transplantation on RILI and other lung injuries has made good progress in recent years. Some of the mechanisms involved have become clearer and MSCs are already being evaluated in clinical trials. However, more questions are likely to arise as advances are made. The role of MSCs as a sword or an accomplice in RILI remains unresolved and more consistent and comparable research is necessary for further clarification that may lead to the development of successful treatment strategies.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (Nos. 30800190 and 81372441) and the Natural Science Foundation of Hubei Province (No. 2011CDB488).

Disclosure

No authors report any conflicts of interest.

References

- Graves PR, Siddiqui F, Anscher MS, Movsas B. Radiation pulmonary toxicity: From mechanisms to management. *Semin Radiat Oncol* 2010; **20**: 201–7.
- You H, Wei L, Sun WL *et al.* The green tea extract epigallocatechin-3-gallate inhibits irradiation-induced pulmonary fibrosis in adult rats. *Int J Mol Med* 2014; **34**: 92–102.
- Rojas M, Xu J, Woods CR *et al.* Bone marrow-derived mesenchymal stem cells in repair of the injured lung. *Am J Respir Cell Mol Biol* 2005; **33**: 145–52.
- Mao YX, Xu JF, Seeley EJ *et al.* Adipose tissue-derived mesenchymal stem cells attenuate pulmonary infection caused by *Pseudomonas aeruginosa* via inhibiting overproduction of prostaglandin E2. *Stem Cells* 2015; **33**: 2331–42.
- Min F, Gao F, Li Q, Liu Z. Therapeutic effect of human umbilical cord mesenchymal stem cells modified by angiotensin-converting enzyme 2 gene on bleomycin-induced lung fibrosis injury. *Mol Med Rep* 2015; **11**: 2387–96.
- Zhao Y, Gillen JR, Harris DA, Kron IL, Murphy MP, Lau CL. Treatment with placenta-derived mesenchymal stem cells mitigates development of bronchiolitis obliterans in a murine model. *J Thorac Cardiovasc Surg* 2014; **147**: 1668–77.e5.
- Steens J, Zuk M, Benchellal M *et al.* In vitro generation of vascular wall-resident multipotent stem cells of mesenchymal nature from murine induced pluripotent stem cells. *Stem Cell Reports* 2017; **8**: 919–32.
- Ding DC, Shyu WC, Lin SZ. Mesenchymal stem cells. *Cell Transplant* 2011; **20**: 5–14.
- Monsel A, Zhu YG, Gennai S, Hao Q, Liu J, Lee JW. Cell-based therapy for acute organ injury: Preclinical evidence and ongoing clinical trials using mesenchymal stem cells. *Anesthesiology* 2014; **121**: 1099–121.
- Dong LH, Jiang YY, Liu YJ *et al.* The anti-fibrotic effects of mesenchymal stem cells on irradiated lungs via stimulating endogenous secretion of HGF and PGE2. *Sci Rep* 2015; **5**: 8713.
- Jiang X, Jiang X, Qu C *et al.* Intravenous delivery of adipose-derived mesenchymal stromal cells attenuates acute radiation-induced lung injury in rats. *Cytotherapy* 2015; **17**: 560–70.
- Wang R, Zhu CZ, Qiao P *et al.* Experimental treatment of radiation pneumonitis with human umbilical cord mesenchymal stem cells. *Asian Pac J Trop Med* 2014; **7**: 262–6.
- Asmussen S, Ito H, Traber DL *et al.* Human mesenchymal stem cells reduce the severity of acute lung injury in a sheep model of bacterial pneumonia. *Thorax* 2014; **69**: 819–25.
- Zhen G, Liu H, Gu N, Zhang H, Xu Y, Zhang Z. Mesenchymal stem cells transplantation protects against rat pulmonary emphysema. *Front Biosci* 2008; **13**: 3415–22.
- Klein D, Schmetter A, Imsak R *et al.* Therapy with multipotent mesenchymal stromal cells protects lungs from radiation-induced injury and reduces the risk of lung metastasis. *Antioxid Redox Signal* 2016; **24**: 53–69.
- Zickri MB, Fadl SG, Metwally HG. Comparative study between intravenous and intraperitoneal stem cell therapy in amiodarone induced lung injury in rat. *Int J Stem Cells* 2014; **7**: 1–11.
- Lalu MM, Moher D, Marshall J *et al.* Efficacy and safety of mesenchymal stromal cells in preclinical models of acute lung injury: A systematic review protocol. *Syst Rev* 2014; **3**: 48.
- Zhao F, Zhang YF, Liu YG *et al.* Therapeutic effects of bone marrow-derived mesenchymal stem cells engraftment on

- bleomycin-induced lung injury in rats. *Transplant Proc* 2008; **40** (5): 1700.
- 19 Ho MS, Mei SH, Stewart DJ. The immunomodulatory and therapeutic effects of mesenchymal stromal cells for acute lung injury and sepsis. *J Cell Physiol* 2015; **230**: 2606–17.
 - 20 Kong QF, Sun B, Bai SS *et al.* Administration of bone marrow stromal cells ameliorates experimental autoimmune myasthenia gravis by altering the balance of Th1/Th2/Th17/Treg cell subsets through the secretion of TGF- β . *J Neuroimmunol* 2009; **207**: 83–91.
 - 21 Foskett AM, Bazhanov N, Ti X, Tiblow A, Bartosh TJ, Prockop DJ. Phase-directed therapy: TSG-6 targeted to early inflammation improves bleomycin-injured lungs. *Am J Physiol Lung Cell Mol Physiol* 2014; **306**: L120–31.
 - 22 Liu L, He H, Liu A *et al.* Therapeutic effects of bone marrow-derived mesenchymal stem cells in models of pulmonary and extrapulmonary acute lung injury. *Cell Transplant* 2015; **24**: 2629–42.
 - 23 Zhu F, Wang J, Qiu X, Li J, Xia Z. Smoke inhalation injury repaired by a bone marrow-derived mesenchymal stem cell paracrine mechanism: Angiogenesis involving the Notch signaling pathway. *J Trauma Acute Care Surg* 2015; **78**: 565–72.
 - 24 Wilson JG, Liu KD, Zhuo H *et al.* Mesenchymal stem (stromal) cells for treatment of ARDS: A phase 1 clinical trial. *Lancet Respir Med* 2015; **3**: 24–32.
 - 25 Zheng G, Huang L, Tong H *et al.* Treatment of acute respiratory distress syndrome with allogeneic adipose-derived mesenchymal stem cells: A randomized, placebo-controlled pilot study. *Respir Res* 2014; **15**: 39.
 - 26 Chang Y, Park SH, Huh JW, Lim CM, Koh Y, Hong SB. Intratracheal administration of umbilical cord blood-derived mesenchymal stem cells in a patient with acute respiratory distress syndrome. *J Korean Med Sci* 2014; **29**: 438–40.
 - 27 Meirelles Lda S, Fontes AM, Covas DT, Caplan AI. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Rev* 2009; **20**: 419–27.
 - 28 He HL, Liu L, Chen QH *et al.* MSCs modified with ACE2 restore endothelial function following LPS challenge by inhibiting the activation of RAS. *J Cell Physiol* 2015; **230**: 691–701.
 - 29 He H, Liu L, Chen Q *et al.* Mesenchymal stem cells overexpressing angiotensin-converting enzyme 2 rescue lipopolysaccharide-induced lung injury. *Cell Transplant* 2014; **24**: 1699–715.
 - 30 Liu F, Gao F, Li Q, Liu Z. The functional study of human umbilical cord mesenchymal stem cells harbouring angiotensin-converting enzyme 2 in rat acute lung ischemia-reperfusion injury model. *Cell Biochem Funct* 2014; **32**: 580–9.
 - 31 Xue J, Li X, Lu Y *et al.* Gene-modified mesenchymal stem cells protect against radiation-induced lung injury. *Mol Ther* 2013; **21**: 456–65.
 - 32 Zhao YF, Luo YM, Xiong W *et al.* Mesenchymal stem cell-based FGF2 gene therapy for acute lung injury induced by lipopolysaccharide in mice. *Eur Rev Med Pharmacol Sci* 2015; **19**: 857–65.
 - 33 Wang H, Yang YF, Zhao L *et al.* Hepatocyte growth factor gene-modified mesenchymal stem cells reduce radiation-induced lung injury. *Hum Gene Ther* 2013; **24**: 343–53.
 - 34 Zhao YF, Xiong W, Wu XL. Mesenchymal stem cell-based developmental endothelial locus-1 gene therapy for acute lung injury induced by lipopolysaccharide in mice. *Mol Med Rep* 2014; **9**: 1583–9.
 - 35 Biernacka A, Dobaczewski M, Frangogiannis NG. TGF- β signaling in fibrosis. *Growth Factors* 2011; **29**: 196–202.
 - 36 Volckaert T, De Langhe SP. Wnt and FGF mediated epithelial-mesenchymal crosstalk during lung development. *Dev Dyn* 2015; **244**: 342–66.
 - 37 Yang JX, Zhang N, Wang HW, Gao P, Yang QP, Wen QP. CXCR4 receptor overexpression in mesenchymal stem cells facilitates treatment of acute lung injury in rats. *J Biol Chem* 2015; **290**: 1994–2006.
 - 38 Duda DG, Kozin SV, Kirkpatrick ND, Xu L, Fukumura D, Jain RK. CXCL12 (SDF1 α)-CXCR4/CXCR7 pathway inhibition: An emerging sensitizer for anticancer therapies. *Clin Cancer Res* 2011; **17**: 2074–80.
 - 39 Sun Z, Wang C, Shi C *et al.* Activated Wnt signaling induces myofibroblast differentiation of mesenchymal stem cells, contributing to pulmonary fibrosis. *Int J Mol Med* 2014; **33**: 1097–109.
 - 40 Dittmer J, Leyh B. Paracrine effects of stem cells in wound healing and cancer progression (Review). *Int J Oncol* 2014; **44**: 1789–98.
 - 41 Akram KM, Samad S, Spiteri MA, Forsyth NR. Mesenchymal stem cells promote alveolar epithelial cell wound repair in vitro through distinct migratory and paracrine mechanisms. *Respir Res* 2013; **14**: 9.
 - 42 Zhu YG, Feng XM, Abbott J *et al.* Human mesenchymal stem cell microvesicles for treatment of Escherichia coli endotoxin-induced acute lung injury in mice. *Stem Cells* 2014; **32**: 116–25.
 - 43 Sdrimas K, Kourembanas S. MSC microvesicles for the treatment of lung disease: A new paradigm for cell-free therapy. *Antioxid Redox Signal* 2014; **21**: 1905–15.
 - 44 Akyurekli C, Le Y, Richardson RB, Fergusson D, Tay J, Allan DS. A systematic review of preclinical studies on the therapeutic potential of mesenchymal stromal cell-derived microvesicles. *Stem Cell Rev* 2015; **11**: 150–60.
 - 45 Shen Q, Chen B, Xiao Z *et al.* Paracrine factors from mesenchymal stem cells attenuate epithelial injury and lung fibrosis. *Mol Med Rep* 2015; **11**: 2831–7.
 - 46 Li J, Huang S, Wu Y *et al.* Paracrine factors from mesenchymal stem cells: A proposed therapeutic tool for acute lung injury and acute respiratory distress syndrome. *Int Wound J* 2014; **11**: 114–21.
 - 47 Quan TE, Cowper S, Wu SP, Bockenstedt LK, Bucala R. Circulating fibrocytes: Collagen-secreting cells of the peripheral blood. *Int J Biochem Cell Biol* 2004; **36**: 598–606.

- 48 Tao L, Huang G, Song H, Chen Y, Chen L. Cancer associated fibroblasts: An essential role in the tumor microenvironment. *Oncol Lett* 2017; **14**: 2611–20.
- 49 Andersson-Sjöland A, de Alba CG, Nihlberg K *et al.* Fibrocytes are a potential source of lung fibroblasts in idiopathic pulmonary fibrosis. *Int J Biochem Cell Biol* 2008; **40**: 2129–40.
- 50 Strieter RM, Keeley EC, Hughes MA, Burdick MD, Mehrad B. The role of circulating mesenchymal progenitor cells (fibrocytes) in the pathogenesis of pulmonary fibrosis. *J Leukoc Biol* 2009; **86**: 1111–8.
- 51 Nakashima T, Liu T, Yu H *et al.* Lung bone marrow-derived hematopoietic progenitor cells enhance pulmonary fibrosis. *Am J Respir Crit Care Med* 2013; **188**: 976–84.
- 52 Marriott S, Baskir RS, Gaskill C *et al.* ABCG2pos lung mesenchymal stem cells are a novel pericyte subpopulation that contributes to fibrotic remodeling. *Am J Physiol Cell Physiol* 2014; **307**: C684–98.
- 53 Shu HK, Yoon Y, Hong S *et al.* Inhibition of the CXCL12/CXCR4-axis as preventive therapy for radiation-induced pulmonary fibrosis. *PLoS One* 2013; **8**: e79768.
- 54 Maharaj S, Shimbori C, Kolb M. Fibrocytes in pulmonary fibrosis: A brief synopsis. *Eur Respir Rev* 2013; **22**: 552–7.
- 55 Phillips RJ, Burdick MD, Hong K *et al.* Circulating fibrocytes traffic to the lungs in response to CXCL12 and mediate fibrosis. *J Clin Invest* 2004; **114**: 438–46.
- 56 Mehrad B, Burdick MD, Zisman DA, Keane MP, Belperio JA, Strieter RM. Circulating peripheral blood fibrocytes in human fibrotic interstitial lung disease. *Biochem Biophys Res Commun* 2007; **353**: 104–8.
- 57 Mehrad B, Burdick MD, Strieter RM. Fibrocyte CXCR4 regulation as a therapeutic target in pulmonary fibrosis. *Int J Biochem Cell Biol* 2009; **41**: 1708–18.
- 58 Hayes M, Masterson C, Devaney J *et al.* Therapeutic efficacy of human mesenchymal stromal cells in the repair of established ventilator-induced lung injury in the rat. *Anesthesiology* 2015; **122**: 363–73.
- 59 Liu L, Mao Q, Chu S *et al.* Intranasal versus intraperitoneal delivery of human umbilical cord tissue-derived cultured mesenchymal stromal cells in a murine model of neonatal lung injury. *Am J Pathol* 2014; **184**: 3344–58.
- 60 Antoniou KM, Papadaki HA, Soufla G *et al.* Investigation of bone marrow mesenchymal stem cells (BM MSCs) involvement in idiopathic pulmonary fibrosis (IPF). *Respir Med* 2010; **104**: 1535–42.
- 61 Xu J, Mora A, Shim H, Stecenko A, Brigham KL, Rojas M. Role of the SDF-1/CXCR4 axis in the pathogenesis of lung injury and fibrosis. *Am J Respir Cell Mol Biol* 2007; **37**: 291–9.
- 62 Sun Z, Gong X, Zhu H *et al.* Inhibition of Wnt/beta-catenin signaling promotes engraftment of mesenchymal stem cells to repair lung injury. *J Cell Physiol* 2014; **229**: 213–24.
- 63 Royce SG, Moodley Y, Samuel CS. Novel therapeutic strategies for lung disorders associated with airway remodelling and fibrosis. *Pharmacol Ther* 2014; **141**: 250–60.