Cyclic tensile strain stimulates CCN2/CTGF expression in human anterior cruciate ligament-derived cells.

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INTRODUCTION
Connective tissue growth factor (CCN2/CTGF) promotes proliferation and differentiation of chondrocytes\textsuperscript{1-3}, and was shown to regulate mesenchymal tissues. However, the expression and function of CCN2/CTGF in ligament-derived cells remain unclear.

Anterior cruciate ligament (ACL) injuries usually occur in the attachments of ligament-to-bone interface region\textsuperscript{4-6}. The interface cells in ACL-to-bone insertion have chondrocytic phenotype\textsuperscript{7-9}. We have previously reported that cyclic tensile strain (CTS) stimulates \(\alpha(1)\) collagen (COL1A1) expression in human ACL-derived cells and has an important role in ligament homeostasis\textsuperscript{10}.

In the present study, we investigated the localization of CTGF/CCN2 in human ACL and CCN2/CTGF expression responding to CTS in ACL-derived cells.

MATERIALS AND METHODS

\textbf{Histology and immunohistochemistry:} We investigated the localization of CCN2/CTGF in human ACL with an anti-CTGF antibody (R&D).

\textbf{Cells and cell culture:} We prepared two types of ACL-derived cells from the midsubstance and ligament-to-bone interface in human ACL.

\textbf{Cyclic tensile strain (CTS):} The midsubstance and interface cells were seeded onto fibronectin-coated stretch chambers. Uni-axial CTS (0.5 Hz, 10% strain) was applied using a STB-LAB device (STREX). RT-PCR and quantitative real-time PCR analyses: The expressions of CCN2/CTGF and COL1A1 genes were assessed by RT-PCR analyses. Relative CCN2/CTGF expression was evaluated by quantitative real-time PCR analysis.

\textbf{ELISA:} The concentration of CCN2/CTGF in conditioned media was measured by using a sandwich ELISA system with two anti-human CCN2/CTGF monoclonal antibodies (MAb 8-64 and 8-86; mouse IgG1, Nichirei).

\textbf{Cell proliferation assay:} Cells were incubated for 48 h in the presence or absence of recombinant CCN2/CTGF (BioVender). Cell proliferation was assessed by WST-1 assay (Roche). Optical density (OD) was measured by Model 550 microplate reader (BioRad) with a test wavelength of 450 nm and a reference wavelength of 630 nm.

RESULTS

CTGF/CCN2 was detected in the interface region of ACL

CCN2/CTGF was observed in the ACL-to-bone interface (1D), but not in the midsubstance region (1C) by an anti-CTGF antibody. CCN2/CTGF was detected in the cells packaged in tibial (1E) and femoral interface (1F) of human ACL.

Interface cells had different features compared with midsubstance cells

The morphology of interface cells was typically characterized by a triangular shape (2A). Despite CCN2/CTGF was present in the tissues of interface region, RT-PCR analyses revealed that CCN2/CTGF expression was remarkably decreased in interface-derived cells (2B), which was efficiently recovered by CTS (2C, D). CTS also increased CCN2/CTGF secretion from interface cells (2E). On the other hand, cultured midsubstance cells constitutively expressed CCN2/CTGF, despite CTGF/CCN2 expression was not detected in the midsubstance region of ACL (2B). CTS treatment did not influence the gene expression of CCN2/CTGF in midsubstance cells (2C, D). However, CCN2/CTGF secretion was increased by CTS in midsubstance cells (2E).

CCN2/CTGF stimulated the proliferation of interface cells

CCN2/CTGF treatment (10 ng/ml) increased the expression of endogenous CCN2/CTGF gene only in interface cells (3A, B). COL1A1 expression was not influenced by CCN2/CTGF treatment in interface cells despite CTGF/CCN2 stimulated COL1A1 expression in midsubstance cells (3C). However, CCN2/CTGF significantly stimulated the proliferation of interface cells in a dose-dependent manner (3D).

DISCUSSION

Our results demonstrated that CCN2/CTGF localized in the ligament-to-bone interface of human ACL. CTS treatment promoted the expression of CCN2/CTGF and COL1A1 in the interface cells. In addition, CCN2/CTGF treatment stimulated endogenous CCN2/CTGF expression and cellular proliferation in the interface cells. These findings suggest that CTS and CCN2/CTGF cooperatively regulate the homeostasis of ACL by inducing cellular proliferation.

SIGNIFICANCE

This study proposes that mechanical stretch-induced CCN2/CTGF would have an important role in enhancing the healing of ligament-to-bone interface after ACL injury.

REFERENCES