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Stromal cell-derived factor 1 promoted migration of adipose-derived stem cells to the wounded area in traumatic rats

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ABSTRACT

Background: Adipose-derived stem cells (ADSCs) were effective in treating wound. Stromal cell-derived factor-1 (SDF-1), a chemokine usually called CXCL12, is well known for its chemotaxis in induction of cell migration. However, little is known about the SDF-1responsible for the complex migration of ADSCs from residence to injured sites.
Objective: Herein, we firstly showed SDF-1 is a major regulator involved in migration of ADSCs during wound repair in *vivo*.
Methods: Trauma in rats was induced by surgical operation. The levels of SDF-1 in wounded tissue were assayed by ELISA. ADSCs were labeled with Green Fluorescent Protein (GFP), and then were transferred to injured rats by intracarotid injection. The plasma levels of ADSCs during wound healing were detected by flow cytometry, and ADSCs in injured tissue were evaluated by bioluminescence imaging in vivo and laser confocal microscopy (LCM), respectively.
Results: ADSCs were successfully labeled with GFP. SDF-1 level reached to the peak value on 24 h after injury and then decreased continuously. Additionally, levels of plasma ADSCs in SDF-1 treated rats reached to the peak value (12%) at d21 after medicine delivery, while those of normal and injured rats showed the peak values of 6.28% and 9.84% at d7 and d21, respectively. Finally, the results of LCM indicated treatment of ectogenic SDF-1 obviously enhanced GFP−ADSCs distribution in wounded tissues.
Conclusion: Our results indicated that SDF-1 treatment obviously promoted the migration and directed distribution of ADSCs in traumatic tissue.

1. Introduction

Tissue defect is a widely clinical phenomenon mainly caused by tumor resection, deep burn and radiation therapy. Currently, the mainstreams of repair proposal used in clinic are disturbed by obvious flaws. For example, wound covering combined with cytokines is unsatisfied for its limited curative effect as well as high medical costs [1]. Tissue flaps transferred to the wound area are prone to induction of secondary tissue injury and cicatrices, while prosthetic filling usually leads a complication of spasm [2,3]. Presently, it is well known that repair in trauma are mainly affected by stem cells (SCs). SCs [4] are multi-potent cell type that enables them to readily differentiate into several different cell types (osteoblasts, chondrocytes, adipocytes and myocytes) under specific culture conditions. To date, SCs have been widely used in treatment of several diseases [5–7]. Adipose-derived stem cells (ADSCs) are most commonly used SCs, mainly because of easy and frequently employed acquisition procedure [8]. ADSCs therapy has been made in the treating wound injury successfully. In 2012, Collawn SS et al. confirmed ADSCs accelerate wound healing in an organotypic raft culture model [9], and later, the other study also concluded that ADSCs transplantation promotes regeneration of expanded skin [10].

Incontrovertibly, curative effect of ADSCs is obviously influenced by migratory efficiency of ADSCs. It is well accepted that transferred process of the cells to the wound area involved migration from normal residential area to circulatory blood, the adhesion to endothelial cells near the injured area oriented by chemotactic molecules, and the adhesive cells transferring through vessel wall and reaching the injured tissue. Stromal cell-derived factor 1 (SDF-1) produced by bone marrow stromal cells (BMSCs) usually is called C-X-C motif chemokine 12 (CXCL12) [11]. SDF-1 is a potent chemotactic cytokine with various biological functions such as

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stem cell mobilization, inflammatory cell infiltration and angiogenesis [11]. Previous studies [12,13] pointed out SDF-1 mediated trafficking and homing of endothelial progenitor cells to injury microenvironment, and increased expression of SDF-1 at wound margin contributed to accumulation of endothelial progenitor cells and acceleration of neovascularization. Importantly, the later published papers [14,15] demonstrated enhanced healing of diabetic wounds by topical administration of ADSCs over-expressing SDF-1, and analogically, combination of SDF-1 with collagen-glycosaminoglycan scaffold accelerates re-epithelialization of dermal wounds in wild-type mice. These results indicate curative effects of ADSCs on wound are affected and controlled by SDF-1. However, the detailed reason as well as its mechanism is still unclear. Here, we firstly showed migratory and distributional process of ADSCs in wound repair is promoted by SDF-1.

2. Materials and methods

2.1. Animals

This study was approved by Ethical Committee on Animal Research of Southern Medical University.

Sprague Dawley (SD) rats (male, 210 ± 10 g) were purchased from Animal Department of Southern Medical University (Guangzhou, China). All animals were maintained in wire bottom cages at 25 °C ± 1 and 50% to 60% humidity under a 12 h–12 h light–dark cycle for at least one week prior to the experiment. The cages were access to standard diet and water was provided ad libitum during the experiment.

2.2. Isolation and preparation of ADSCs

ADSCs were prepared as described previously [16]. Briefly, the inguinal fat pads of anesthetized rats were removed and washed with PBS. Subsequently, the tissue were minced and incubated for 1 h. Rinse the tissue in PBS for 5 min followed by digestion with 0.15% collagenase and shaking for 30 min at 37 °C. Later on, an equal volume of control medium was added to neutralize the collagenase. The cell suspension was centrifuged at 1500 rpm for 5 min and the cell was re-suspended. The cultured medium was replaced every 3 days and the non-adherent cells were discarded.

2.3. Lentivirus packaging and transfection of ADSCs [17,18]

A lentivirus packaging system was obtained from GeneCopoeia (USA). Virus particles were packaged in 293FT cells and condensed by ultracentrifugation. Briefly, when 293FT (Shanghai cell bank of Chinese academy of sciences) had grown to a density of 80–90%, the cells were added with the mixture of plasmid (containing 1.5ugpsPAX.2 and 1.5ugpMD2.G) and 15 μl lipo2000 followed by replacement of medium with DMEM containing 10% fetal calf serum (FCS). The supernatant was collected at 48 h following infection, and then centrifuged (1500 rpm, 10 min, 4 °C) and passed through a 0.45 μm filter. After a new centrifugation (6000 rpm, 10 min, 4 °C), the pellet was dissolved in serum-free culture medium, aliquoted and stored at −80 °C prior to further use.

The isolated ADSCs were seeded into 24-well plates (5×10^4cells/well) with 10% FCS at 37 °C in an atmosphere containing 5% CO_2. When ADSCs reached 70% confluence, lentivirus was added to the wells based on a multiplicity of infection (MOI) of 20. The medium was replaced 24 h following the infection and the fluorescence intensity was measured after 96 h. Non-infected ADSCs were used as negative controls. Two groups were harvested by 0.25% trypsin (Hyclone, Logan, UT, USA), digested for 2 min at room temperature and resuspended in PBS after 96 h infection. The Green Fluorescent Protein (GFP)-expressing cells were observed by fluorescence microscope and the transduction efficiency was determined by fluorescence-activated cell-sorting analysis (FACS; LSR II, BD, CA, USA).

2.4. Induction of traumatic rats and injection of GFP labeled ADSCs

The wound in rats were induced by previously published method [19]. Briefly, the skin of anesthetized rats was shaved using an electrical shaver and disinfected with 75% alcohol. A uniform trauma area with 2 cm diameter was sheared from the back of the rats, and skin tension was kept constant during the procedure. To evaluate effect of SDF-1 on migration and distribution of ADSCs during wound healing. Three animal models were set in present study. Group 1(traumatic rats) received intradermal injection of SDF-1(1 μg) in wound sites at 0, 1, 2, 3 and 4 d, while Group 2(traumatic rats) and Group 3(normal rats) received PBS with the same manner. Meanwhile, all Groups were treated with GFP-ADSCs (0.5 ml, 1.0×10^7 cells) by carotid artery injection.

2.5. SDF-1 levels in wounded skin

When the wounded animals were prepared, several rats were killed and the traumatic tissues were removed. The tissue was lysed in lysate followed by homogenization at 4 °C for 1 h. Subsequently, homogenate was centrifuged (3000 rpm, 4 °C) for 20 min and were removed. The levels of SDF-1 in homogenate were assayed by ELISA (USCN Life Science, Wuhan, China).

2.6. Observation of labeled ADSCs in rats by live-imaging technique

To evaluate effect of SDF-1 on migration and distribution of ADSCs, a Vivo Imaging System IVIS™ (Xenogen, Allameda, CA) was used to observe the ADSCs. The process was carried out based on the method described previously. Briefly, the anesthetized rats received an intraperitoneal injection of firefly D-luciferin (Gold Biotechnology), and were imaged 10 min later with a cooled charge-coupled device (CCD) camera (IVIS; PerkinElmer). All rats was observed at 24 h、3d、7d、14d、21d after injection of ADSCs.

2.7. Observation of labeled ADSCs in wounded tissue by laser confocal microscopy

Traumatic tissues were removed at 24 h、3d、7d、14d、21d after injection of ADSCs. The tissue was frozen rapidly in dry ice to generate 8 μm-thick cryosections, and then stored at −70 °C. Before observation, the frozen sections were thawed for 30 s and fixed in cold acetone. Images were captured using a Zeiss LSM510 laser scanning confocal microscope (LCM) equipped with an argon laser giving 488 nm (blue excitation, green emission), a helium/neon laser giving 543 nm (green excitation, red emission) and a helium neon laser giving 633 nm (green excitation, infrared emission). Sections were analyzed at 63X oil immersion objective (Zeiss plan-Apochromat NA 1.4) and at 40X objective (Zeiss plan neo fluar N.A 0.75). Sequential images were merged and false colored using Zeiss LCM image browser software to produce a composite multicolor image.

2.8. Quantification of plasma ADSCs by flow cytometry

At 24 h、3d、7d、14d、21d after injection of ADSCs, the rats were killed, and the blood was collected in anticoagulative tube. Subsequently, the blood was transferred and added with 1 ml RBC Lysing solution followed by centrifugation (5 min, 4 °C, 1500 rpm). The supernatant was collected and then resuspended in PBS.