Synovial Wnt and WISP1 induce OA-like cartilage damage by skewing of TGF-beta signaling from Smad 2/3 towards Smad 1/5/8 phosphorylation

ABSTRACT INTRODUCTION:
Although damage of cartilage and bone are the main features of osteoarthritis (OA), a large proportion of the OA patients also show significant involvement of the synovium. However, the consequences of this synovial involvement are largely unknown. We found strong up-regulation of canonical Wnts 2b and 16 specifically in the synovium and Wnt-1 induced signaling protein 1 (WISP1), a downstream protein, in both the synovium and cartilage of two experimental murine OA models. Polymorphisms of genes from the Wnt/β-catenin signaling pathway, which is very important for the development of cartilage, have been implicated in OA incidence. Modulation of the β-catenin pathway leads to OA-like changes in cartilage. In addition, TGF-β signaling is critical in cartilage maintenance. It has been shown that TGF-β signals via both ALK5 and ALK1 and downstream via Smad 2/3 and Smad 1/5/8 respectively. Whereas TGF-β signaling via the anabolic ALK5 pathway results in preservation of the chondrocyte phenotype, signaling via the ALK1 pathway results in chondrocyte hypertrophy. The aim of the present study is to investigate the potency of canonical Wnts, produced in the synovium, to induce OA-like cartilage damage and whether canonical Wnts skew TGF-β signaling from the protective Smad 2/3 pathway to the Smad 1/5/8 pathway, which can induce chondrocyte hypertrophy.

METHODS:
In vivo synovial overexpression of genes from the canonical Wnt signaling pathway was achieved by intra-articular injection of adenoviral vectors in the right knee joints of C57Bl6 mice. Joint patholgy was assessed by histology at several time points after injection. Gene expression was analyzed by qPCR after overexpression of Wnt genes in isolated human chondrocytes. In vitro chondrocytes were stimulated with WISP1 and/or the canonical Wnt3a with or without addition of TGF-β. Western blot analysis was done to detect phosphorylation of Smad 2/3 and Smad 1/5/8.

RESULTS SECTION:
To determine if synovial overexpression of canonical Wnts leads to cartilage damage, adenoviral vectors for Wnt8a and 16 were injected in murine knee joints. These adenoviral vectors are too large to penetrate cartilage, and therefore specifically target synovial cells. At day 7 after overexpression a highly significant induction of OA pathology was found at the medial margin of the medial tibial plateau, where the first damage is often found in murine experimental OA models. The incidence was 92% (n=12) for 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.

Because of their relatively small size, Wnts and WISP1 proteins can penetrate the articular cartilage and possibly alter chondrocyte phenotype. Synovial Wnt8a and Wnt16 overexpression led to β-catenin accumulation in chondrocytes, a tell-tale sign of canonical Wnt signaling, indicating diffusion of Wnts to the cartilage. Moreover, overexpression of canonical Wnts and WISP1 in human chondrocytes led to a significant increase of collagen type I and a significant decrease in type II collagen expression, suggesting a loss of chondrocyte phenotype. Pre-incubation with Wnt3a or WISP1 alone or Wnt3a + WISP1 together resulted in a shift from TGF-β-induced phosphorylation of Smad 2/3 towards Smad 1/5/8 phosphorylation. This implies that Wnt3a and WISP1 cause a shift towards dominant TGF-β signaling via the hypertrophy-inducing ALK1 pathway.

DISCUSSION:
Canonical Wnts produced in the synovium may play an important role in OA pathology by inducing β-catenin signaling in the cartilage followed by cartilage damage. Synovial overexpression of canonical Wnts, as is found in experimental OA, may lead to chondrocyte phenotype changes, probably via modulation of the important TGF-β signaling pathway. This underlines that synovial Wnt/WISP1 expression may 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 role of members from the Wnt signaling pathway in the pathology of OA will provide new insights that might help us choosing new targets for the development of OA therapy.