INTRODUCTION:
Anterior cruciate ligament (ACL) and medial collateral ligament (MCL) are the major stabilizers of knee joint. The deficiency of these ligaments could result in the early development of osteoarthritis. The cellular activities responding to growth factors have important roles in ligament healings. ACL has a poor healing potential rather than MCL after injuries. To accelerate the ligament healing, we systematically investigated the proliferation, migration, adhesion, and matrix synthesis responding to several growth factors in ACL and MCL cells. Cellular responses including proliferation and migration depend on the interaction between adhesion molecule and extracellular matrix (ECM). Integrins consisting of α and β subunits primarily mediate cell adhesion by recognizing ECM substrates and cell surface proteins. Adhesion strengths on type I and III collagens, the major components of ligaments, are different in ACL and MCL cells. However, the cellular responses on type I collagen are still unclear in ligament cells. Here, we analyzed the integrin α2, which is the major subunit recognizing type I collagen, mediated cellular behavior in ACL and MCL cells.

In the present study, we demonstrate that basic fibroblast growth factor (bFGF) and growth and differentiation factor (GDF)-5 strongly enhance cell migration and collagen synthesis in ligament cells, respectively.

MATERIALS AND METHODS:
Cells and cell culture: Cells were isolated from ACL and MCL of ten-week-old Japanese white rabbits. Cells between passage 3 and 6 were used for experiments.

Cell proliferation assays: To investigate the effect of several growth factors in proliferation, ACL and MCL cells were seeded onto 96-well plates and incubated for 12 h and the media were replaced with serum-free DMEM. Recombinant human bFGF and recombinant human bone morphogenetic protein (BMP)-2, and recombinant mouse GDF-5, -7 were added as growth factors. These plates were incubated for 48 h prior to addition of WST-1.

Cell migration assays: Cell migration assays were performed in a modified Boyden chamber. The membrane coated with rat tail type I collagen was placed over the bottom chamber filled with a medium containing each growth factor (bFGF, BMP-2, GDF-5, and -7). Cells were added to the upper chamber. The assembled chemotaxis chamber was incubated for 8 h to allow cells for migration through the membrane. The total number of cells with nuclei that migrated per well was counted.

RT-PCR and image analyses: Cultured ACL and MCL cells were treated with the following growth factors for 8 h: bFGF, BMP-2, GDF-5 or -7 (10 ng/ml). RNA samples were reverse-transcribed. The cDNAs underwent PCR amplification in the presence of each specific primer for rabbit α1 chain of type I collagen (Col1a1), Col3a1, and glyceraldehyde-3-phosphate dehydrogenase (G3pdh).

Immunohistochemistry: ACL and MCL cells were harvested on type I collagen-coated slides for 12 h. Then, the cells were treated with bFGF, BMP-2, GEF-5, or -7 (100 ng/ml) for 8 h. To investigate the cellular attachments responding to each growth factor, we observed the distribution of integrins and actin fibers.

RESULTS:
bFGF is the most effective stimulator for the proliferation of ACL and MCL cells.

The cell proliferation of bFGF-treated ACL and MCL were increased approximately 1.7-times higher than that of untreated cell (Fig. 1A and B). The MCL proliferation responding to each growth factor was slightly higher than ACL. These data suggested that bFGF might have a strong potential to induce the healing of ACL and MCL.

The migration activity of MCL cells is higher than that of ACL cells.

The migrations of ACL cells responding to growth factor stimulations were slower than that of MCL cells (Fig. 2). The increase of migrated MCL was approximately 5-times higher than that of ACL in response to bFGF or GDF-5 stimulation. These findings prompted us to investigate the cellular attachment of ACL and MCL responding to the stimulation of growth factors.

DISCUSSION:
The healing potential of ACL injury is different from that of MCL injury. However, adequate information to explain the difference between ACL and MCL healings has not been obtained. The activity of cell migration is greatly influenced by cellular attachment. The expression of fibronectin-binding integrin α5β1 is higher in MCL cells than ACL cells. The present study demonstrated that the integrin α2-dependent adhesion responding to bFGF and GDF-5 had a key role to activate the cellular response. These findings suggest that the combination therapy with bFGF and GDF-5 may contribute to the treatment of ligament injury.

REFERENCES:

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