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Extracellular superoxide dismutase increased the therapeutic potential of human mesenchymal stromal cells in radiation pulmonary fibrosis

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Abstract

Background aims. Pulmonary fibrosis induced by irradiation is a significant problem of radiotherapy in cancer patients. Extracellular superoxide dismutase (SOD3) is found to be predominantly and highly expressed in the extracellular matrix of lung and plays a pivotal role against oxidative damage. Early administration of mesenchymal stromal cells (MSCs) has been demonstrated to reduce fibrosis of damaged lung. However, injection of MSCs at a later stage would be involved in fibrosis development. The present study aimed to determine whether injection of human umbilical cord–derived MSCs (UC-MSCs) over-expressing SOD3 at the established fibrosis stage would have beneficial effects in a mice model of radiation pulmonary fibrosis. *Methods.* Herein, pulmonary fibrosis in mice was induced using Cobalt-60 (^{60}Co) irradiator with 20 Gy, followed by intravenous injection of UC-MSCs, transduced or not to express SOD3 at 2 h (early delivery) and 60 day (late delivery) post-irradiation, respectively. *Results.* Our results demonstrated that the early administration of UC-MSCs could attenuate the microscopic damage, reduce collagen deposition, inhibit (myo)fibroblast proliferation, reduce inflammatory cell infiltration, protect alveolar type II (AE2) cell injury, prevent oxidative stress and increase antioxidant status, and reduce profibrotic cytokine level in serum. Furthermore, the early treatment with SOD3-infected UC-MSCs resulted in better improvement. However, we failed to observe the therapeutic effects of UC-MSCs, transduced to express SOD3, during established fibrosis. *Conclusion.* Altogether, our results demonstrated that the early treatment with UC-MSCs alone significantly reduced radiation pulmonary fibrosis in mice through paracrine effects, with further improvement by administration of SOD3-infected UC-MSCs, suggesting that SOD3-infected UC-MSCs may be a potential cell-based gene therapy to treat clinical radiation pulmonary fibrosis.

Key Words: *extracellular superoxide dismutase, human umbilical cord–derived mesenchymal stromal cells, oxidative damage, radiation pulmonary fibrosis, reactive oxygen species*

Introduction

Alveolitis/pneumonitis, an acute phase of radiation-induced lung disease, and its subsequent late/chronic manifestation of radiation pulmonary fibrosis (RPF) are common complications of radiotherapy of chest wall or intrathoracic malignancies, radiotherapy prior to bone marrow transplantation or peripheral blood stem cell transplantation and occupational exposures to high levels of radiation resulting from nuclear reactor accidents [1]. Clinical studies showed that among the patients with treatment of radiotherapy for cancers, the incidence of symptomatic radiation-induced pneumonitis ranges from 5% to 15% [2] and may be as high as 43% [2,3].

RPF, which is characterized by inflammatory cell infiltration, fibroblast proliferation and excessive deposition of extracellular matrix (ECM) proteins in lung parenchyma [4], is a chronic, progressive and fatal interstitial pulmonary disease with a poor prognosis, a high mortality rate and ineffective response to available medical therapies [5]. Thus, many innovative strategies for repair of injured lung have been developed and tested in the past decades. Notably, cell therapy has raised and gained special attention as a new alternative to stimulate lung repair [6].

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Multipotent mesenchymal stromal cells (MSCs) are primitive cells originating from the mesodermal germ layer and are classically described to give rise to connective tissues, skeletal muscle cells and cells of the vascular system [7]. MSCs may exhibit immunosuppressive properties and have been suggested to be "immune-privileged," and thus are protected from rejection, potentially permitting their use in allotransplantation. It seems certain that MSCs have the capacity to localize to injured lung and differentiate into specific cell types [8,9]. Some reports argue that the therapeutic effects of MSCs in lung fibrosis are mainly mediated by paracrine actions, including stimulation of endogenous repair, angiogenesis and arteriogenesis, attenuating remodeling and reducing apoptosis [10]. Recent reports suggest that MSCs may also promote lung repair through activation of endogenous distal lung airway progenitor cell populations [11], and Cx43-dependent alveolar attachment and mitochondrial transfer [12]. Of interest, injection of murine MSCs, but not human MSCs, differentiated into osteosarcomas in the injured lung. Therefore, human MSCs appear to be more feasible and safer than murine MSCs in the treatment for lung injury [13]. Human MSCs [14] and conditioned medium from human MSCs [15] have been demonstrated to reduce fibrosis in the bleomycin-induced pulmonary fibrosis. However, injection of MSCs at later stage after irradiation would be involved in fibrosis development [5,16].

MSCs can be transduced through several transfection or transduction approaches and are also increasingly described as vehicles for delivery of therapeutic genes and proteins [6]. It is well documented that MSCs transfected with angiopoietin-1 resulted in nearly complete reversal of lipopolysaccharides (LPS)-induced increases in lung permeability [17], and transfected with keratinocyte growth factor (KGF) reduced the histological hallmarks of bleomycin-induced fibrosis, induced proliferation of alveolar type II cells and decreased collagen levels within the lungs [18]. Extracellular superoxide dismutase (SOD3) is a principal enzymatic scavenger of the superoxide anion in extracellular spaces. SOD3 is expressed in especially high levels in mammalian lungs where it is bound to the extracellular matrix through a positively charged heparin/matrix-binding domain [19]. Targeted over-expression of human SOD3 in the lungs of mice significantly protects these mice against bleomycin-induced lung injury, whereas enhanced bleomycin-induced pulmonary damage occurs in mice lacking SOD3 [20–22]. It seems that loss of SOD3 may enhance oxidative stress and injury in the bleomycin-induced lung injury model.

In the present study, we hypothesized that the combined delivery of human umbilical cord–derived mesenchymal stromal cells (UC-MSCs) and SOD3 using gene transfer approach at the established fibrosis stage may show potential beneficial effects on RPF. To our knowledge, this is the first study to assess and highlight the combined cell and gene therapy for treatment of RPF.

Methods

Animals

Eight-week-old female C57BL/6 mice were housed according to the guidelines of the United States National Institutes of Health (NIH) and the local committee for the care and use of laboratory animals. The mice were housed five mice per cage prior to irradiation and one mouse per cage after irradiation. The mice were maintained under standard environmental conditions (temperature 22 ± 2°C, humidity 55 ± 5% and 12-h light/12-h dark cycle), fed a normal pellet diet and provided water *ad libitum* in the cages. All of the experimental procedures involving animals and their care were carried out in accordance with the Guide for the Care and Use of Laboratory Animals published by the NIH, and approved by the local committee for animal use. Every effort was made to minimize the number of animals used as well as their suffering.

Isolation, characterization and differentiation of UC-MSCs

UC-MSCs were collected from the human umbilical cord with informed consent of the mother. The collection was performed in accordance with the ethical standards of the local ethics committee. UC-MSCs were successfully isolated from the Wharton's jelly (WJ) of umbilical cords according to described methods in our previous report [23]. Briefly, fresh umbilical cords were collected after obtaining consent from the mothers. The umbilical cords were rinsed in phosphate-buffered saline (PBS) until the cord blood was cleared, and the blood vessels were removed. The remaining WJ tissue was cut into 1–2 mm^3 pieces and placed in six-well plates in the presence of 0.1% collagenase type II (Sigma) in PBS at 37°C for 1 h. Ten percent fetal bovine serum (FBS, Invitrogen) was then added to stop the digestion. The dissociated mesenchymal cells were dispersed in 10% FBS–Dulbecco's Modified Eagle's Medium (DMEM) and further cultured until well-developed colonies of the fibroblast-like cells reached 80% confluence. Then, the cultures were trypsinized with 0.25% trypsin–ethylenediaminetetraacetic acid (EDTA; Invitrogen) and passaged into new flasks for further expansion. The multipotent differentiation capacity of the UC-MSCs was confirmed by their differentiation into adipocytes, chondroblasts and