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Intravenous delivery of adipose-derived mesenchymal stromal cells attenuates acute radiation-induced lung injury in rats

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Abstract

Background aims. Radiation-induced lung injury (RILI) commonly occurs in patients with thoracic cancer. However, an effective treatment option has not yet been established. Adipose-derived mesenchymal stromal cells (Ad-MSCs) have significant potential for clinical use, but their role in RILI is currently unknown. We aimed to evaluate the therapeutic capacity of Ad-MSCs to heal acute RILI in rats. *Methods.* Sprague-Dawley rats were used in this study. Rat Ad-MSCs were delivered through the tail veins of rats 2 h after thorax irradiation. Lung histopathologic findings, pulmonary levels of inflammatory cytokines (interleukin [IL]-1, IL-6, IL-10 and tumor necrosis factor-α), pro-fibrotic factors (transforming growth factor [TGF]-β1, connective tissue growth factor, α-smooth muscle actin and type 1 collagen), pro- or anti-apoptotic mediators (Bcl-2, Bax and caspase-3) and the multifunctional factor hepatocyte growth factor were evaluated after Ad-MSC transplant. *Results.* Intravenous delivery of Ad-MSCs attenuated acute RILI. Further studies showed that Ad-MSCs had anti-inflammation and anti-fibrotic effects and maintained lung epithelium integrity, as indicated by reduced serum levels of the pro-inflammatory cytokines IL-1, IL-6 and tumor necrosis factor-α, increased levels of the anti-inflammatory cytokine IL-10, and downregulated transforming growth factor -β1, α-smooth muscle actin and type 1 collagen levels in irradiated lung tissues. Ad-MSCs also regulated the expression of pro- and anti-apoptotic mediators (Bcl-2, Bax and caspase-3) to protect lung cells from apoptosis. *Conclusions.* Intravenous Ad-MSC delivery attenuated acute RILI through anti-inflammation, anti-fibrosis and anti-apoptosis mechanisms.

Key Words: *adipose tissue, cell-based transplantation, mesenchymal stromal cells, radiation-induced lung injury*

Introduction

Radiation-induced lung injury (RILI) is a common major obstacle in thoracic cancer radiotherapy [1,2]. The clinical incidence of radiation-induced pneumonitis ranges from 5% to 10% [3,4]. RILI develops through a complex pathological process, resulting in excessive inflammation or extracellular matrix deposition in the lung interstitium, ultimately leading to impaired lung function and respiratory failure [5]. Thus, it is important to alleviate RILI to improve tumor control and the patient's quality of life. The current primary approach to manage RILI is hormone treatments to temporarily suppress inflammation [2]. However, there is no known effective therapeutic strategy for RILI.

Mesenchymal stromal cells (MSCs) have significant clinical potential [6–8]. Recent studies have demonstrated that engrafted stem cells have beneficial effects in injured lung tissues [9,10]. Radiation exposure of the lung leads to fibrosis, and fibrosis is a complex pathology driven by numerous biological factors, such as chronic inflammation and hypoxia. MSC anti-fibrotic functions have been described in various organs [11]. MSC cellular therapy would be an ideal approach to treat RILI. However, the clinical use of bone marrow–derived MSCs has presented problems, including pain, morbidity and low cell number on harvest [12]. Adipose-derived mesenchymal stromal cells (Ad-MSCs) have also been widely studied because of their ease of isolation, abundant distribution and low immunogenicity [13,14]. In addition, Ad-MSCs have been shown to have expansion capacity superior to that of bone marrow–derived MSCs *in vitro* [15,16]. Therefore,

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Ad-MSCs are a potential candidate for autologous transplant in lung disease. Moreover, autologous Ad-MSC application is free from ethical or security issues.

The therapeutic potential and usefulness of Ad-MSC delivery in RILI treatment has not been studied. We studied the effects of intravenous (IV) delivery of Ad-MSCs on acute RILI in a Sprague-Dawley rat model.

Methods

Ethical approval

Sprague-Dawley rats (female; weight, 180–220 g) were purchased from the Laboratory Animal Center of the Academy of Military Medical Sciences (Beijing, China). All experiments involving animal subjects were performed in accordance with guidelines approved by our local animal care and use committee.

Ad-MSC isolation, culture and characterization

Fat tissue was obtained from subcutaneous adipose tissue in the inguinal groove of female Sprague-Dawley rats under sterile conditions. Ad-MSCs were separated by a 200-mesh strainer and suspended in complete medium (90% Dulbecco's modified Eagle's medium-low glucose/F-12+10% fetal bovine serum) (Gibco) and grown with 5% CO_2 in a humidified atmosphere at 37°C, as described previously [17]. Cells were passaged every 2 to 3 days, and were then harvested at passage 3 for characterization, identification and IV delivery.

The phenotype of Ad-MSCs was evaluated by means of flow cytometry analysis with the use of anti-rat cluster of differentiation (CD)11b-phycoerythrin (PE), CD29-PE, CD44–fluorescein isothiocyanate (FITC) and CD45–antigen-presenting cells. Mouse immunoglobulin G1–FITC and PE were used as isotype controls. All antibodies were purchased from eBioscience. Cells were harvested at passage 3 and plated into six-well plates. Adipogenic, osteogenic and chrondrogenic medium (Trevigen) was added to each well under standard culture conditions according to the manufacturer's protocol. Cells in the remaining wells served as controls. Adipogenesis, osteogenesis and chondrogenesis were confirmed by oil red O, alizarin red and alcian blue staining, respectively.

Experimental design

Rats received a single local dose of radiation with 15 Gy of x-rays to the right thorax (160 kV, 25 mA, 1.25 Gy/min, RS-2000 Pro Biological Irradiator, Rad-Source). The remainder of the body was shielded by lead strips.

Ninety rats were used in the study. Animals were randomly divided into three groups ($n = 30$ per group). After thoracic irradiation, animals received a 0.5-mL saline injection (radiation+phosphate-buffered saline [PBS] group), 5×10^6 Ad-MSCs in a 0.5-mL saline injection (radiation+MSC group), or were used as normal controls. Animals were injected through the tail vein within 2 h after irradiation. Peripheral blood and lung tissues were collected from rats on days 1, 3, 7, 14 and 28 for analysis.

Tracing experiments

The cell tracker CM-Dil (Invitrogen Inc) was dissolved in dimethyl sulfoxide (Invitrogen) and added to passage 3 Ad-MSCs according to the manufacturer's protocol. A total of 5×10^6 cells were intravenously injected into irradiated and normal rats. The distribution of labeled Ad-MSCs was determined by use of the Xenogen (IVIS, Lumina, Caliper Life Sciences) *in vivo* optical imaging technique, and frozen lung sections were examined my means of fluorescence microscopy (Olympus).

Histopathology

For histological examination, irradiated lung tissue was fixed in 10% neutral-buffered formalin for 48 h, paraffin-embedded and sectioned at an average thickness of 5 μm. The sections were stained with hematoxylin and eosin and Masson's trichrome. The sections were semi-quantitatively analyzed by two blinded observers using five random images per group.

Enzyme-linked immunosorbent assay

Serum was collected from the peripheral blood by centrifugation. IL-1, IL-6, IL-10, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β1 and hepatocyte growth factor (HGF) (R&D Systems) levels were measured by enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions. For hydroxyproline analysis, the lung tissues were homogenized and assayed by use of hydroxyproline ELISA kits (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions.

Immunohistochemistry

Paraffin-embedded lung sections were de-waxed and rehydrated before antigen retrieval. The sections were incubated in 0.3% H_2O_2 to block endogenous