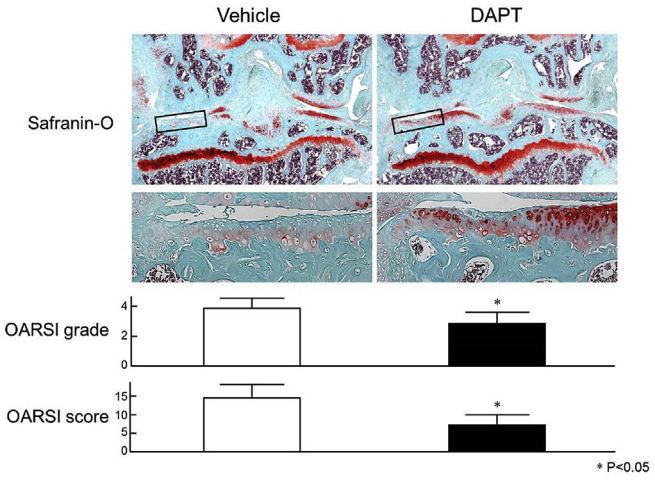
β-glycerophosphate, and 10 μg/mL ascorbic acid) for 2 weeks. To assess their hypertrophic differentiation, we quantitated markers of hypertrophic chondrocytes by real-time RT-PCR and performed ALP and Alizarin red S stainings. For *in vivo* analyses, we created a surgically induced OA model by resecting the medial collateral ligament and the medial meniscus in the knee joints of 8-week-old mice. Intraarticular administration of DAPT was performed twice a week for 10 weeks after surgical induction. For each administration, we injected 10 μL of 2.5 μM DAPT solution, which was prepared by diluting 25 mM DAPT in DMSO with PBS at 1:10,000. OA severity was quantified by the OARSI histopathology grade 10 weeks after surgery. Expressions of marker proteins were determined by immunofluorescence.

Results: In the primary culture of mouse articular chondrocytes with differentiation medium, DAPT treatment suppressed expression of *Mmp13, Vegfa,* and *Hes1* and inhibited mineralization in a dose-dependent manner, while it induced *Col2a1* expression. These results were compatible with those from the Notch ICD overexpression experiments. Intraarticular administration of DAPT inhibited articular cartilage degradation of the mouse experimental OA model. OARSI grading confirmed that DAPT caused significant resistance to OA progression. Immunofluorescence showed reduced expression of Mmp13, Vegfa and Hes1 in the articular carilage with DAPT administration.

Conclusion: Inhibition of Notch signaling by intraarticular administration of DAPT prevents OA progression in the mouse experimental model. Notch-related molecules including the Notch ligands, Adam and γ-secretase may be candidate therapeutic targets for OA prevention.



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WNT16 SUPPORTS THE PHENOTYPE OF THE SUPERFICIAL ZONE CELLS IN CARTILAGE

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Purpose: During embryonic skeletogenesis WNT16 is a specific marker of the joint interzone which will give rise to the joint and the permanent articular cartilage. In adult joints WNT16 is no longer expressed, but it becomes rapidly upregulated in the articular cartilage following injury and in osteoarthritis (OA).

The superficial zone of the articular cartilage contains chondrogenic progenitor cells which specifically express WNT16 and Lubricin and are essential for the long-term maintenance of cartilage homeostasis. Lubricin in adulthood is essential for joint lubrication and in its absence, mice develop spontaneous OA.

This study tests the hypothesis that WNT16 contributes to cartilage homeostasis following injury by supporting the survival, phenotypic stability, and function of the superficial zone progenitor cells.

Methods: Superficial zone cells (SZC) were isolated from the knees of neonatal wnt16-/- and wild-type mice by a sequential digestion with trypsin and collagenase followed by a selection procedure based on adhesion to fibronectin. Deep zone chondrocytes (DZC) were obtained by further digestion of the joints with collagenase overnight.

Results: Upon isolation, in wild type mice, SZC were characterized by the expression of Lubricin and WNT16 as previously reported, whereas DZC expressed Matrilin-1. Chondrocyte marker Sox9 and extracellular matrix components such as Aggrecan and Collagen2A1 were more expressed in the DZC as expected.

Lubricin expression was decreased in wnt16-/- and heterozygous SZC compared to those from wild type mice. Exogenous WNT16 dose-dependently increased Lubricin expression in wnt16-/- SFZ cells and primary bovine chondrocytes. WNT16 treatment also dose-dependently increased the canonical target gene Axin2 and correlated with the TOP-FLASH reporter assay. Importantly, DKK1 prevented the WNT16-induced Lubricin upregulation, thereby demonstrating that modulation of the gene occurs through the canonical pathway. Interestingly, WNT-3A was a much stronger activator of the canonical WNT pathway, but it hardly activated Lubricin expression, thereby suggesting that other, yet unknown, aspects of WNT16 signalling are essential for its capacity to upregulate Lubricin.

Sox9 expression was increased in wnt16-/- SZC and lost upon WNT16 stimulation in these cells as well as bovine primary chondrocytes. wnt16-/- SZC also had a higher content of sulphated proteoglycans when cultured in micromass, (as measured with alcian blue).

Therefore WNT16 maintains the progenitor phenotype of superficial zone cells and in its absence, these cells acquire a phenotype more similar to that of the deep zone chondrocytes.

Conclusion: We have discovered that WNT16 supports Lubricin expression through activation of the canonical WNT pathway and that WNT16 supports the maintenance of the specific phenotype of the superficial zone cells.

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HEDGEHOG SIGNALING REGULATES CHOLESTEROL HOMEOSTASIS IN OSTEOARTHRITIS

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Current evidence suggests that mechanical, genetic, and metabolic factors play a role in the pathogenesis of osteoarthritis (OA). Hedgehog (Hh) signaling is known to regulate chondrocyte differentiation, and to be activated in human and murine OA. Since Hh signaling regulates Gli-mediated gene expression, we identified Hh target genes that are expressed in chondrocytes. Microarray analyses were performed to detect changes in gene expression when the Hh pathway was modulated in human OA cartilage samples. Results from the Affymetrix Human Gene 1.0 ST microarray were analyzed for differentially expressed genes from five patient samples. Using Ingenuity® Pathway analysis, several genes known to be involved in sterol homeostasis were found to be upregulated with Hh inhibition. To investigate the function of these genes in cartilage, mice with chondrocyte-specific cholesterol accumulation were generated. This was achieved by excising Insig1 and Insig2, major negative regulators of cholesterol homeostasis, under Col2a1 regulatory elements. With aging or surgically induced joint instability, mice with chondrocyte-specific cholesterol accumulation developed more severe OA than control littermates. They expressed markers of chondrocyte hypertrophy in the articular cartilage, including type X collagen. Statin treatment to inhibit cholesterol production rescued this phenotype and reduced the severity of OA. Genetic manipulation of Hedgehog signaling in these mice suggests that Hh signaling is modulating the phenotype by regulating sterol homeostasis. Here we identified novel Hh target genes in chondrocytes that regulate intracellular cholesterol levels, and found that cholesterol dysregulation in the chondrocytes predisposes to OA. These data suggest that pharmacologic correction of intra-articular sterol imbalance can be used as a treatment for osteoarthritis.

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EXPRESSION AND FUNCTION OF CCAAT/ENHANCER-BINDING PROTEIN FAMILY IN CHONDROCYTES

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