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DKK1, FRZB, GREM1 PREVENT IL1β INDUCED ARTICULAR CARTILAGE DEGRADATION

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Abstract:

Purpose:

Osteoarthritis (OA) is a multifactorial disease characterized by progressive degradation of articular cartilage leading to loss of joint function. It affects several million people in the world. Currently there is no cure for OA. In a subset of patients OA is associated with hypertrophic differentiation of articular chondrocytes. This process normally occurs in the growth plate. Healthy articular cartilage is protected against hypertrophy. Our group identified DKK1, FRZB (WNT antagonists) and GREM1 (BMP antagonist) as the natural brakes on hypertrophic differentiation and regulation of the maintenance of the articular phenotype. Moreover, we previously demonstrated that decreased production of DKK1, FRZB, GREM1 in human chondrocytes has been associated with cartilage hypertrophy in OA. In this project, we hypothesize that DKK1, FRZB, GREM1 are the gatekeepers for the maintenance of homeostasis in articular cartilage. To prove this, we investigated whether knockdown of DKK1, FRZB and GREM1 is sufficient to cause hypertrophic differentiation in healthy articular chondrocytes. Furthermore, we investigated the influence of catabolic factors such as interleukin-1β (IL-1β) on the gene transcription levels of DKK1, FRZB, GREM1. We found that the presence of DKK1, FRZB and GREM1 is sufficient to inhibit the catabolic effects of IL-1β.

Methods:

Human primary chondrocytes (hCh) were isolated from cartilage as described previously by our group. We isolated a neutralizing VhH antibody against DKK1 from a llama VhH library and used this to block DKK1 activity in human primary chondrocytes cultured in monolayer. The chondrocytes were cultured in chondrocyte proliferation medium containing 5 µg/ml neutralizing antibody for 1 week. Normal IgG served as a negative control. The neutralizing function of the antibody was tested before use. Isolated chondrocytes were exposed to 10ng/ml of recombinant human IL-1β, and 200ng/ml of each of DKK1, FRZB, GREM1 (all recombinant proteins are from R&D systems). Cells received no medium refreshment after stimulation and were cultured up to 48 hours.

Results:

In the presence of recombinant DKK1 protein the mRNA expression of the WNT target gene AXIN2 was downregulated. In presence of both DKK1 and the DKK1 neutralizing antibody, AXIN2 mRNA was upregulated suggesting that the WNT signal pathway activity was (re)activated. MMP1, 3 and 13 as well as RUNX2 were upregulated in cells with DKK1 knockdown. The expression of Col10 could not be detected. This 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.

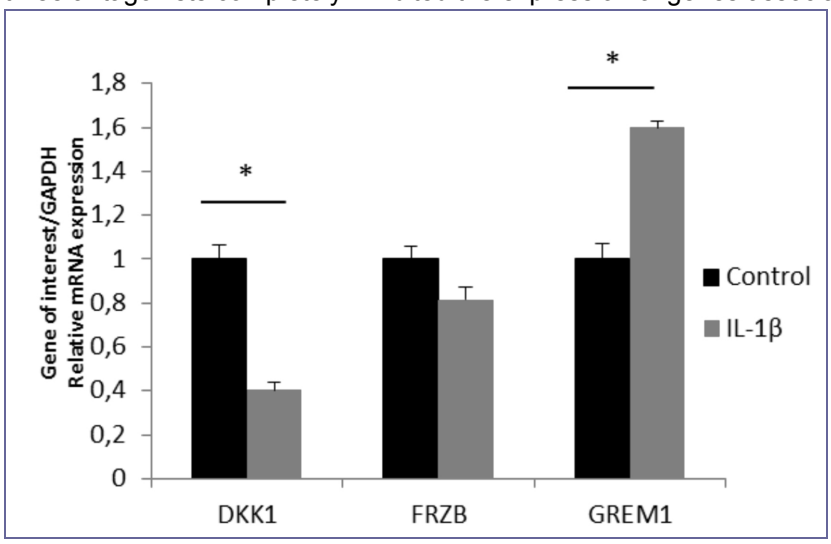

Figure 1. Effects of IL1β on the mRNA expression of DKK1, FRZB, GREM1. Human chondrocytes received a single dose of 10ng/ml IL-1β. Antagonists mRNA expression was measured by qRT-PCR. * P<0.05. Student T-test.

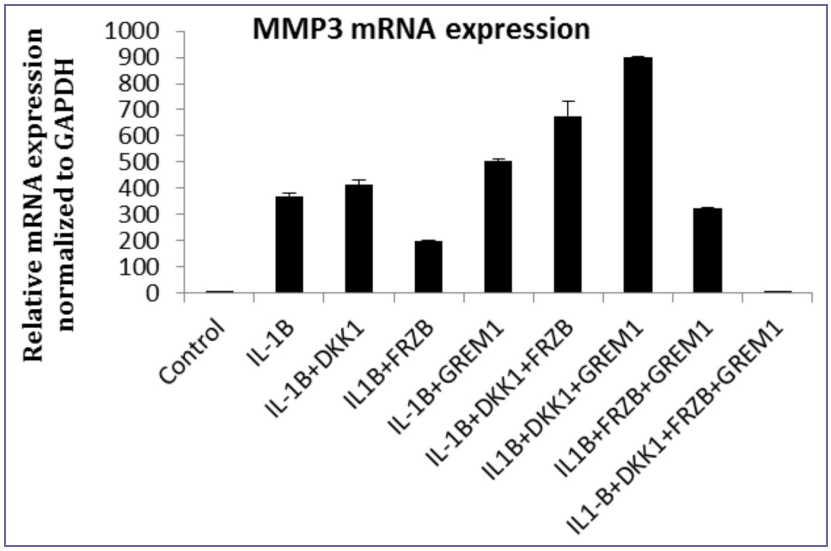

Figure 2. Chondrocytes in monolayer cultures were exposed to 10ng/ml of recombinant human IL-1β, and 200ng/ml each of DKK1, FRZB, GREM1 for 48 hours. MMP expression was measured by qRT-PCR.

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