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Experimental treatment of radiation pneumonitis with human umbilical cord mesenchymal stem cells

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ABSTRACT

Objective: To evaluate of the curative effect of human umbilical cord mesenchymal stem cells (hUC-MSCs) on rat acute radiation pneumonitis. **Methods:** Fourty rats were randomly divided into control group, radiation group, stem cell prevention group, stem cell treatment group and prednisone treatment group. All rats except those in the control group were radiated with X ray to establish the acute radiation pneumonitis damage model. The hUC-MSCs cultured *in vitro* was administrated to the rats of the prevention group via tail vein (1×10^6 cells/kg BW) 24 h before the radiation, while the same administration was performed in the rats of the treatment group 24 h after the radiation. After 24 h post the radiation, the rats in the radiation group were given 0.4 mL physiological saline, and those in the prednisone group were given 1 mg/kg prednisone. All rats were observed and executed 72 h after the radiation to detect lung histological changes. **Results:** After the administration of hUC-MSCs, the survival status of the rats in the prevention group and treatment group was obviously better than that in the control group. As shown by the histological staining, the morphology, proliferation activity and bronchial state of lung tissues were better in the prevention group and treatment group than in the control group. **Conclusion:** The hUC-MSCs have definite therapeutic effects on acute radiation pneumonitis in rats.

1. Introduction

Radiation pneumonitis refers to the inflammatory reaction caused by injuries of the normal lung tissue within the radiation field after radiotherapy of lung cancer, esophageal cancer, breast cancer, malignant lymphoma or other chest and neck tumors. Usually, irritating dry cough appears as the main symptom of acute patients[1], while for chronic patients, lung infection can be easily stimulated due to their weak immunity, further generating extensive lung fibrosis[2] and causing damage of the respiratory function and even lethal respiratory failure. Clinically, adrenocortical hormone is more often used for the treatment of radiation pneumonitis[3] with good curative effects but many side effects. Currently, there is still no effective means to prevent and treat acute and chronic radiation lung injury.

Human umbilical cord mesenchymal stem cells (hUC-MSCs) are a kind of stem cell characterized by self-renewal, proliferation and multi-directional differentiation potency[4]. Presently, it is widely used for the treatment of multiple organ injury[5] clinically with the advantages of convenience, extensive sources, sufficient quantity, low immunogenicity, no ethical controversy, fast proliferation, strong differentiation ability and extensive application prospect. In this experiment, the curative effect of hUC-MSCs on rat acute radiation pneumonitis was observed, providing a clinical basis for the prevention and treatment of radiation-induced diseases.

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2. Materials and methods

2.1. Materials

Mouse anti-human CD34-PE, CD45-PE, CD29-PE, CD44-PE, CD73-PE, CD90-PE, and CD105-PE antibodies were purchased from American Cell Signaling Technology Co., Ltd. The Swedish Elekta Precise linear accelerator and FACSCanto II flow cytometer were purchased from Becton Dickinson Medical Instrument Co., Ltd. TCS-SPZ inverted fluorescence confocal microscopy was purchased from German Leica Company.

2.2. Animal grouping and radiation

Fourty healthy male Wistar rats at the age of 2 months and with weight of (200±20) g were provided by the Laboratory Animal Research Center of Shandong University and randomly divided into five groups, namely, the control group, radiation group, stem cell prevention group, stem cell treatment group and prednisone treatment group, eight rats in each group.

The rats except those in the control group were fixed on the plastic foam board by rubber bands, with chest exposed. They were given chloral hydrate (4-5 mL/kg BW) for peritoneal injection anesthesia. The Swedish Elekta Precise linear accelerator (X-ray) was used for irradiation performed under the following conditions: source skin distance, 120 cm; absorbed dose rate, 300 mu/min; radiation field, 10 cm × 20 cm; X-ray energy, 6 mv; and total effective biological dose, 8 GY[6].

2.3. In vitro culture of hUC-MSCs

The hUC-MSCs were cultured *in vitro*[7]. In this aseptic operation, 30-40 cm long healthy neonatal umbilical cord was put into aseptic saline solution containing 0.1% penicillin and streptomycin. Before the experiment, the umbilical cord was rinsed with 75% (v/v) ethanol and cut into 1.5-2.0 cm pieces. After peeling of umbilical cord skin and artery blood vessel, these pieces were washed 2-3 times in the saline solution, cut into pieces, and then put into a centrifuge tube. Then, 2 times volume of 0.2% Type I collagen enzyme and 100 μL of 0.1% penicillin and streptomycin were added, followed by overnight digestion at 37 ℃. In the next morning, 2.5 g/L trypsin with 0.5 g/L EDTA was added, followed by further digestion for 30 min with 37 ℃. Subsequently, the digestion products were fully diluted with 8 times volume of saline solution, mixed well and put into centrifuge tubes separately, followed by centrifugation at 3 000 r/min for 20 min. They were washed again with normal saline and centrifuged at 2 000 r/min for 10 min. The supernatants were discarded and the cell pellet was resuspended in a certain amount of culture medium.

The cells were inoculated at a density of 1×10^6 cells/cm^2 and incubated in 5% (v/v) CO_2 at 37 ℃. Digestion and passage were performed when the convergence area reached 90%. The mixture containing PBS and 2.5 g/L trypsin with 0.5 g/L EDTA at a volume ratio of 1:4 was used for digestion. When most of the cells became round, culture medium was added to stop digestion. The cell suspension was transferred into centrifuge tubes and centrifuged at 1 500 r/min for 10 min. Then, the cells were collected and diluted with culture medium for cell count. The cells were inoculated at a density of 3 000-6 000 cells/cm^2 in an ncubator with 5% (v/v) CO_2 at 37 ℃. Generally, the cells of the first five generations were used for the experiment.

2.4. Flow cytometry

After digestion, the hUC-MSCs suspension was centrifuged at 1 300 r/min for 8 min. Then the cells were resuspended in PBS and made into single cell suspension by gently blowing. Later, 15 μL of mouse anti-human CD34-PE, CD45-PE, CD29-PE, CD44-PE, CD73-PE, CD90-PE and CD105-PE antibody were added into each tube containing 50 μL single cell suspension respectively and incubated at room temperature in darkness for 30 min. Then, 1 mL PBS was added into each tube and centrifugated at 1 500 r/min for 6 min. After removal of the supernatants, 2 mL PBS was added into each tube and centrifugated again at 1 500 r/min for 6 min. After the supernatants were discarded, the cell pellets were finally resupended in 400 μL PBS and detected by flow cytometry.

2.5. Administration and observation

The rats in the stem cell prevention group were injected with 0.4 mL of hUC-MSCs suspension (1×10^6 cells/kg BW) via tail vein 24 h before the radiation, while the same administration was done in the rats of the stem cell treatment group 24 h after the radiation. The rats in the radiation group and prednisone treatment group were given normal saline (0.4 mL per rat) and prednisone (1 mg/kg·d) via tail vein 24 h after the radiation, respectively.

The rats were fed in separate cages under the same conditions, and their mental states and manure were observated. They were weighted and killed 72 h after radiation. Under aseptic conditions, the right lung tissue was selected to make slices for HE staining and immunohistochemical staining, and the results were observed under an inverted microscope.