Mechanical stretch-mediated CCN2 up-regulation in meniscus cells

Takaaki Tanaka1, Takayuki Furumatsu1, Emi Matsumoto1, Zhichao Lu1, Tomoko Kanazawa1, Satoshi Kubota2, Masaharu Takigawa2, Toshifumi Ozaki1

1Dept. of Orthopaedic Surgery, Okayama University Graduate School, 2Department of Biochemistry and Molecular Dentistry, Okayama University Graduate School

[Objective] The intrinsic zone-specific properties of the menisci are determined by biomechanical environments, such as tension, compression, and shear stress. In this study, we examined mechanical stretch-dependent expression of multifunctional growth factor CCN2/CTGF, and investigated the role of CCN2 in human meniscus cells.

[Methods] Meniscus cells were isolated from macroscopically intact lateral menisci. Inner and outer meniscus cells were prepared from the inner and outer halves of menisci. Uni-axial cyclic tensile strain (CTS) was applied using a STB-140 system. CTS-induced expression of CCN2 and COL1A1 was assessed by quantitative real-time PCR analysis. The distribution of CCN2 and Smad2/3 in stretched cells was investigated by immunohistochemical analysis. Smad2/3-dependent CCN2 transactivation was measured by luciferase reporter assay. The relationship between Smad2/3 and CTS-induced CCN2 transcription was investigated by chromatin immunoprecipitation.

[Results] CTS stimulated gene expression of CCN2 and COL1A1 in inner meniscus cells, but not in outer meniscus cells. Recombinant CCN2 increased COL1A1 expression only in inner meniscus cells. CCN2 synthesis and nuclear translocalization of phosphorylated Smad2/3 in inner meniscus cells were stimulated by CTS. The CCN2 promoter activity was synergistically enhanced by overexpressed Smad3 in stretched inner meniscus cells, but was not by Smad2. Chromatin immunoprecipitation revealed that CTS increased the association between Smad3 and the Smad-binding element on the CCN2 proximal promoter in inner meniscus cells.

[Conclusion] Our results suggest that stretch-induced Smad3 phosphorylation and CCN2 transactivation may have crucial roles in preserving the feature of the human inner meniscus.