Mechanical Stretch Stimulates Integrin αVβ3-mediated Collagen Expression in Human Anterior Cruciate Ligament Cells

INTRODUCTION:
The injury of anterior cruciate ligament (ACL) is frequent in sports activity. However, a ruptured ACL does not cure by a conservative treatment. Mechanical stimuli are important for ligament homeostasis including the proliferation and type I collagen expression in tendon fibroblasts. We hypothesized that mechanical stretch plays an important role in the process of ligament healing.

ACL has the three histological zones consisting of round, fusiform, and ovoid cells. Here, we isolated the midsubstance and interface cells from human ACL. In addition, we investigated the collagen gene expression and integrin distribution responding to mechanical stretch in two types of ACL cells. We demonstrated that mechanical stretch increased integrin αVβ3-mediated COL1A1 expression in the interface cells, rather than that in middle substance cells. These results suggest that non-stretched interface cells might lose their healing potentials in ACL injuries.

MATERIALS AND METHODS:
Cells: Human ACL cells were harvested from ligament samples donated by patients who underwent total knee arthroplasties with informed consents (n=5). Interface cells were isolated from the 5-mm end of ACL. Midsubstance cells were cultured from the middle part of ACL.

Mechanical stretch: Midsubstance and interface cells were seeded onto fibronectin-coated stretch chambers. Uniaxial cyclic stretch was applied on cells at 0.5 Hz with 7% length stretch for 2h using ST140.

RT-PCR: RNA samples derived from both cells were reverse-transcribed, and cDNAs underwent PCR amplification.

Quantitative real-time RT-PCR analysis: Quantitative real-time RT-PCR analysis was performed using LightCycler-FastStart DNA Master SYBR Green I kit. The expression value was calculated as a relative mRNA level.

Immunohistochemistry: To investigate the cellular attachments responding to mechanical stretch, we observed the distribution of integrins and actin fibers in both cells. Stretched cells were incubated with an anti-integrin α5, β1, and αVβ3 antibody for 1 h. Alexa Fluor 488-conjugated antibody, Alexa Fluor 568-conjugated phalloidin, and Hoechst 33342 were used for detections.

Functional blocking assay: To inhibit the function of integrin α5 and αVβ3 in stretching experiments, anti-human integrin α5 and αVβ3 functional blocking antibodies were used. Cells were incubated with each antibody for 10 min before seeding on chambers. Cells were allowed to attach for 2 h, then mechanical stretch was applied for 2 h. RNAs were immediately collected after stretching experiments.

RESULTS:
Non-stretched ACL cells loose their phenotypes.

COL1A1 and COL3A1 expressions were maintained in the tissue RNAs of interface zones. However, these expressions were totally inhibited in cultured interface cells (Fig. 1, I). COL3A1 gene was abnormally detected in cultured midsubstance cells (Fig. 1, M). Integrin αV and β3 gene expressions were decreased in cultured interface cells (Fig. 1, I). These results indicated that the phenotypes of ACL cells were changed in usual cultured conditions.

Mechanical stretch reproduces the expression of collagen genes in cultured ACL cells.

Mechanical stretch stimulated the COL1A1 expression of midsubstance and interface cells up to 6- and 14-fold level of non-stretched cells, respectively. The COL1A1 expression was also augmented by cyclic stretching in both cells (Fig. 2, A and B). In addition, the mechanical stretch increased the integrin αV expression in cultured midsubstance and interface cells (Fig. 3). These findings suggested that mechanical stretch-dependent integrin expressions might have key roles to maintain the collagen gene expressions and the phenotypes of ACL cells.

Stretch-activated collagen gene expressions depend on the integrin αVβ3-mediated cellular adhesions.

To assess the role of integrin for stretch-induced collagen gene expression, we performed functional blocking assays using anti-integrin antibodies. Stretch-activated COL1A1 and COL3A1 expressions were inhibited by the functional blocking antibody for integrin αVβ3 in the both ACL cells (Fig. 4). However, the functional blocking for integrin α5 did not suppress the stretch-induced COL1A1 and COL3A1 expressions in both ACL cells (Fig. 4). These results suggested that stretch-activated intracellular signals for collagen syntheses might be transduced by integrin αVβ3, not by integrin α5, in human ACL cells.

Mechanical stretch activates the integrin αVβ3-mediated stress fiber formation on fibronectin.

We analyzed the relationships among mechanical stretch, cellular adhesion, and stress fiber formation in ACL cells. Integrin αVβ3 was shifted to the peripheral edge of cells by stretching treatments (Fig. 5A). Integrin αVβ3, green signals. In addition, mechanical stretch increased the integrin αVβ3-dependent stress fiber formation in both ACL cells on fibronectin (Fig. 5A, red signals). Integrin αVβ3 also localized with phosphorylated FAK in stretched ACL cells (Fig. 5B).

DISCUSSION:
Several authors have reported that cyclic stretch stimulates collagen gene expression in ligament cells. However, the stretch-mediated regulation of collagen expression is still unknown. In the present study, we demonstrated that mechanical stretch reproduced the expression of collagen genes in cultured ACL cells. Moreover, the expression of integrin αVβ3 was increased in stretched ACL cells. These findings suggest that integrin αVβ3-mediated stretch signals might be necessary for ACL healings by activating collagen gene expression.

REFERENCES:
4) Kim SG et al. (2002) Cell Struct Funct. 27, 139-144.