**Increased expression of Hypoxia-inducible factor-2α in osteoarthritic meniscus cells**

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**INTRODUCTION:** Osteoarthritis (OA) is a common disease characterized by a progressive degradation of articular cartilage leading to loss of joint function. The underlying process leading to cartilage destruction in OA is poorly understood. Recently, some articles reported that endochondral ossification plays a crucial role in OA development, and expression of transcriptional factor HIF-2α causes endochondral ossification by transactivation of its related gene, COL10A1, MMP13, VEGF and other osteogenic factors. 

Knee menisci have essential roles to play load transfer and distribution during joint motion, and its degenerative change initiates degeneration of the adjacent articular cartilage leading to knee OA. Although the meniscal degeneration is associated with knee OA, the precise cellular mechanisms underlying meniscal degeneration remain unclear. Previous reports have indicated the morphologic changes of meniscus cells during OA progression. And, a few studies have reported the expression of type X collagen in OA meniscus, which is observed in OA cartilage. However as far as we know, expression of HIF-2α in meniscus cells has not been reported. The objectives of this study are to evaluate whether HIF-2α is up-regulated and cause transactivation of endochondral ossification in human meniscus related molecules in OA meniscus.

**METHOD:** After informed consent was obtained and in accordance with the institutional review board of Nagoya University Graduate School of Medicine, Human menisc and articular cartilage were obtained from 6 knee joints with OA who were undergoing total knee arthroplasty, and human non-OA meniscus were obtained from 3 knee joints with discoid meniscus who were undergoing partial meniscectomy. Cell isolation and culture: Meniscus cells and chondrocytes were isolated by enzymatic digestion. Primary meniscus cells and chondrocytes and HEK293 were used for comparing gene expression levels. The cells were seeded and cultured in DMEM and HAM F-12 mixture plus 10% FBS and 1% penicillin.

**qRT-PCR:** Meniscus cells and chondrocytes obtained just after isolation were analyzed for mRNA expression of HIF2α, MMP-13, COL10A1, VEGF, RUNX2 by real time RT-PCR. Histology and immunohistochemistry: Meniscus and cartilage tissue were sectioned into 6μm-thick and performed Safranin O staining. Immunohistochemistry staining was performed with anti-human HIF2α, COL10A1, MMP-13 IgG (abcam). Treatment with interleukin-1β (IL-1β): Total RNA was isolated from meniscus cells treated IL-1β (5ng/ml) for 12h and analyzed for mRNA expression.

**Small interfering RNA:** Meniscus cells were transfected for 6h with siRNA specific to HIF2α or Non-silencing siRNA (Sigma-Aldrich) by the Lipofection method using lipofectamine 2000 (Invitrogen). Transfected cells were exposed to IL-1β (5ng or 10ng/ml) for 12h. Then total RNA was isolated for RT-PCR.

We evaluated the statistical significance of data using Student’s t-test. We considered a P value of 0.05 to be significant. The results are shown as mean ± SD (error bars).

**RESULTS:** The mRNA expression of HIF2α in OA meniscus cells was higher than that of OA chondrocytes. The mRNA expression of MMP-13 and COL10A1 in OA meniscus cells were similar to those of OA chondrocytes (Fig.1.). The mRNA expression of HIF2α and endochondral ossification related gene except for RUNX2, were much higher in OA meniscus cells than non-OA meniscus cells. And HIF2α and its related gene expression were significantly up-regulated in OA meniscus cells treated with IL-1β in comparison with control (Fig.2.). Immunohistochemistry in OA meniscus showed strong positive staining for HIF2α in both nuclei and cytoplasm. Positive staining for MMP-13 and type X collagen were observed in cytoplasm and superficial layers stained weakly with safranin O. (Fig.3.). The expression of HIF2α mRNA was suppressed by siRNA specific to HIF2α, and siRNA-mediated knockdown of HIF2α also reduced mRNA expression of MMP-13 and COL10A1 (Fig.4.).

**DISCUSSION:** In this study, we have shown that HIF-2α was also expressed in OA meniscus as well as in OA cartilage, and it was induced by IL-1β. According to a previous study, COL10A1, MMP-13 and VEGF are the direct transcriptional targets of HIF-2α. Our immunohistochemistry demonstrated the similar expressions pattern of HIF-2α and MMP-13 and type X collagen in degenerated layers. These results indicate that HIF-2α may act as a crucial mediator of matrix destruction not only in articular cartilage, but also in OA meniscus. Our result raises the possibility that HIF-2α, highly expressed in OA meniscus, causes meniscal matrix degradation by transactivation of endochondral ossification related gene. Therefore HIF-2α may be one of the therapeutic targets to abrogate matrix destruction in both joint cartilage and meniscus during OA progression. However, future study is required to explore the specific mechanisms of HIF2α activation and its downstream effects in meniscus.

**SIGNIFICANCE:** This study is the first to directly demonstrate HIF-2α expression in human OA menisci. Inhibitors of HIF-2α may have therapeutic benefit in both cartilage and meniscal degradation in OA.