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 healing.

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.

Our objective in this study is to clarify the different abilities of ACL and MCL fibroblasts by investigating the cellular behavior and the integrin α2-mediated migration in response to growth factors.

MATERIALS AND METHODS:
Cells and cell culture: Cells were isolated from ACL and MCL of 10-week-old Japanese white rabbits. Cells between passage 3 and 6 were used for experiments.

Cell proliferation assays: To investigate the effects 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 basic fibroblast growth factor (bFGF) and bone morphogenetic protein (BMP)-2, and recombinant mouse GDF-5, -7 were added. These plates were incubated for 48 h prior to addition of WST-1.

RT-PCR and image analyses: Cultured ACL and MCL cells were treated with 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, integrin α2, and glyceraldehyde-3-phosphate dehydrogenase (G3pdh).

Cell migration and functional blocking 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 chamber was incubated for 8 h to allow cells for migration through the membrane.

In functional blocking assays, an anti-integrin α2 antibody or a control mouse IgG was added to the bottom chamber at a concentration of 10 μg/ml. The total number of cells with nuclei that migrated per well was counted.

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, GDF-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 F-actin fibers.

RESULTS:
bFGF is the strong stimulator for the proliferation of ACL and MCL cells: The proliferation of bFGF-treated cells was increased approximately 1.7-times higher than that of untreated ACL and MCL cells (Fig. 1).

GDF-5 increases the Coll1a1 expression in ligament fibroblasts: Coll1a1 and Col3a1 expressions were stimulated by GDF-5 and -7 treatments in ACL cells (Fig. 2A). In MCL, Coll1a1 expression was strongly induced by GDF-5 (Fig. 2B). These findings indicated that GDF-5 had a strong potential for ECM syntheses in ACL and MCL cells.

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. 3). The increase of migrated MCL was approximately 5-times higher than that of ACL in response to bFGF or GDF-5 stimulation.

DISCUSSION:
The present study demonstrates that MCL fibroblasts have a stronger migration activity in response to bFGF and GDF-5/7, than ACL fibroblasts, by modulating the integrin α2 expression and the integrin α2-mediated cellular migration on type I collagen. In addition, GDF-5 enhances the Coll1a1 expression, especially in MCL cells. Our results suggest that the different healing potentials between ACL and MCL injuries may be caused by the integrin α2-mediated cellular migration and the Coll1a1 expression responding to growth factors.

REFERENCES: