기계적 자극이 중간엽줄기세포의 거동에 미치는 영향

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Modulation of Bone Marrow-derived Mesenchymal Stem Cells Growth by Mechanical Tension

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INTRODUCTION

To support and enhance the *in vitro* growth and activity of MSCs, the cell culture medium may be supplemented with various proteins and factors to mimic the physiological environment in which cells optimally proliferate and differentiate. The majority of studies have been restricted to chemical factors, such as growth factors, cytokines, and hormones, but it was recently reported that mechanical forces also play a central role in the physiology of a wide variety of tissues. Mechanical deformations of the cell or matrix are recognized by specific interactions between extracellular matrix (ECM) molecules and membrane proteins, including the family of transmembrane proteins known as integrins. Several investigators have reported that cyclic mechanical stretch increases the production of ECM molecules, especially collagen, in fibroblasts cultured on flexible membranes, and changes in morphology. The application of mechanical force also influences cell proliferation, differentiation, and gene expression in a wide variety of cell types. However, little is known concerning the effects of low levels of mechanical stretch on the *in vitro* behavior of MSCs.

MATERIALS AND METHODS

Bone marrow aspirates were obtained from the iliac crest of healthy donors with the approval of the patients themselves and the Institutional Review Board of St. Mary's Hospital, Catholic University. Bone marrow aspirates were collected in a syringe containing 10,000 IU heparin in order to prevent

coagulation. The mononuclear cell fraction was isolated by Ficoll (0.77 g/ml) density gradient centrifugation. Mononuclear cells were plated into tissue culture flasks in expansion medium at a density of 10^5 cells/cm^2. The expansion medium contained Dulbecco's Modified Eagle Medium (DMEM, low glucose; Invitrogen Co.) and 10% fetal bovine serum (FBS; Cambrex Co.). Upon reaching 80% confluency, the cells were trypsinized with 0.25% trypsin/ 1 mM EDTA (Sigma) and replated at a density of about 9000 cells/cm^2. The cells were expanded for 2 to 6 passages.

Dynamic mechanical strain was applied to cell monolayers using the Flexercell System (Flexcell International Co., PA, USA) and six-well Bioflex® plates (Flexcell International Co., PA, USA), which possess flexible silicone membrane bottoms. The Bioflex® plates were coated with 0.25% type I atel-collagen (0.5% collagen : 70% ethanol =1: 1) to compensate for the difficulties in cell adhesion and growth resulting from their hydrophobic surface property. To mechanically load the cells, the Bioflex® plate-attached silicon membrane was pushed by anchors placed beneath the Bioflex® plate. During training culture, a cyclic vacuum was used to draw the flexible silicone membrane, resulting in cell stretch. The release of the vacuum relaxed the membranes and returned them to their pre-strained condition. The cells were subjected to 5, 8, 10 or 15% elongation stretch for 5 s followed by 25 s of relaxation (2 cycles/min) for 14 days. Control cells were cultured on the same plates without cyclic strain.

The MSCs (at the 5^{th} passage) were seeded on a Bioflex® plate (flexible-bottom silicone plates, 35-mm 6-well) coated with type I atel-collagen (Bioland Co., Korea) at a density of 1×10^4 cells/well and cultured for 14 days in the growth medium. The growth medium was DMEM (low glucose; Invitrogen Co.) containing 10% FBS, antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin and 1 μg/ml amphotericin B, Invitrogen Co.), 4 mM L-glutamine, 25 mM ascorbate-2-phosphate (Sigma), and 1 ng/ml basic fibroblast growth factor (bFGF, Camarllo). The experimental group was divided into five groups: no stretch group, 5% strained group, 8% strained group, 10% strained group, and 15% strained group. Cell number was measured by 3-[4,5-dimethylthiazol-2-yl]- 2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma) staining. Bioflex® plates were incubated at desired time points in MTT solution (0.5 mg/ml MTT in cell culture medium) at 37 ℃ / 5% CO_2 for 2 h. The intense purple colored formazan derivative formed during active cell metabolism was eluted and dissolved in 95% iso-propanol containing 0.04 N HCl and the absorbance measured at 570 nm.

LDH activity was measured using an LDH-LQ kit (Asan Pharmaceutical Inc. Korea). Total intracellular soluble collagen was measured using a Sircol™ Collagen Assay Kit (Bioassay Inc.). The total intracellular sulfated GAG content was measured using a Blyscan™ Sulfated Glycosaminoglycans Assay Kit (Bioassay Inc.). Antibodies against human antigens CD90 were purchased from BD Sciences (San Jose, CA, USA) and antibody against CD105 was purchased from Ancell (Bayport, MN, USA). A total of $5x10^5$ cells were resuspended in 200 ㎕ of PBS and incubated with fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibodies for 20

min at room temperature (or for 45 min at 4℃). The fluorescence intensity of the cells was evaluated by a flow cytometer (FACScan; BD Sciences Inc.), and the data were analyzed using the CELLQUEST software (BD Sciences).

RESULTS AND DISCUSSION

The initial seeding cell number was the same (1×10^4 cells) in each group. After three days of culture, the cell number was determined to be 1.82×10^4 cells in the no stretch group, 2.82×10^4 cells in the 5% strained group, 2.65×10^4 cells in the 8% strained group, 2.74×10^4 cells in the 10% strained group, and 2.75×10^4 cells in the 15% strained group. At this point, the PDL values were 0.86 (no stretch), 1.50 (5% strained group), 1.41 (8% strained group), 1.46 (10% strained group), and 1.46 (15% strained group), respectively. After 14 days of culture, the cell number was determined to be 15.34×10^4 cells in the no streteh group, 14.61×10^4 cells in the 5% strained group, 15.86×10^4 cells in the 8% strained group, 14.93×10^4 cells in the 10% strained group, and 15.56×10^4 cells in the 15% strained group. At this point, the PDL values were 3.94 (no stretch), 3.87 (5% strained group), 3.99 (8% strained group), 3.90 (10% strained group), and 3.96 (15% strained group), respectively. This result showed that mechanical stretch (5-15% strained groups) significantly increased cell proliferation in the experimental groups as compared to the control (no stretch) during the initial days (3 days) of culture. The proliferation of MSCs under various strain groups (5-15% strain) showed significant improvement in comparison to the control group (no stretch). However the improvement was only transient because there was no difference after 14 days of culture. There was no morphological change or improvement in total cell number observed with the naked eye in comparison to the control group. And a uniform directional cell alignment formed in experimental group (5-15% strain) except for control group (no stretch).

LDH is a cytoplasmic catalytic enzyme related to the reversible conversion between pyruvic acid and lactic acid. LDH is released through the cell membrane of a cytotoxic cell. Therefore, less LDH release means less cytotoxicity. The media were collected and analyzed after 14 days of culture in order to examine the state of cellular cytotoxicity according to various degrees of mechanical strain. The LDH levels in all experimental groups were similar to those in the control (no stretch) group. This result showed that mechanical strain had no effect on cytotoxicity.

Collagen and GAG are the main components of the ECM that are involved in both cell proliferation and differentiation. Analysis of the intracellular collagen and GAG content was performed after 14 days of culture. Mechanical stretch increased collagen production, except for in the 15% strain group, but did not increase GAG production. Collagen production in the 5% strained group was significantly increased in comparison to the no stretch group. These results showed that the appropriate level of mechanical stretch (5-10% strain) increased collagen synthesis but did not increase GAG synthesis.

To determine whether or not mechanical strain altered MSC surface antigen expression, FACS analysis was performed for CD90 and CD105, which are known markers of MSCs. The MSCs in the A group were cultured using a static culture condition and served as the control group. With the exception of the 15% strain group, CD90 and CD105 were highly expressed on the cell surface and remained unchanged after culture, suggesting that appropriate levels of mechanical stretch (5-10% strain) did not induce the alterations in MSC surface antigen expression.

In this study, We found that mechanical strain improved the proliferation of bone marrow-derived MSCs during short-term culture (after 3 days of culture). The frequency of mechanical strain applied (0.03 Hz) was significantly weaker than in other studies. Many studies have shown that mechanical stretching induces various cellular responses in many types of cells and that the frequency- and duration-dependent effects of cyclic strain could also modulate various cellular responses. However, the cellular responses to mechanical stimuli are not completely understood. Therefore, it is not clear which molecular mechanisms are related to the response. Thus, further investigation is necessary in order to obtain a more complete understanding of the molecular biological mechanism underlying the cellular responses to mechanical stimuli.

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