and differentiation was induced by keeping the cells for 21 days in α-MEM supplemented with 10% FBS, 1% sodium pyruvate, 1% antibiotic-antimycotic, 10mM β-glycerophosphate (BGP) and 50μg/μl ascorbic acid-2-phosphate (AA). Alkaline phosphatase activity (ALP) was measured and mineralization was analyzed by alizarin red staining. Gene expression of extracellular matrix genes, genes linked to mineralization and TGFβ superfamily genes was analyzed by RT-qPCR. Intracellular signaling pathways (PKCα, ERK1/2, SMAD1/5/8, p38MAPK) were investigated by Western blot. HPDCS were cultured for 21 days in DMEM (+ the same supplements as for MC3T3 differentiation and 100nM dexamethasone). HUVECS were stimulated in EGM-2 medium with 200 ng/ml of BMP6, to trigger endothelial to mesenchymal transition. Then cells were cultured as hPDCS. hPDCs and HUVECS were cultured with supernatants harvested from control, Smoc2+ and ΔCaBD MC3T3 cells.

Results: Mineralization and ALP activity was reduced in Smoc2+ MC3T3 cells compared to controls. Gene expression analysis showed an overall altered differentiation when overexpressing Smoc2. The activation status of PKCα, ERK1/2, p38MAPK and SMAD1/5/8 was significantly modified in Smoc2+ cells. On the other hand, we could not observe an effect on osteogenesis when silencing Smoc2. ΔCaBD cells however exhibited less of the inhibiting effects of Smoc2+ cells. Moreover we could observe partial restoration after addition of extracellular calcium to the culture medium of differentiating Smoc2+ cells. Alizarin red staining and ALP activity was reduced in hPDCs cultured in the presence of Smoc2+ supernatant compared to controls. However, hPDCs stimulated with ΔCaBD supernatants exhibited a lesser decrease of these markers compared to Smoc2+. Alizarin red quantification showed the same effect for HUVECs. In ATDC5 cells, Smoc2 overexpression altered mRNA level of chondrogenic markers and reduced Wnt and BMP signaling. Smoc2 silencing enhanced chondrogenic differentiation by increasing BMP signaling.

Conclusions: SMOC2 can regulate osteogenesis and chondrogenesis. In the osteoblast cell line silencing of Smoc2 did not affect osteogenesis suggesting a limited endogenous role. SMOC2 appears to exert its effects through interaction with calcium and by interfering with BMP and Wnt signaling. SMOC2 or its calcium domain could therefore have beneficial effects on disorders associated with excessive mineralization, including osteoarthritis and cardiovascular disease.

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DKK1 AND FRZB ARE NECESSARY FOR CHONDROCYTE (RE) DIFFERENTIATION AND PREVENTION OF CELL HYPERTROPHY IN 3D CULTURES OF HUMAN CHONDROCYTES AND HUMAN MESENCHYMAL STEM CELLS

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Purpose: Autologous chondrocytes and mesenchymal stem cells (MSCs) in either monoculture or co-culture, for the enhancement of chondrogenesis, show great promise for treatment of cartilage disorders. However, maintaining the chondrogenic phenotype and avoiding hypertrophy of chondrogenic differentiating MSCs remain a big challenge in these cell-based strategies. Previously, we identified Dickkopf 1 homolog (DKK1) and frizzled-related protein (FRZB) as key factors in controlling the articular chondrocyte phenotype. DKK1 and FRZB are naturally occurring antagonists of the WNT signaling pathway. The expression of DKK1 and FRZB is low in cultured human mesenchymal stem cells (hMSCs) and high in cultured human articular chondrocytes (hChs). We have previously shown that addition of either DKK1 or FRZB blocks hypertrophy in chondrogenically differentiating hMSCs and that expression of DKK1 and FRZB is lost in OA. We hypothesized that DKK1 and FRZB are necessary for chondrogenesis and for preventing cell hypertrophy in chondrocyte terminal differentiation.

Methods: To block DKK1 and FRZB the variable domain of single chain heavy chain only antibodies (VHH) was used to neutralize DKK1 and FRZB. We tested our hypothesis using 3D pellet cultures of three relevant human cell based systems: isolated hChs, isolated hMSCs and co-culture of hChs with hMSCs. The effects were analyzed using real-time quantitative polymerase chain reaction (qPCR), histology, and immunohistochemistry (IHC).

Results: In the presence of DKK1 and FRZB neutralizing VHH, glycosaminoglycan (GAG) deposition and Collagen type II staining were significantly reduced in redifferentiating chondrocytes (Figure 1 A and B) and in chondrogenic differentiating hMSCs, indicating loss of chondrogenic potential. Upon neutralization of DKK1 and FRZB in co-cultures, the cells in pellets showed hypertrophic differentiation as indicated by matrix mineralization, apoptosis and significantly increased expression of the hypertrophic markers, COL10A1, MMP13 and RUNX2. This is indicative of terminal differentiation.

Conclusions: DKK1 and FRZB are necessary for multiple steps during chondrogenesis: first DKK1 and FRZB are necessary for the initial steps of chondrogenic differentiation of hMSCs and in redifferentiation of cultured chondrocytes, and secondly in preventing chondrocyte hypertrophy in terminal differentiation of articular chondrocytes (Figure 2).

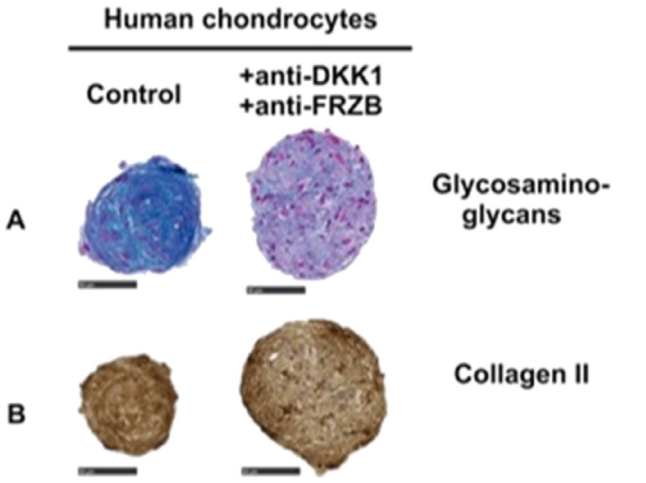

Figure 1. Blocking of DKK1 and FRZB inhibits chondrocyte re-differentiation. Histological analysis of mid-sagittal sections of the chondrocyte pellets using Alcian blue staining for GAGs (A) and immunohistochemistry staining for collagen type II (B).

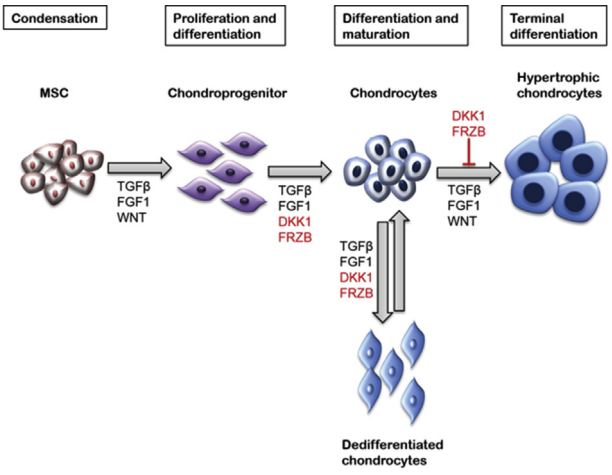

Figure 2. DKK1 and FRZB are necessary for multiple steps during chondrogenesis to ensure chondrogenesis and to prevent cell hypertrophy in terminal differentiation.

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DIET-INDUCED OBESITY MAY INITIATE ADVANCED GLYCATION END-PRODUCT-MEDIATED INFLAMMATION IN ARTICULAR CARTILAGE

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