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CXCL12/CXCR4 axis promotes mesenchymal stem cell mobilization to burn wounds and contributes to wound repair

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ABSTRACT

Background: Bone marrow–derived mesenchymal stem cells (BM-MSCs) play a crucial role in tissue repair. Their role in thermal burn wound regeneration and the relevant mechanism, however, is rarely studied.

Methods: BM-MSCs from green fluorescent protein transgenic male mice were transfused to irradiated recipient female C57BL/6 mice. Twenty-one days later, the female mice were inflicted with burn wounds. The size of the burned area was measured by an *in vivo* fluorescence imaging system, and BM-MSC chemotaxis and epithelialization were estimated by fluorescence *in situ* hybridization and immunofluorescence technology. The expression of CXCL12 and CXCR4 in the wound margin was detected by enzyme-linked immunosorbent assay and immunohistochemistry. The importance of CXCL12/CXCR4 signaling in BM-MSC chemotaxis was further estimated by blocking CXCR4 *in vivo* and *in vitro*.

Results: *In vivo* imaging results showed that BM-MSCs migrated to the injured margins. Fluorescence *in situ* hybridization and immunofluorescence technology revealed that Y chromosome–positive cells derived from green fluorescent protein transgenic mice were detected to be colocalized with keratin protein. Enzyme-linked immunosorbent assay revealed increased levels of CXCL12 and CXCR4 protein in the wound sites of BM-MSC-treated chimeric mice after burn. Immunohistochemistry also disclosed that CXCL12 levels were elevated at postburn day 7 compared with day 0. Furthermore, pretreatment of the BM-MSCs with the CXCR4 antagonist AMD3100 significantly inhibited the mobilization of BM-MSCs *in vitro* and *in vivo*, which attenuated wound closure.

Conclusion: BM-MSC migration to the burned margins promotes the epithelialization of the wound, and mobilization of BM-MSCs is mediated by CXCL12/CXCR4 signaling.

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1. Introduction

Bone marrow–derived mesenchymal stem cells (BM-MSCs) are a subset of nonhematopoietic cells in bone marrow characterized by their capacity for self-renewal and differentiation into multiple cell types, including osteoblasts, adipocytes, and chondrocytes [1]. It has been widely accepted that tissue repair of injuries is primarily advanced by bone marrow stem cells that migrate to the site of damage and undergo differentiation, promoting structural and functional repair [2]. Studies have demonstrated that intravenous delivery of BM-MSCs leads to their migration to the injured site of bone or cartilage fracture [3], myocardial infarction [4], and ischemic cerebral injury [5].

BM-MSCs can mobilize and migrate to target sites to participate in tissue repair and regeneration. Many studies have focused on the mechanisms of how BM-MSCs migrate to injured tissues, and some key molecules involved in several signaling pathways have been reported to participate in this process, such as the CXCL12/CXCR4 pathway, which mediates BM-MSC migration and enhances wound healing, damage repair, and regeneration. CXCL12 (also known as SDF-1) is a member of a large family that promotes chemotaxis. It was first identified as a lymphocyte- and monocyte-specific chemoattractant under both normal and inflammatory conditions [6]. Wynn *et al.* demonstrated that CXCR4, the receptor for CXCL12, was highly expressed in BM-MSCs and that the CXCL12/CXCR4 axis was involved in the migration of BM-MSCs [7]. The CXCR4 antagonist significantly inhibited the chemotaxis of BM-MSCs to CXCL12 [8]. In addition, the CXCL12/CXCR4 pathway was shown to mediate the homing of transplanted BM-MSCs to injured sites in the brain, and it was shown that BM-MSCs migrated toward a CXCL12 gradient in a dose-dependent manner [5]. These studies suggest that the CXCL12/CXCR4 axis is required for BM-MSC migration.

Burn injuries, which are characterized by heat-induced tissue coagulation at the time of injury, constitute a worldwide public health problem [9]. Compared with incisional wounds, burn wounds heal more slowly because of edema, extensive necrosis, and relative hypoxia of the burn wound [10]. Furthermore, it has been well established that large burn injuries induce systemic immune dysfunction, which endangers the patient's life [11,12]. Advances in the field of burn wound healing remain limited but are necessary. BM-MSCs have shown the capacity to accelerate the healing process of incisional wounds [13]. However, to date, there have been few studies regarding the role of BM-MSCs in the repair process of burns, and the related mechanism is even less understood. In the present study, we investigated the detailed localization and effects of BM-MSCs in burn wound healing using a deep burn model of chimeric C57BL/6 mice and examined the role of CXCL12/CXCR4 signaling in BM-MSC migration. Our results showed that the transplanted BM-MSCs primarily distributed in the hair follicles and epidermis of the wound margin, accelerating the epithelialization of the wound. The CXCL12/CXCR4 axis promoted BM-MSC mobilization to burn wounds, which suggests that the CXCL12/CXCR4 axis would be a novel therapeutic target for the treatment of burn wounds.

2. Materials and methods

2.1. Irradiation and transplantation

All animal experiments were approved by the Animal Care and Use Committee of the Third Military Medical University and were performed in compliance with the "Guide for the Care and Use of Laboratory Animals" published by the National Institutes of Health. Eight-week-old female C57BL/6 mice were obtained from the Third Military Medical University. They were housed in autoclaved cages and treated with antibiotics (280 mg erythromycin and 320 mg gentamicin sulfate per liter of deionized drinking water) for 10 d before irradiation and 2 wk after irradiation. Recipient mice were exposed to 10 Gy whole-body irradiation using a Cobalt-60 source (Theratron-780 model; MDS Nordion, Ottawa, ON, Canada), which is a method that has been widely used to destroy marrow [14,15]. Four hours after irradiation, 1×10^6 BM-MSCs from male green fluorescent protein (GFP) transgenic C57BL/6 mice (Cyagen Biosciences, Guangzhou, China) were injected into the tail vein of female recipient mice.

2.2. Quantitative real-time polymerase chain reaction (RT-PCR) for confirmation of the chimeric model

Mouse chimerism was evaluated by detecting the male-specific gene Sry in the peripheral blood harvested from recipient mice 20 d after BM-MSC transplantation. The expression of Sry from the peripheral blood of male mice was used as a positive control and that from wild-type female mice as a negative control. Genomic DNA was extracted from mouse blood with the DNeasy Blood and Tissue Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. Quantitative real-time PCR was performed as described previously [16]. The primer sequences were as follows: Sry (forward), 5′-GGAGGCACAGA GATTGAAGA-3′; Sry (reverse), 5′-ACTCCAGTCTTGCCTGTATG; GAPDH (forward), 5′-ACCCATCAC CATCTTCCAGGAG-3′; and GAPDH (reverse), 5′-GAAGGGGCGGAGATGATGA C-3′.

2.3. Animal burn model

A burn injury model was established 21 d after BM-MSC transplantation, as described previously [17]. Briefly, the mice were anesthetized by intraperitoneal injection of pentobarbital sodium (75 mg/kg). Then, a total of 1.75 cm^2 body surface area of the mouse dorsum was exposed to a 100°C water bath for 8 s. On days 1, 3, 7, 14, 21, and 28, the entire wound area, including the adjacent 2-mm skin margins, was collected for further analysis of fluorescence *in situ* hybridization (FISH), immunofluorescence (IF), enzyme-linked immunosorbent assay (ELISA), and immunohistochemistry (IHC).

2.4. In vivo fluorescence imaging

In vivo fluorescence imaging was performed on days 1, 3, 7, 14, 21, and 28 post wounding using a Maestro *In Vivo* Imaging System (Cambridge Research & Instrumentation, Boston, MA). The excitation filter for GFP was 445–490 nm. The tunable