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Human umbilical cord mesenchymal stem cells improve irradiation-induced skin ulcers healing of rat models

Zhongshan Liu[a,b,1], Daojiang Yu[a,1], Jianwei Xu[c], Xiujie Li[a], Xianyao Wang[d], Zhixu He[c,*], Tianlan Zhao[a,**]

[a] Department of Plastic, The Second Affiliated Hospital of Soochow University, Suzhou, China
[b] Department of the Burns and Plastic, The Affiliated Hospital of Guizhou Medical University, Guiyang, China
[c] The Stem Cell and Tissue Engineering Research Center, Guizhou Medical University, China
[d] Department of Cell Biology, Jiangsu Key Laboratory of Stem Cell Research, Suzhou, China

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ABSTRACT

Irradiation-induced skin ulcers can be resultant from nuclear accident or reaction to radiation therapy of tumor and is intractable for healing. Human umbilical cord mesenchymal stem cells (hUC-MSCs) have been considered to be the potential therapeutic tools for tissue regeneration. However, the underlying mechanisms are still not well understood. This study aims to investigate the effects of hUC-MSCs on irradiation-induced skin ulcers healing and the related mechanisms. The ulcers were induced by irradiating the skin of adult SD rats. The ulcers of SD rats were treated with vehicle or hUC-MSCs donated from mother giving birth. The ulcer healing was measured by imaging the healing rate and the H&E staining. CD31 and VEGF expression was measured with immunohistochemistry assay. iTRAQ proteomics analysis was used to analyze the signaling pathway. The results showed that hUC-MSCs improved healing of irradiation-induced skin ulcers *in vivo* using a rat model of skin ulcer. Transplantation of hUC-MSCs promoted keratin generation and keratinocytes proliferation of ulcer areas. Furthermore, the results demonstrated that hUC-MSCs increased expression of CD31 and VEGF in ulcers and promoted neovascularization. iTRAQ proteomics analysis results indicated that PI3K/Akt signaling pathway involved in hUC-MSCs-mediated repairing of irradiation-induced skin ulcer. In conclusion, human umbilical cord mesenchymal stem cells promoted neovascularization and re-epithelization, and improved healing of irradiation-induced skin ulcers. This healing improvement may be conducted through activating the PI3K/Akt signaling pathway, however, which needs to be proven by the further investigations.

1. Introduction

Irradiation-induced skin ulcers could be resultant of radiation therapy of malignant tumor, nuclear accident at ordinary time or nuclear explosion in war, and brings patients huge pain because of repetitive recurrence, resistance to therapy and even canceration [1–4]. Till now, tumor radiotherapy is a major cause of skin ulcers, accounting for 8.4% of non-healing wounds. However, the therapeutic effect of on the radiation-induced skin ulcers is insufficient, and the mechanism for ulcer formation is not clear. Although transplantation is an ideal therapeutic strategy, autogenous skin sources are seriously deficient, and xenogenic skin sources have been limited after the execution of the Regulation of Human Organ Transplantation. Therefore, novel and effective therapies for promoting the healing of severe radioactive skin ulcer are in large requirement.

Mesenchymal stem cells (MSCs) derived from multiple human tissues, such as bone marrow, adipose tissue, umbilical cord blood and the umbilical cord [5], all of which show critical potential in clinic through replacement and paracrine effects [6,7]. Compared to the other original MSCs, the human umbilical cord MSCs (hUC-MSCs) take advantages in short amplification time, high proliferation rate, higher safety and convenience [8–14]. It has been reported that hUC-MSCs can be recruited to wound areas and promote the functional recovery of patients with burns by paracrine effect [6,15–20]. However, it is not clear whether hUC-MSCs can promote the healing of radiation-induced ulcers and what is the mechanism. Therefore, the aim of the present study is to evaluate the effect of hUC-MSC on radioactive skin ulcer and its potential mechanisms.

* Corresponding author at: The Stem Cell and Tissue Engineering Research Center, Guizhou Medical University, University City of Anxin Distinct, Guiyang, 550025, China.
** Corresponding author at: Department of Plastics, The Second Affiliated Hospital of Soochow University, 1055 Sanxiang Road, Suzhou, 215004, China.
E-mail addresses: hzx@gmc.edu.cn (Z. He), tianlanzhao1965@yeah.net (T. Zhao).
[1] These authors contributed equally to this study.

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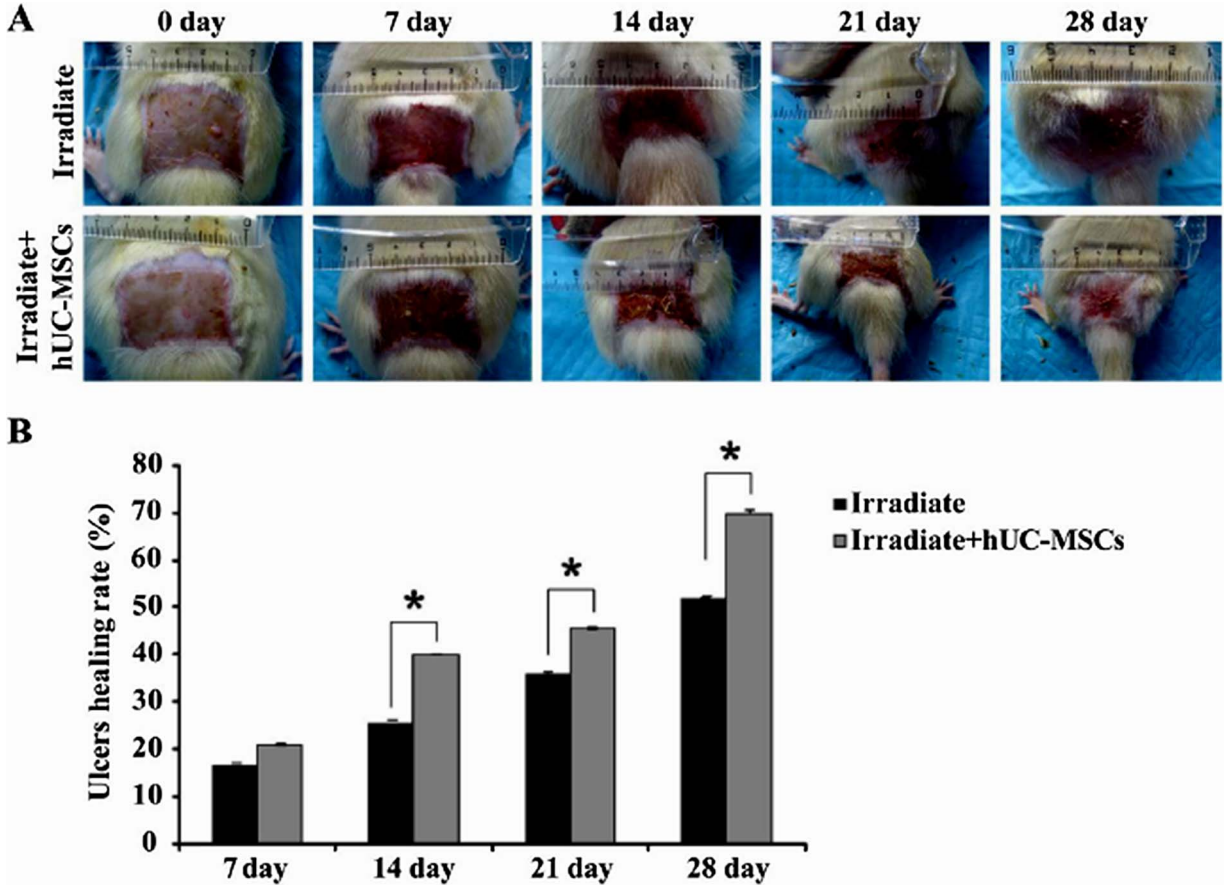

Fig. 1. hUC-MSCs promoted recovery of irradiation-induced severe ulcers. (A) Representative images of skin ulcers at different times after hUC-MSC transplantation. (B) Statistical analysis of ulcers healing rate at 7, 14, 21, and 28 days after transplantation was presented in the histogram. * $p < 0.05$ *vs.* irradiation group, $^{\#}$ $p < 0.05$ *vs.* Sham group.

2. Materials and methods

2.1. Animals and experimental protocol

All animal experiments were approved by the Research Ethics Board of Guizhou Medical University. Adult male Sprague-Dawley rats (200–250 g) were purchased from the Experimental Animal Center of Guizhou Medical University of China and were randomly divided into 3 groups (n = 36), including Sham group (n = 12), irradiation group (n = 12) and irradiation plus hUC-MSCs group (n = 12). The animals were kept on a 12–12 h light-dark cycle in the Centre for Laboratory Animal Science at Guizhou Medical University. Under anesthesia with intraperitoneal injection of 50 mg/kg pentobarbital, the animals in the latter 2 groups were irradiated with an MBR-1505R2 irradiator (Hitachi Medical Corporation, Tokyo, Japan) through a single electron beam delivered by a Clinac iX linear accelerator (Varian Medical System, Inc. Palo Alto, CA, USA). The total irradiated area for each buttock was 4.5 cm × 4.0 cm = 18 cm^2, and the total irradiation dose was 45 Gy β ray for 7.5 min for one time. Except for the irradiated area, animals were protected by a 1 cm filter of lead. After 21 days of, ulcers were created and the rats were housed individually to prevent gnawing of ulcers and other potentially damaging interactions.

2.2. Isolation, culture, and labeling of hUC-MSCs

hUC-MSCs were generously donated by 3 mothers who labored full-term healthy fetuses via caesarean delivery (gestation age, 39–40 weeks) and signed the informed consent with ethical approval from the affiliated hospital of Guizhou Medical University. HUC-MSCs were isolated, expanded *in vitro* and characterized as Zhang et al. described [21]. The cells were plated in 10 cm dish at a density of 2×10^6 cells and cultured in expansion medium containing high-glucose Dulbecco's modified Eagle's medium (Gibco, Rockville, MD, USA), 10% fetal bovine serum (Gibco, Rockville, MD, USA), 100 units/ml penicillin (Gibco, Rockville, MD, USA), and 100 μg/ml streptomycin (Gibco, Rockville, MD, USA) under a humidified atmosphere of 5% CO2 at 37 ℃. Culture medium was changed every 3 or 4 days. The cells were passaged upon reaching 90% confluence. All experiments were performed using cells in 3–4 passages. Two days before transplantation, hUC-MSCs were labeled with green fluorescent protein (GFP) using an adenoviral strategy.

2.3. Cell transplantation

The rats in the irradiation plus transplantation of hUC-MSC group received a multi-point subcutaneous injection of 2×10^6 GFP-labeled hUC-MSCs (1 ml) at week 3 after irradiation. The rats in the other 2 groups received a multi-point subcutaneous injection of PBS (1 ml) at the same time.

2.4. Images capture and assay

To evaluate the development of radiation-induced skin ulcers, skin ulcers images were obtained and the healing time and healing rate of the ulcers were evaluated at 7, 14, 21 and 28 days post the transplantation. Ulcer areas were measured photographically every week after hUC-MSCs transplantation, and the rate of ulcer closure was calculated as follows: Healing rate (%) = (original defect area-unepithelialised area)/original defect area × 100%. Quantitative measurements of the ulcer area were assessed using Image Pro Plus 5.1 image analysis