Increased expression of Wnt5a/ROR2 signaling is associated with chondrogenesis in meniscus degeneration

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INTRODUCTION: Meniscus degeneration is commonly seen in the knee osteoarthritis (OA) and prevention of meniscus degeneration has gained great interest. The Wnt signaling pathway has been suggested to influence the regenerative capacity of meniscus [1]. However, no other study investigated how the expression of the Wnt signal change during the degeneration. In addition, change of progenitor cell phenotype in the degenerated meniscus is unclear. The purpose of this study is to test the hypothesis that the non-canonical Wnt signaling pathway is associated with chondrogenesis in meniscus degeneration.

METHODS: Twenty OA meniscus and four non-OA meniscus were collected at the time of total knee arthroplasty and used for the study. Degeneration was histologically assessed using a grading system. Each slide was immunohistochemically assessed to detect the expression of several markers related to hyaline chondrocyte: SOX9, collagen type II, collagen type XI, and aggrecan, and those related with Wnt signaling: Wnt3a, Wnt5a, βcatenin, ROR2, and TCF7. Furthermore, real-time PCR and western blot were performed to examine the expression of Wnt signaling. This study was approved by the Ethics Committee of the Yokohama City University (no. B191100008) and informed consent was obtained from all patients.

RESULTS SECTION: Immunohistochemistry showed significant correlations between the degeneration score and the percentage of positive cells with SOX9 (r=0.73, P=0.0002), collagen type II (r=0.73, P=0.0002), collagen type XI (r = 0.86, P＜0.0001), aggrecan (r = 0.84, P＜0.0001), Wnt5a (r = 0.80, P ＜0.0001), ROR2 (r = 0.85, P＜0.0001), or TCF7 (r=-0.58, P=0.0073) (Figure.1). The real-time PCR revealed that *WNT5A* (P=0.006), *ROR2* (P=0.012) showed higher and *TCF7* (P=0.012), *AXIN2* (P=0.042) showed lower expression level in OA meniscus compared to non-OA meniscus. The OA meniscus showed significantly higher expression level of Wnt5a (P=0.006), and significantly lower expression level of AXIN2 (P=0.042) and TCF7 (P=0.006) than the non-OA meniscus. No significant differences between the OA and the non-OA meniscus were found in the expression levels of *WNT3*, *WNT4*, *WNT9A*, *CTNNB*, and *LRP6* (Figure.2). The western blot revealed that the OA meniscus showed significantly higher expression level of Wnt5a (P=0.006), and significantly lower expression level of AXIN2 (P=0.042) and TCF7 (P=0.006) than the non-OA meniscus as well. There was no significant difference in βcatenin expression level (Figure.3).

DISCUSSION: The key findings of this study were that the expression levels of Wnt5a/ROR2 increased and the expression levels of the downstream target of Wnt/βcatenin signal decreased in the degenerated meniscus. The study also found significant correlations between the degeneration score and the percentage of positive cells with SOX9, collagen type II, collagen type XI, aggrecan, Wnt5a, ROR2, or TCF7. The increased expression of Wnt5a promoted chondrogenesis with antagonizing the Wnt/βcatenin signaling. These findings suggest that chondrogenic differentiation is crucial in the progression of meniscus degeneration and that the non-canonical Wnt signaling pathway has a potential role in this process. Wnt5a/Ror2 signaling is involved in inhibiting canonical Wnt signaling at the level of TCF/LEF-mediated transcription [2]. This study supported the initial hypothesis that the non-canonical Wnt signaling pathway, Wnt5a/ROR2 signaling, is associated with chondrogenesis in meniscus degeneration.

SIGNIFICANCE/CLINICAL RELEVANCE: The study provides new insights into the mechanisms involved in the development of OA and may help researchers develop new therapies that target the Wnt signaling pathway to prevent or slow down the degeneration of meniscus.

RFERENCE: [1] Später D, et al. Eur. Cell. Mater. 2006;12:71-80. [2] Yuan Y, et al. Int. J. Mol. Med. 2011;27(1):63-69.

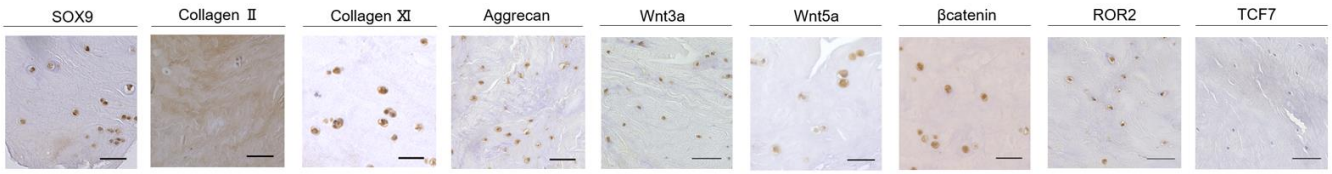

Figure.1 Immunohistochemistry of chondrogenic markers and Wnt signaling components in human OA meniscus. Scale bar = 50 mm.

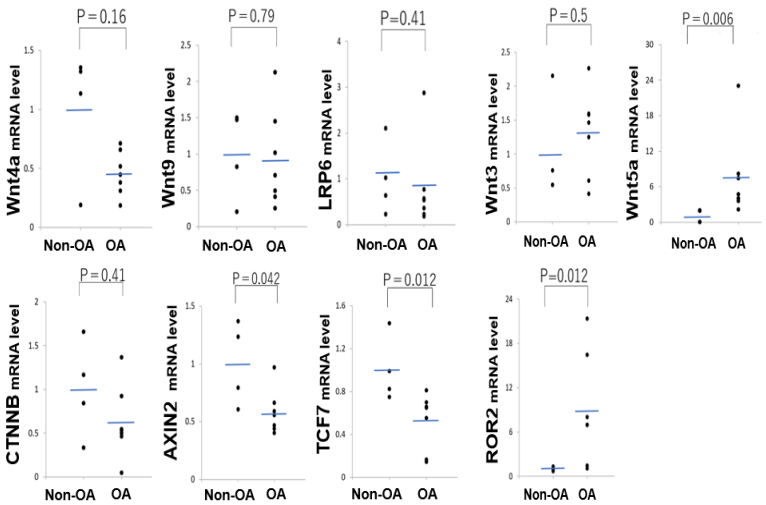

Figure.2 Dot plot graphs for expression levels of genes of the Wnt signaling component.

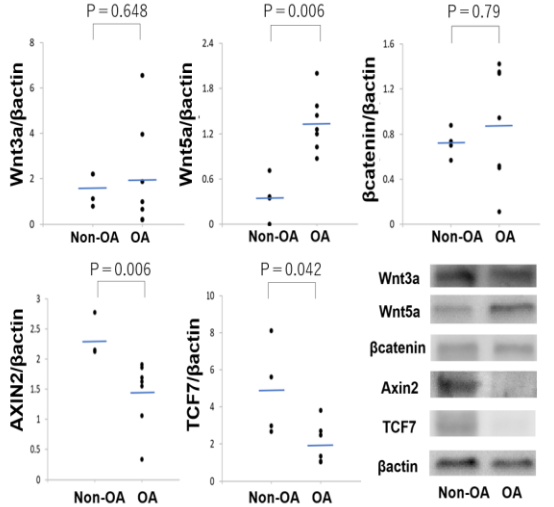

Figure.3 Western blot analysis of proteins related to the Wnt signaling.