indicates that knockdown of DKK1 alone is not sufficient to result in hypertrophic differentiation of articular chondrocytes.

After addition of 10 ng/ml IL-1β DKK1 and FRZB mRNA levels were decreased while expression of GREM1 mRNA was increased (figure 1). IL-1β is a potent activator of MMP expression in human chondrocytes and has been implicated in cartilage degradation in OA. As expected, IL-1β potently induced expression of MMP1, MMP3, and MMP13 at the mRNA level. Co-stimulation with IL-1β and DKK1 or GREM1 further increased IL-1β-induced MMP expression. Especially MMP3 was increased significantly. Surprisingly, the addition of DKK1, FRZB and GREM1 simultaneously in the presence of IL-1β reduced MMP gene expression to undetectable levels (figure 2). This suggests that the combination of three antagonists can effectively counteract IL-1β induced catabolic activity in human chondrocytes.

Conclusions: Hypertrophic differentiation may play a role in early and late stage OA. Inhibition of hypertrophy by DKK1, FRZB and GREM1 might be a therapeutic target to slow down further OA progression. DKK1 plays a crucial part in preventing chondrocytes from hypertrophy. Furthermore, we found that the expression of DKK1, FRZB, GREM1 in human primary chondrocytes was affected by the osteoarthritis associated factor IL-1β. We also provided evidence that, even in the presence of IL-1β, the combination of three antagonists completely inhibited the expression of genes associated with chondrocyte hypertrophy and OA.
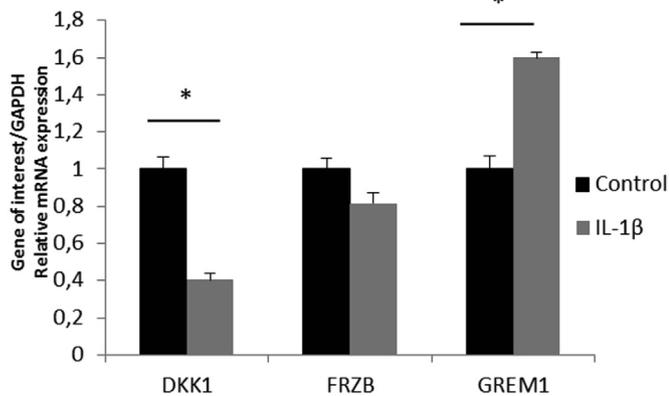

Figure 1. Effects of IL1β on the mRNA expression of DKK1, FRZB, GREM1. Human chondrocytes received a single dose of 10ng/ml IL-1β. Antagonists mRNA expression was measured by qRT-PCR. * P < 0.05. Student T-test.
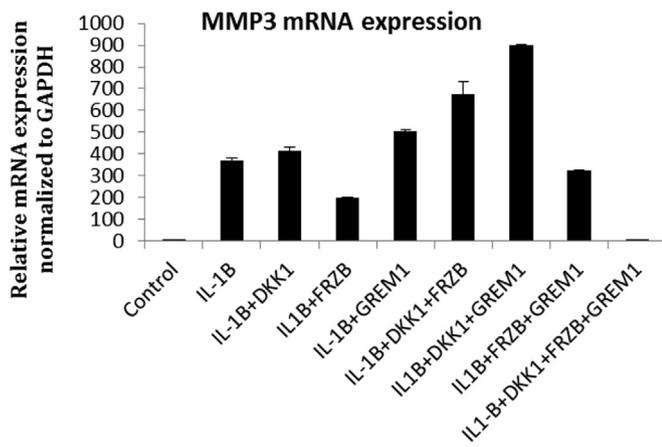

Figure 2. Chondrocytes in monolayer cultures were exposed to 10ng/ml of recombinant human IL-1β, and 200ng/ml each of DKK1, FRZB, GREM1 for 48 hours. MMP expression was measured by qRT-PCR.

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AN ECHO IN BIOLOGY: VALIDATING THE EXECUTABLE CHONDROCYTE

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Purpose: Computational modeling of biological networks permits comprehensive analysis of cells and tissues to define molecular phenotypes and novel hypotheses. We recently presented ANIMO (Analysis of Networks with Interactive Modeling), an intuitive software tool for modeling molecular networks for use by biologists. We used ANIMO to generate a computational model of articular cartilage, ECHO.

Over 1.5 million people in the Netherlands suffer from osteoarthritis (OA) in one or more joints. OA is a painful, disabling disease and currently cannot be cured. In a subset of OA patients, joint cartilage is replaced by bone via endochondral ossification. This is a natural process in growing long bones, where transient growth plate cartilage is replaced by bone. In contrast, healthy joint cartilage is permanent as it is protected against bone formation. Stable joint cartilage is under control of master transcription factor SOX9, whereas bone formation is controlled by RUNX2. The processes that regulate the switch between a SOX9+ state and a RUNX+ state are poorly understood, which greatly hampers the development of successful therapies.

Methods: Based on a large-scale literature study and our own experiments, we recently developed ECHO (Executable Chondrocyte), a computational model of the key processes that regulate expression and activity of SOX9 and RUNX2. Simulations in ECHO were performed to investigate the robustness of the chondrocyte network.

To validate ECHO predictions, we used FRAP to measure mobility of SOX9 and RUNX2, which we have shown to be a faithful readout of their activity. Primary chondrocytes of 2 OA donors were tested at passage 2 after isolation. In these donors we observed little interdonor variation in SOX9 mobility. Using lipofectamine LTX we obtained 40-70% transfection efficiencies in primary human chondrocytes even at low passages.

Results: In its unperturbed form, ECHO displays two stable states in which activities of SOX9 and RUNX2 are mutually exclusive. We tested the hypothesis that addition of WNT (performed with a few mouse clicks) will change permanent into transient cartilage by inducing hypertrophy. Indeed, when we add WNT, a known regulator of bone formation, the permanent or SOX9+ state changes to a transient or RUNX2+ state. However, it is known that healthy articular cartilage is resistant to hypertrophic differentiation. Our group has previously found that this was probably due to the secretion of DKK1, FRZB and GREM1. We therefore added nodes to ECHO representing DKK1, FRZB and GREM1 (figure 1). GREM1 and DKK1 are able to stabilize the permanent cartilage or SOX9+ state even after addition of WNT to ECHO. We observed that in our model activation of WNT leads to a switch from a SOX9+ state to a RUNX2+ state. To prove that WNT/β-catenin signaling can directly regulate SOX9 function, we investigated the response of SOX9 mobility to WNT3A in live primary chondrocytes. Addition of WNT3A to human chondrocytes transfected with SOX9-GFP resulted in a significant decrease of the immobile SOX9 fraction from 53% to 34% within 15 minutes after addition. A direct correlation between the elevated levels of β-catenin after WNT addition and the activity of SOX9 indicates that β-catenin can directly change the mobility by complex formation with SOX9.

Conclusions: Using ECHO we predicted the stimuli that prevents hypertrophic differentiation differentiation of articular cartilage, and tested this experimentally with FRAP using SOX9 and RUNX2 mobility as a read-out.
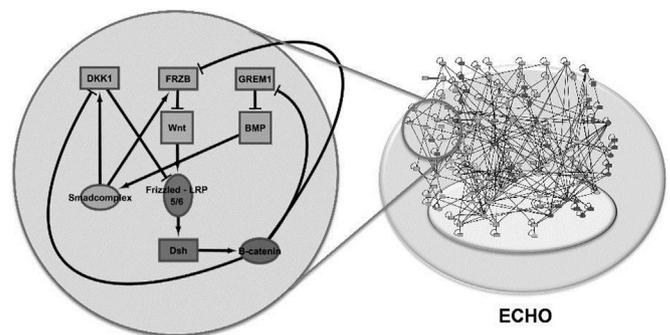

Figure 1. A complex network of many signal transduction pathways determines the development of either transient or permanent cartilage. ECHO consists of 123 nodes with 354 interactions. We can perform in silico experiments using the complete network, or investigate detailed processes in smaller areas of the network.