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Enhanced wound healing by topical administration of mesenchymal stem cells transfected with stromal cell-derived factor-1

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ABSTRACT

The objective of this study was to investigate the ability of mesenchymal stem cells (MSC) genetically engineered with stromal cell-derived factor-1 (SDF-1) to heal skin wounds. When transfected with SDF-1 plasmid DNA, MSC which were isolated from the bone marrow of rats, secreted SDF-1 for 7 days. In vitro cell migration assay revealed that the SDF-1-engineered MSC (SDF-MSC) enhanced the migration of MSC and dermal fibroblasts to a significantly greater extent than MSC. The SDF-MSC secreted vascular endothelial growth factor, hepatocyte growth factor, and interleukin 6 at a significantly high level. A skin defect model of rats was prepared and MSC and SDF-MSC were applied to the wound to evaluate wound healing in terms of wound size and histological examinations. The wound size decreased significantly faster with SDF-MSC treatment than with MSC and PBS treatments. The length of the neoepithelium and the number of blood vessels newly formed were significantly larger. A cell-tracing experiment with fluorescently labeled cells demonstrated that the percent survival of SDF-MSC in the tissue treated was significantly high compared with that of MSC. It was concluded that SDF-1 genetic engineering is a promising way to promote the wound healing activity of MSC for a skin defect.

1. Introduction

Wound healing is a complex biological process comprising three phases: inflammation, tissue formation including angiogenesis and granulation, and tissue remodeling [1]. Both acute and chronic wounds continue to be a major clinical problem. Non-healing wounds may result from a variety of causes, including large defects, diabetes, ischemia, and complications from radiation therapy. Multiple impairments in cellular responses, including the production of growth factors and cytokines, the recruitment of cells into injured tissues, contribute to non-healing wounds [1,2]. Various strategies, including growth factor [2–5], gene [6–8], and stem cell therapies [9–11], have been used to enhance the healing of non-healing wounds. Stem cell-based therapy is an attractive approach for the treatment of wounds with multiple impairments.

Mesenchymal stem cells (MSC), which are referred to as multipotent stromal progenitor cells, have been shown to promote tissue repair in numerous studies [9–13]. Transplantation of MSC can improve wound healing through cell differentiation and the release of paracrine factors [9–11,14]. However, the poor viability of MSC at the transplanted site often decreases their therapeutic potential [15,16]. It is important to improve the survival of transplanted MSC and enhance the secretion of factors and their biological functions in vivo.

Genetic engineering of MSC is a potential method to break through these problems. Much research has reported the therapeutic effects of MSC genetically engineered using viral vectors [8,17,18], but the strategy using viral vectors is not available for clinical cell therapy. It is necessary for clinical cell therapy to use non-viral vectors of gene transfection.

We have developed a spermine-pullulan of a non-viral vector for the gene transfection of MSC [19,20]. The spermine-pullulan is a cationic carrier of spermine-introduced pullulan which can interact with negatively charged plasmid DNA, and can be internalized into MSC by way of a sugar-specific-asialo-protein receptor that is expressed on the surface of MSC [21]. As a result, the complex of spermine-pullulan and plasmid DNA has been demonstrated to be feasible in gene transfection with low cytotoxicity and high specificity for MSC [19–21]. We have previously reported that MSC genetically engineered with spermin-pullulan showed better survival of cells and their therapeutic potential in vivo [21,22].

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Stromal cell-derived factor-1 (SDF-1, CXCL12) is known as a chemokine that plays a central role in normal wound healing [7]. SDF-1 functions in inflammation reactions, leukocyte development, and the mobilization and recruitment of stem and progenitor cells including hematopoietic stem cells (HSC) and MSC, and other CXCR4 (SDF-1 receptor)-expressing cells [23,24]. Consequently, SDF-1 contributes to cell-based vascularization and skin regeneration [25]. Moreover, SDF-1 increased the survival and growth of CXCR4-expressing stem cells, such as MSC, both in vitro [26] and in vivo [18,27]. Some researchers have reported that the local administration of SDF-1 enhanced wound healing [4,7,17,23].

In this study, we evaluated the therapeutic effect of local administration of MSC genetically engineered with SDF-1 on a full-thickness skin defect model of rats. The MSC were transfected with the complex of spermine-pullulan and SDF-1 plasmid DNA. When treated with SDF-1-engineered MSC, wound healing of skin defects was assessed in terms of size and histological examinations. In addition, we examined the mechanism of the therapeutic effect with MSC genetically engineered in terms of cell migration and growth factor secretion.

2. Materials and methods

2.1. Materials

Pullulan with an average molecular weight of 47,300 was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Spermine was purchased from Sigma Chemical Co. (St. Louis, MO). N,N′-carbonyldiimidazole (CDI) and dehydrated dimethyl sulfoxide were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and used as obtained.

2.2. MSC isolation and culture

MSC culture was performed according to the method previously described [21]. Briefly, 3-wk-old Fisher 344 rats (Japan SLC, Inc., Shizuoka, Japan) were sacrificed, and the bone marrow was harvested by flushing femurs and tibiae with alpha-minimum essential medium (α-MEM; Invitrogen Corporation, Ltd., Carlsbad, CA) supplemented with 15 vol% bovine fetal calf serum (FCS) and 1 wt% penicillin–streptomycin (control medium). The bone marrow cell mixture was placed in a 10 cm tissue culture dish (430167; Corning Inc., Cambridge, MA) and cultured in the control medium at 37 °C in 5% CO_2 and 95% air atmospheric condition. The medium was refreshed on the third day after isolation to remove non-adherent cells and continuously refreshed every 3 days. Cells of 2–3 passages in a 70–80% confluent condition were used for the following experiments.

2.3. Dermal fibroblast isolation and culture

Dermal fibroblasts were obtained from 3-wk-old Fisher 344 rats as previously described [28]. Briefly, the skin specimens were cut into small pieces with surgical scissors and placed in a 10 cm tissue culture dish. The skin specimens were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Invitrogen Corp.) supplemented with 10 vol% FCS and 1 wt% penicillin–streptomycin at 37 °C in 5% CO_2 and 95% air atmospheric condition, and the medium were changed every 3 or 4 days. Outgrowing fibroblasts were dissociated with 0.25 wt% trypsin-EDTA (Invitrogen Corp.) and passaged. Passages 3–5 were used for the following experiments.

2.4. Preparation of plasmid DNA

The plasmid DNA used was a plasmid coding human SDF-1 alpha (pORF-hSDF-1α; InvivoGen, San Diego, CA). The plasmid DNA was propagated in *Escherichia coli* (strain DH5α) and purified by the QIAGEN plasmid Mega kit (Qiagen K.K., Tokyo, Japan) according to the manufacturers' instructions. Both the yield and purity of plasmid DNA were evaluated by UV spectroscopy (DU800 spectrometer; Beckman Coulter, Germany). The absorbance ratio at wavelengths of 260–280 nm for plasmid DNA solution was measured to be between 1.8 and 2.0.

2.5. Preparation of cationized pullulan

Spermine was chemically introduced into the hydroxyl groups of pullulan by a CDI activation method [20]. Briefly, 9.28×10^{-3} mol of spermine and 1.39×10^{-3} mol of CDI were added to 50 ml dehydrated dimethyl sulfoxide containing 50 mg pullulan. Following agitation at room temperature for 20 h, the reaction mixture was dialyzed against double-distilled water (DDW) for 2 days. Then, the solution dialyzed was freeze-dried to obtain samples of spermine-introduced pullulan (spermine-pullulan). When determined by conventional elemental analysis, the molar percentage of spermine introduced into the hydroxyl groups of polysaccharides was 11.0 mol%.

2.6. Preparation of spermine-pullulan–plasmid DNA complexes

Polyion complexes (PIC) were prepared by mixing spermine-pullulan and the SDF-1 plasmid in aqueous solution. Briefly, spermine-pullulan was dissolved in DDW and mixed with an equal volume of PBS containing 100 μg plasmid DNA (100 μg/ml), and then left for 15 min at room temperature. The PIC composition was calculated on the basis of the nitrogen number of spermine-pullulan (N) per the phosphorus number of plasmid DNA (P) (N/P ratio). In this study, the PIC were prepared at the N/P ratio of 3.0 [22].

2.7. Gene transfection of MSC and the time course of SDF-1 secretion

MSC (1×10^4 cells/cm^2) were cultured with spermine-pullulan–plasmid DNA complexes at the plasmid DNA dose of 0.5 mg/cm^2 at 37 °C for 4 h in OPTI MEM (Invitrogen Corp.) to allow cells to be genetically engineered. Then, the medium was changed to the control medium, and MSC genetically engineered with SDF-1 plasmid DNA (SDF-MSC) were incubated further for 24 h. The level of human SDF-1 expressed in the culture medium ($n = 3$) was measured by ELISA (Human CXCL12/SDF-1α ELISA Quantikine Kit; R&D Systems Inc., Minneapolis, MN). The total protein of each well was determined with BCA Protein Assay Reagent (Thermo Fisher Scientific, Inc., Waltham, MA) to normalize the influence of the number variance of cells.

To investigate the time course of SDF-1 secreted from MSC and SDF-MSC, the cells were cultured for 7 days in control medium, which was collected each day and replaced. The amount of SDF-1 in the medium was determined by the ELISA ($n = 3$).

2.8. Transmembrane migration assay

To evaluate the effect of MSC and SDF-MSC on MSC and dermal fibroblasts migration, a transmembrane migration assay was performed using 24-well Transwell inserts (#3422; Corning Corp.). MSC were seeded in the bottom chamber at a density of 1×10^4 cells/cm^2 and cultured for 24 h in the control medium. MSC were divided into two groups with MSC and SDF-MSC. In the SDF-MSC group, MSC were transfected as described above and the medium (control medium or DMEM with 10 vol% FBS and 1 wt% penicillin–streptomycin) was replaced 24 h after transfection. In the MSC group, the medium (control medium or DMEM with 10 vol% FBS and 1 wt% penicillin–streptomycin) was replaced similarly. Then, 6.5 mm-diameter Transwell chambers (upper chamber) with polycarbonate membrane inserts (8 μm pore size) were placed in each group and well. MSC in the control medium or dermal fibroblasts in DMEM with 10 vol % FBS and 1 wt% penicillin–streptomycin were seeded into the upper compartment of the upper chamber (1×10^4 cells/well), followed by incubation at 37 °C for 24 h. Then, the number of cells that had migrated through the membrane to the lower compartment of the upper chamber was counted after their trypsinization ($n = 6$). The control bottom chambers contain medium (control medium or DMEM with 10 vol% FBS and 1 wt% penicillin–streptomycin) without cells.

2.9. Secretion of growth factors and cytokines from MSC and SDF-MSC

To evaluate the secretion of growth factors and cytokines, the conditioned medium of MSC and SDF-MSC was analyzed with commercial ELISA kits. MSC and SDF-MSC were cultured in a 12-well plate for 72 h and the conditioned medium was collected ($n = 4$). The growth factor and cytokine levels in the conditioned medium were measured by ELISA (Rat VEGF, Rat HGF, Rat IL-6, Rat TGB-β, Human FGF2, Rat PDGF-bb ELISA Quantikine Kit; R&D Systems Inc.). The total protein in each well was determined with BCA Protein Assay Reagent (Thermo Fisher Scientific, Inc.) to normalize the influence of the number variance of cells.

2.10. Wound healing model and cells transplantation

Eight-wk-old Fisher 344 rats ($n = 18$, 6 rats in each group) were purchased from Japan SLC (Japan SLC, Inc.) and each rat was anesthetized by intraperitoneal injection of 50 mg/kg pentobarbital (Abbot Laboratories, North Chicago, IL). After shaving and depilating the rats, two 15-mm full-thickness skin defects including the panniculus carnosus were created on each side of the dorsal midline. The rats were divided into three groups and MSC or SDF-MSC (1.0×10^6 cells) in 500 μl PBS or 500 μl PBS alone were homogeneously administered into the subcutaneous tissue around the wound defect. The wound was covered with polyurethane film (Tegaderm; 3M HealthCare, Borken, Germany).

2.11. Wound analysis

The skin wounds were photographed on different days after treatment with MSC and SDF-MSC or PBS. The wound area was analyzed by tracing the wound