CHEMOKINES OF HUMAN BMSCs FOR ARTICULAR CARTILAGE REGENERATION

1Park, M S; 1Kim, Y H; 1Lee, J W
1Brain Korea 21 Project for Medical Science, 1Department of Orthopaedic Surgery, Yonsei University College of Medicine, Seoul, South Korea
liwos@yuhs.ac

Introduction

Articular cartilage has a limited capacity for self-regeneration after injury. Bone marrow-derived mesenchymal stem cells (BMSCs) are good sources of repair since they can migrate directly to the injury site and differentiate to articular chondrocytes. The healing process is more effective if the cartilage defect is connected to the bone marrow. However, chondral defects do not heal as effectively due to the lack of sufficient reparative chondrocytes at the defect site and migration of surrounding chondrocytes and BMSCs to the injury site. Therefore, rapid induction of migration of a large number of BMSCs to the injury site can be an effective strategy for cartilage regeneration. Chemokines induce cells to migrate as quickly as in a few hours. The expression of chemokines is also induced by pro-inflammatory cytokines, such as IL-1β and TNF-α. Therefore, discovering the effective chemokines for the recruitment of BMSCs to the cartilage defects is required.

The purpose of this study was to investigate the chemokines inducing chemotaxis of human BMSCs for articular cartilage regeneration.

Materials and Methods

-Cell Culture:
Human BMSCs were cultured in DMEM-low glucose medium containing 10% of FBS and 1% of antibiotics and the medium was replaced every 3 days.

-Treatment of Cytokines:
After serum starvation of human BMSCs for 24 hours, human IL-1β or human TNF-α was added to DMEM-low glucose medium without FBS. At 4, 24, and 48 hours of IL-1β or TNF-α treatment, cells were harvested.

-RT-PCR:
Total DNA was isolated from BMSCs using an RNeasy kit (Qiagen, USA). RT-PCR was performed to evaluate expression of 19 chemokine receptors and their ligands.

-Reverse Dot Blot Hybridization:
To evaluate changes in expression of chemokine receptors, sample DNA of chemokine receptors were amplified using RT-PCR and spotted onto a nylon membrane in a Bio-Dot SF apparatus (Bio-Rad, Italy). Membranes were hybridized in 10% dCTP labeled cDNA of IL-1β or TNF-α-treated BMSCs. And then, the signals of radioisotope-labeled samples were detected by fluorescent image analyzer (FLA-7000, Fujifilm). Images of detected signals were quantitated with a densitometer.

-Cell Proliferation and Differentiation Assay:
After serum-starvation of BMSCs, IL-8, SDF-1, MCP-1, MIP-3α were treated to BMSCs. DMEM-LG containing 10% FBS and MTT solution were added and incubated at 37°C for 4 hours. The intracellular formazan was solubilized by DMSO (Sigma). The absorbance of the produced formazan was measured at 570 nm and each measurement was performed in triplicate. To differentiate, the BMSCs were grown in osteogenic or chondrogenic differentiation media for 14 or 21 days.

-Wound Healing Assay and Live Cell Tracking:
BMSCs were grown to confluency in silicon culture-inserts for wound healing assay (Bidi, Munich, Germany) attached on tissue culture plates. After serum-starvation of BMSCs, culture-inserts were removed. IL-8, SDF-1, MCP-1, MIP-3α, were treated to BMSCs. Migrated cells into wounded area were counted at 0.1, 3, 5, 7, 9, 12, 24 and 48 hours. Cell counting was performed in triplicate by three observers. To calculate the velocity of cell migration, we tracked the migrating cells using microscopy (CK-41, Olympus, Tokyo, Japan), CCD camera (KP-D20BU, HITACH, Tokyo, Japan), capture software (TOMORO ver. FG 2.9.19, Olympus) and calculated using image J (Bidi, Munich, Germany).

-Chemotaxis Assay:
To analyze migration of MSCs in vitro, a transwell assay with 24-well culture insert system containing 8 μm pores (Falcon, Dickinson and Company, Heidelberg, Germany) was used. Each insert used 10⁵ cells. After incubation for 6 hours, the cells were stained with crystal violet dye. After the un migrated cells on the membrane were wiped with cotton brush, stained cells were counted. Cell counting was performed in triplicate by three observers. In vivo chemotaxis of human BMSCs was performed with xExplore Optix system (ART Advanced Research Technologies Inc., Montreal, Canada). Briefly, After subcutaneous implantation of PLGA scaffolds (3Dtx4(Timm disk) containing saline or chemokines into the back of nude mice, NIR-labeled human BMSCs (1x10⁶ cells) were tail-injected. Signals of NIR-labeled human BMSCs were detected with xExplore Optix system at every 3 days.

Results

Increased chemokine receptors and ligands by IL-1β or TNF-α in BMSCs

To examine the expression of chemokines and cytokine receptors in unstimulated human BMSCs, we performed RT-PCR of 19 chemokine receptors with human BMSCs from 3 donors. CCR2, CCR6, CCR7, CCR8, CCR10, CXCR1, CXCR5, CXCR7 were expressed in unstimulated MSCs obtained from 3 donors. To examine the changes in the expression of chemokine receptors by pro-inflammatory cytokines, we performed reverse dot-blotting with radioisotope-labeled cDNA of human BMSCs stimulated by IL-1β or TNF-α for 4, 24, and 48 hours. In stimulated BMSCs, CCR2 and MCP-1; CCR6 and MIP-3α; CXCR1 and IL-8; SDF-1 were increased.

Collectively, in both RT-PCR and reverse dot-blotting, three receptors (CCR2, CCR6, CXCR2) and four ligands (MCP-1, MIP-3α, IL-8, and SDF-1) were expressed in unstimulated BMSCs and were increased in stimulated BMSCs by inflammatory cytokines.

The effect of chemokines on cell proliferation and differentiation

To test the effect on the cell proliferation and differentiation, we performed MTT assay and osteo- or chondro-specific staining assay using four chemokines (IL-8, MCP-1, SDF-1 and MIP-3α). According to the results, cell proliferation and osteo- or chondrogenic differentiation of BMSCs were not affected by four chemokines.

Chemotaxis of BMSCs using selected chemokines in vitro and in vivo

We examined in vitro chemotactic capacity using MCP-1, MIP-3α, IL-8 and SDF-1 in human BMSCs. In wound healing assay and live cell tracking using four chemokines such as IL-8, MCP-1, SDF-1 and MIP-3α. In transwell assay, chemotaxis of BMSCs in vitro was significantly increased by IL-8 (3.3 fold) and MIP-3α (3.4 fold). In the animal test, tail-injected human BMSCs were significantly recruited toward IL-8 and MIP3α in vivo (fig 1).

Figure 1 chemotaxis of BMSCs in vitro (left) and in vivo (right).

Discussion

We have made an in vitro cartilage-injury condition using IL-1β or TNF-α, and investigated on the increased expression of chemokine receptors in human BMSCs. According to the results, IL-8 and MIP3α were induced recruitment of human BMSCs. Conclusively we suggest that IL-8 and MIP-3α are useful inducing-factors for injured-articular cartilage regeneration.

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