BCP crystals induce hypertrophic differentiation of chondrocytes by activating canonical WNT signaling

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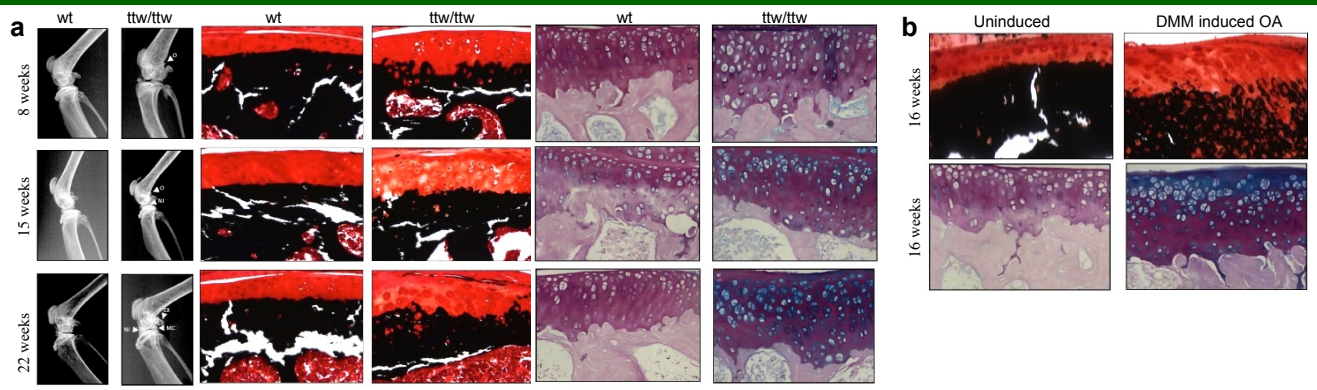
Background

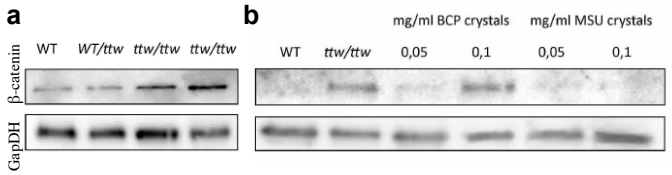
Calcification of cartilage is a common finding during osteoarthritis (OA) and is directly linked to the severity of cartilage degradation. We have found in a previous study that basic calcium phosphate (BCP) crystal calcification is present in murine and human OA cartilage. The observed cartilage changes resemble aspects of endochondral ossification. In this study we aim to investigate the effect of BCP crystals on articular cartilage matrix changes.

Figure 1: Changes in articular cartilage matrix in ttw/ ttw mice



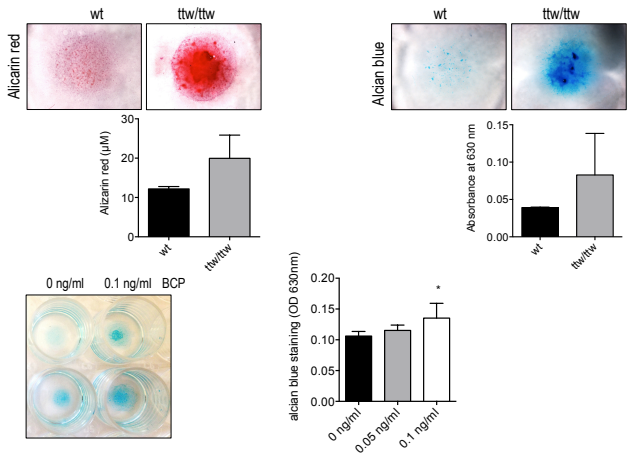
A: Time course of articular cartilage changes in ttw/ ttw mice compared to wt mice demonstrated by X-ray, Safranin-O/ von Kossa staining and Alcian blue/ PAS staining pH 1.
B: Safranin-O/ von Kossa staining and Alcian/ PAS staining pH 1 of wt articular cartilage w/o induction of OA using the DMM model.

Figure 3: Canonical WNT signalling is activated by BCP crystals



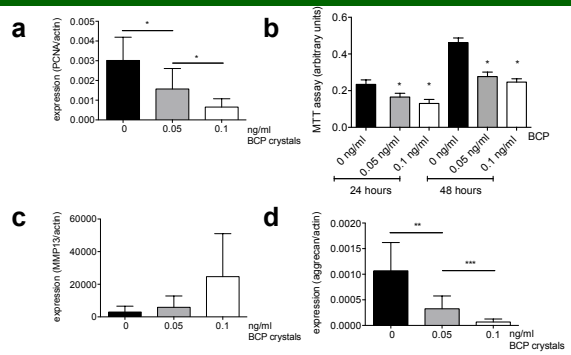
A: Higher amounts of β- catenin were detected indicating an activation of canonical Wnt signalling in ttw/ttw chondrocytes
B: Activation of canonical WNT signalling seems to be specific for BCP crystals.

Figure 2: Matrix production is altered in ttw/ttw articular cartilage



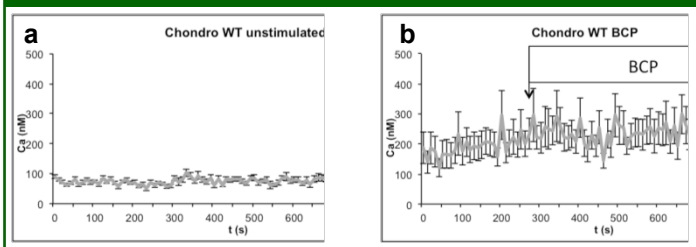
Matrix production in micro masses cultures of wt and ttw/ ttw chondrocyte cultures.

Figure 4: Effects of BCP crystals on the chondrocyte phenotype



A, C and D: Quantitative RT-PCR of different chondrocytic marker genes. BCP crystals seem to induce hypertrophic differentiation of chondrocytes. B: MTT-assay shows decreased chondrocyte viability upon BCP stimulation

Figure 5: Ca^{2+} levels are in creased upon BCP stimulation



A: Fura-2 calcium measurement showed basal calcium levels in wt chondrocytes of 80 nM.
B: Chondrocytes pre-treated with BCP crystals show calcium oscillations and increased basal calcium levels of 150 nM. Further stimulation with BCP did not change the calcium signalling significantly.

Conclusion

The calcification of articular cartilage seems to be associated with activation of canonical WNT signalling and subsequent hypertrophic differentiation of chondrocytes. Our data support the notion that OA is characterized by the re-initiation of developmental programms associated with endochondral ossification.

Methods

The tip-toe walking (ttw/ttw) mouse that carries a mutation in the *enpp1* gene encoding for NPP1 was used as a natural model of OA. Using von Kossa staining of knee joint sections we assessed the calcification of articular cartilage and the severity of OA using the Mankin-Score over a time course from 6 to 22 weeks and compared the results to DMM induced OA. We analysed the influence of BCP crystals on chondrocyte phenotype using quantitative RT-PCR for the marker genes MMP13, PCNA and aggrecan. We compared these findings with data from ttw/ttw micromass cultures. The influence of BCP crystals on matrix composition in vitro was investigated in micro mass cultures with alcian blue and alizarin red staining. Using Western Blot for β- catenin and pCamKII we investigated the activation of WNT signalling. Using FURA-2 measurements we investigated the effects of BCP on Ca2+ mobilization in chondrocytes.