Wisp1 Aggravates Osteoarthritis By Modulation Of TGF-β Signaling And Positive Regulation Of Canonical Wnt Signaling

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Introduction: The majority of osteoarthritis (OA) patients show synovial activation, which is thought to be involved in joint destruction. Previously, we found increased expression of various members of the Wnt signaling in the synovium in two experimental OA mouse models. In addition, we found WISP1, a key downstream target of the canonical Wnt signaling, to be significantly increased both in the synovium and cartilage of these experimental OA models and in human OA cartilage. One of the most well-known functions of Wnt signaling is the embryonic development of the tissues that are present in articular joints. It is generally believed that some of the processes that take place during embryonic development are turned on again during OA, making Wnt signaling an alluring pathway to study during OA. The involvement of Wnt signaling in OA incidence has been implicated mainly via activation of the β-catenin-dependent canonical signaling pathway. Modulation of β-catenin leads to OA-like changes in the cartilage. However, the role of the downstream protein WISP1 in the initiation and progression of the main hallmarks of OA is not known. These main hallmarks include degeneration of the articular cartilage, inflammation and fibrogenesis in the synovium, sclerosis of the subchondral bone and ectopic bone formation at the joints margins and in the ligaments of the joint. In the present study, we investigated the role of WISP1 during OA by inducing various experimental OA models in WISP1 knockout mice.

Methods: Pathway analysis of microarray data from the synovium of both a collagenase-induced OA (CIOA) and a destabilization of the medial meniscus (DMM) mouse model time course was performed using DAVID bioinformatics software. Experimental OA was induced by either CIOA or DMM in WISP1 knockout mice and their WT controls and OA pathology was allowed to develop for 42 and 56 days respectively. Afterwards, joint pathology was assessed by histology after Safo/FastGreen staining. Immunohistochemical staining was used to determine the effects of WISP1 on Smad phosphorylation and β-catenin.
accumulation. Microarray analysis was performed on the synovial tissue of patients enrolled in the CHECK study. This cohort study is a multicenter based initiative of the Dutch Arthritis Foundation to follow the progression of OA symptoms in patients that visited their general practitioner with early complaints of knee or hip pain.

**Results:** Previous studies in our lab using pathway analysis of microarray data from synovial tissue with DAVID showed enrichment of the Wnt signaling pathway in both the CIOA and DMM at various time points after induction of the OA models. In addition, microarray analysis of synovial tissue obtained from patients in the CHECK cohort of early OA patients showed a strong correlation between WISP1 expression and damage at baseline. Moreover, WISP1 expression levels were correlated with the progression of OA between baseline and the five-year follow-up measurement, as defined by a decrease of joint space width of at least 1 mm and progression of osteophyte formation of at least 4x in size. In order to further pinpoint the role of WISP1 as a central downstream mediator of the canonical Wnt signaling pathway in the etiopathology of OA, we induced both the CIOA and DMM model in WISP1 KO mice. These OA models are both dependent on instability of the tibio-femoral joint, but differ in other characteristics, like inflammation of the synovial membrane, fibrosis and ectopic bone formation. After the development of OA pathology for 42 and 56 days respectively, we found significantly decreased cartilage damage in the tibio-femoral joints of the WISP1 KO mice as compared with the WT mice. Balanced TGF-β-induced Smad signaling is crucial for the homeostasis of the cartilage. Therefore, we stained our sections for phosphorylated Smad 2/3 in order to determine if WISP1 could affect this balance. We found that Smad 2/3 signaling was increased in the WISP1 KO mice, suggesting that increased WISP1 expression during OA decreases Smad 2/3 signaling, which has been shown to be beneficial for chondrocyte homeostasis. In addition, recent data showed that WISP1 might not only act as a downstream regulator of canonical Wnt signaling, but might also be involved in the accumulation of β-catenin, thereby positively regulating canonical Wnt signaling that could further aggravate the OA pathology. To investigate if this mechanism was present in our OA models, we stained our sections for β-catenin and found that absence of WISP1 indeed resulted in decreased levels of β-catenin accumulation in the cartilage.

**Discussion:** Overexpression of WISP1 in the synovium and cartilage, as is found in OA conditions, may play an important role in OA pathology via modulation of TGF-β signaling and via a positive feedback mechanism on canonical Wnt signaling. Because the Wnt signaling pathway is both extremely complex and tightly regulated and its involvement in many processes in the body has been shown, the more upstream Wnt signaling pathway will be difficult to
target without undesired side-effects. WISP1 is a downstream mediator of canonical Wnt signaling and therefore, targeting WISP1 may more specifically address the events that take place during OA without interfering with other processes. This highlights the potential for WISP1 expression to be a potential target for OA therapy.

**Significance:** The canonical Wnt signaling pathway is thought to play a role in the development of OA pathology, possibly via increased expression of WISP1. Therefore, increasing our knowledge about the mechanism how members of the canonical Wnt signaling pathway, and WISP1 in particular, contribute to the development of OA will provide new insights that might help us choosing new targets for the development of OA therapy.

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