Synovial Wnt And Wisp1 Expression Induces Expression Of Cartilage-degrading Metalloproteinases In The Synovium

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Introduction: Many osteoarthritis (OA) patients show significant synovial involvement, although the consequences for OA pathology are largely unknown. The synovium mainly consists of fibroblasts, macrophages and we see monocytes in the early phases of our OA models. Previously, we found strongly increased expression of Wnt2b, Wnt16 and WISP1, a downstream protein of canonical Wnt signaling, in knee joints in two murine OA models. Wnt signaling has been implicated in OA incidence through activation of the β-catenin-dependent canonical Wnt signaling pathway. Modulation of this β-catenin pathway, leads to OA-like changes in cartilage. However, the role of the synovium in the induction of OA pathology under the influence of Wnt signaling is still unclear. In the present study, we investigated the potential of Wnt signaling to increase the expression of cartilage-degrading enzymes in the synovium.

Methods: Pathway analysis of microarray data from the synovium of a collagenase-induced OA mouse model was done using DAVID software. In vivo synovial overexpression of genes from the canonical Wnt signaling pathway was achieved by intra-articular injection of adenoviral vectors. Joint pathology was assessed by histology at several time points after injection. Human OA synovial tissue was collected from joint replacement surgery and either stimulated directly or used for outgrowth of OA fibroblasts. Monocytes were isolated fromuffy coats from healthy donors and stimulated directly or after differentiation into M1 or M2 macrophages. Gene expression was analyzed by qPCR. Protein expression was measured in culture supernatants by Luminex.

Results: Earlier studies in our lab demonstrated that pathway analysis using DAVID bioinformatics showed that the Wnt signaling pathway was enriched in the synovium during experimental OA. Many people consider OA to be a recapitulation of developmental processes in the joints. It is known that Wnt signaling is of the utmost importance during the joint development. Together, this points to Wnt signaling as an interesting pathway to study in OA. To determine the effects of Wnt signaling on the expression of cartilage-degrading enzymes in synovial tissue, we stimulated human OA synovial specimen with Wnt3a or its downstream protein WISP1. This resulted in increased expression of MMP3, MMP9 and MMP13, whereas expression of the MMP inhibitors TIMP1 and 3 was not altered. Next, we investigated which cell-type in the synovium might have caused the increased MMP expression. Stimulation of human synovial OA fibroblasts with Wnt3a or WISP1 increased the expression of both MMP3 and MMP13, whereas the TIMP expression was not altered. In contrast, stimulation of both M1 and M2 macrophages with either Wnt3a or its downstream protein WISP1 did not result in increased expression of MMPs. In addition, we stimulated monocytes, which are present in the synovium in the early phases of our OA mouse models. Stimulation of primary human monocytes with Wnt3a or WISP1 strongly increased the expression of MMP3, MMP9 and MMP13. Expression levels of TIMP1 and 3 were not altered. Next, we hypothesized that if increased Wnt signaling was present in OA synovial tissue and stimulation of synovial tissue with members of the Wnt signaling pathway increased the expression of MMPs, we should be able to decrease the expression of MMPs by blocking the Wnt signaling pathway. Inhibition of Wnt signaling by both FrzB and DKK-1, a specific inhibitor for canonical Wnt signaling, led to decreased expression of MMP3, MMP9 and MMP13 in human synovial specimen. To determine if synovial overexpression of members of the Wnt signaling pathway leads to cartilage damage in vivo, we injected adenoviral vectors for Wnt8a, Wnt16 into murine knee joints. These vectors specifically target synovial cells but do not penetrate into the cartilage, due to their size. A strongly enhanced expression of RNA of the genes that were overexpressed was found in the synovium. Seven days after overexpression, we found a significant induction of OA pathology at the medial margin of the medial tibial plateau, a preferential site for damage in experimental OA. Lesions were found in 92% (n=12) of the knee joints after Wnt8a overexpression compared to 17% (N=12) for the control virus and 80% (N=5) for Wnt16 overexpression, but only 20% (N=5) for the control virus.

Discussion: Canonical Wnts produced in the synovium may play an important role in OA pathology. Stimulation of human OA synovium with Wnts and WISP1 increases the expression of MMPs, which was mainly found in fibroblasts and monocytes, but not in macrophages. In addition, synovium-specific overexpression of Wnt signaling members, as is found in experimental OA, induces cartilage damage in vivo. This underlines synovial Wnt/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. Therefore, increasing our knowledge about the mechanism how members of the canonical Wnt signaling pathway 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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Figure 1. In vivo overexpression of members of the Wnt signaling pathway causes early OA-like cartilage damage.