Mechanical stretch enhances COL2A1 expression on chromatin by inducing SOX9 nuclear translocalization in inner meniscus cells

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[Introduction]
The meniscus is a fibrocartilaginous tissue that plays an important role in controlling complex biomechanics of the knee such as tension, compression, and shear stress.1 Proper functioning of the meniscus depends on the composition and organization of its extracellular matrix (ECM).2 We have previously demonstrated that human inner meniscus cells show ovoid and chondrocytic morphologies although slender and fibroblastic cells are obtained from the outer halves of human menisci.3 In vitro differentiation analyses have revealed that inner meniscus cells maintain higher chondrogenic potential such as the expression of Sry-type HMG box (SOX) 9 and α(II) collagen (COL2A1) genes compared with outer meniscus cells.4 In the present study, we further investigated the precise function of SOX9 under stretching condition in human meniscus cells. The SOX9-related transcriptional complex has an important role in the epigenetic regulation of chondrogenesis. We hypothesized that SOX9 may epigenetically regulate COL2A1 expression in response to mechanical stretch. This study aims to clarify the cross-talk among mechanical stretch, SOX9, and SOX9-regulated transcription on chromatin in chondrocytic inner meniscus cells.

[Materials and methods]
Cells and cell culture: Institutional Review Board approval and informed consent were obtained before all the experimental studies. Macroscopically intact lateral menisci were obtained at total knee arthroplasty in patients suffering from medial compartment osteoarthritis of the knee. Inner and outer meniscus cells were prepared from the inner and outer halves of the lateral meniscal samples, respectively. Stretching experiments: Inner and outer meniscus cells were seeded onto stretch chambers coated with rat tail type I collagen. Uni-axial cyclic stretch strain (CTS: 0.5 Hz, 5% strain) was applied using a STX-140 system for 2, 4, and 8 h. Non-stretched meniscus cells cultured on stretch chambers were used as controls. Cells, RNAs, and proteins were immediately collected after stretching experiments. RT-PCR and quantitative real-time PCR: Expressions of COL2A1, SOX5/6/9, COL9A1, COL1A1, and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were assessed by PCR analyses. Amplification data of G3PDH were used for control. Relative mRNA levels were normalized with the level of non-stretched inner meniscus cells. Immunohistochemistry, Western blot (WB), and immunoprecipitation (IP): The deposition of type II collagen in human meniscal tissues was assessed by immunohistochemical analyses. The nuclear translocation and phosphorylation of SOX9 after CTS treatment were evaluated by anti-SOX9 and anti-phospho-SOX9 antibodies. The fraction immunoprecipitated with a mouse anti-SOX9 antibody was assessed by WB using a rabbit anti-p-SOX9 antibody (IP-WB). Chromatin IP: Meniscal DNA fragments immunoprecipitated with endogenous SOX9 were purified after CTS treatments. PCR reactions were performed using the primer sets to amplify the promoter and SOX9-binding enhancer of COL2A1 gene.4

[Results]
Type II collagen deposition and SOX9 production were detected only in the inner half of human meniscus
Type II collagen was detected only in the inner meniscal ECM (1A, brown). SOX9 was observed in the inner meniscus (1D, open arrow heads). However, the signals immunostained by anti-type II collagen and anti-SOX9 antibodies were not detected in the outer meniscus (1B, 1E).

Mechanical stretch stimulated the gene expression of COL2A1 and SOX9 in inner meniscus cells
CTS treatments increased the expression of COL2A1 in inner meniscus cells up to a 2.6-fold level of controls (2A, 2B). SOX9 gene expression was enhanced up to a 4-fold level of controls by 4-h-CTS in inner meniscus cells (2A, 2C). SOX5/6/9 expressions were increased in stretched inner meniscus cells (2A). However, expression of COL2A1 and SOX5/6/9 was not detected by CTS in outer meniscus cells (2A-C).

Mechanical stretch activated nuclear translocation and phosphorylation of SOX9 in inner meniscus cells
CTS treatments induced the nuclear translocation of endogenous SOX9 in inner meniscus cells (3A, green). CTS also enhanced SOX9 phosphorylation in inner meniscus cells (3B, green). However, SOX9 and p-SOX9 were not increased by CTS in outer cells (3C, 3D).

Mechanical stretch induced the recruitment of phosphorylated SOX9 on the COL2A1 enhancer in inner meniscus cells
WB analyses revealed that CTS induced the phosphorylation of SOX9 in inner meniscal cells (4A, p-SOX9). IP/WB analyses revealed that p-SOX9 was also increased by CTS (4B). Chromatin IP analyses revealed that CTS treatments increased the association between SOX9 and the conserved SOX9-binding site on the COL2A1 enhancer in inner meniscus cells (4C). Amplified fragments were detected in the SOX9-IP fraction of stretched meniscus cells, but were not observed in that of non-stretched cells (4C). No fragment was observed in chromatin IP analyses using the primer set for the COL2A1 promoter region lacking SOX9-binding ability.

[Discussion]
Our results indicate that inner and outer meniscus cells have different properties in mechanical stretch-induced SOX9 and COL2A1 expression. Further studies will be required to understand the relationship between stretch-activated intracellular signaling and p-SOX9. The present study demonstrates important evidence that mechanical stretch epigenetically regulates COL2A1 expression on the chromatin in inner meniscus cells.

[Significance]
Mechanical stretch would contribute to achieve a new clinical approach that enhances the healing of meniscal injury in inner avascular zones by inducing SOX9 phosphorylation and type II collagen production.

[References]