BAPX1/NKX3.2; a Novel Chondrocyte Hypertrophy Molecular Switch in Osteoarthritis

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Introduction: Osteoarthritis (OA) is the most common degenerative joint disorder and presents with degradation of articular cartilage, leading to loss of joint mobility and function, accompanied by chronic pain. Recently we showed that Bone Morphogenetic Protein 7 (BMP-7) is able to suppress chondrocyte hypertrophic differentiation from progenitor cells and that the transcriptional repressor Bapx1/Nkx3.2 plays a key role in this action [1]. As OA involves a shift of the articular chondrocyte phenotype towards hypertrophic differentiation and mineralization, we hypothesized that impaired Bapx1/Nkx3.2 function and concomitant loss of hypertrophic protection of the articular chondrocyte is central in the changing chondrocyte phenotype that drives OA.

Methods: Healthy and OA human articular chondrocytes (HACs) were isolated from healthy human knee cartilage or from OA cartilage from total knee arthroplasty. HACs were cultured until passage 2 and used in experiments. HACs were exposed to BMP-7 (1 nM), IL-1β (10 ng/ml), TNFα (20 ng/ml) or OA synovial fluid (20% (v/v)). Loss- and gain-of-function experiments were performed by RNAi using siRNA duplexes targeting Bapx1/Nkx3.2 and by transiently overexpressing FLAG-Bapx1/Nkx3.2 by transfection of cloned vectors. Mouse experimental models for OA were used (DMM- and collagenase-induced OA) and (immuno)histochemistry was applied on knee joint tissue sections. Gene- and protein expression of Bapx1/Nkx3.2 and important chondrogenic-, hypertrophic-, cartilage degrading- and inflammatory mediators were determined by RT-qPCR and immunoblotting. ALP activity was determined using a colorimetric assay and PGE2 levels were measured by EIA.

Results: Exposure of healthy HACs to BMP-7 lead to a pronounced increased expression of Sox9, Col2a1 and Acan expression. At the same time, expression of hypertrophy associated genes Runx2, Col10a1 and ALP was suppressed, suggesting the existence of a general pro-chondrogenic and hypertrophy-suppressive action of BMP-7 in chondrocytic cells. Interestingly, BMP-7 significantly increased Bapx1/Nkx3.2 mRNA levels. Knocking-down Bapx1/Nkx3.2 in healthy HACs did not significantly influence the expression levels of Sox9, Col2a1 and Acan, but led to a major hypertrophic shift in the HAC phenotype. This was evidenced by increased expression of Runx2, Col10a1 and ALP. Overexpression of Bapx1/Nkx3.2 again did not change expression levels of Sox9, Col2a1 and Acan. However, it decreased the expression of Runx2, Col10a1 and ALP in healthy HACs suggesting that Bapx1/Nkx3.2 fulfills a central regulating role in balancing the chondrocyte phenotype with specific regulatory functions in controlling the chondrocytes’ hypertrophic phenotype.

As OA involves a phenotypic shift of the articular chondrocyte from a chondrogenic to a hypertrophic phenotype, we questioned whether Bapx1/Nkx3.2 expression follows the chondrocyte’s phenotype in OA. We found that the expression of Bapx1/Nkx3.2 was significantly lower in OA HACs and IL1β- or TNFα-treated HACs as compared to healthy non-treated HACs. Furthermore we found that
Bapx1/Nkx3.2 was predominantly expressed in the upper zone of mouse knee joint articular cartilage. In mouse knee joints subjected to experimental OA (DMM and collagenase-induced OA) we observed decreased levels of Bapx1/Nkx3.2 in the upper zone of knee joint articular cartilage. Overexpression of FLAG-Bapx1/Nkx3.2 in OA HACs did not significantly change the expression of chondrogenic genes, but caused significant downregulation of the expression of Runx2, Col10α1, ALP, MMP13, ADAMTS5, COX-2 and IL-6. In addition, we mimicked an OA inflammatory environment by exposing healthy HACs to OA synovial fluid (SF) and determined whether overexpression of FLAG-Bapx1/Nkx3.2 was able to protect healthy HACs against the katabolic effects of SF exposure. In agreement with its katabolic properties, SF exposure lead to decreased Bapx1/Nkx3.2 expression and overexpression of FLAG-Bapx1/Nkx3.2 protected the HACs against the effects of SF exposure. Together these data show that overexpression of Bapx1/Nkx3.2 in OA HACs and in healthy HACs exposed to an OA synovial fluid environment is able to change the OA-associated chondrocyte hypertrophic phenotype to a more chondrogenic phenotype and counteract the expression of important OA related katabolic genes.

Next we investigated whether the hypertrophy-suppressive action of Bapx1/Nkx3.2 can be extracellularly induced in OA HACs by BMP-7. BMP-7 exposure of OA HACs and healthy HACs which were exposed to IL1β or TNFα almost completely rescued the chondrocyte hypertrophic phenotype back to mRNA expression levels that were detected in healthy HACs. Analysis of Bapx1/Nkx3.2 levels revealed that BMP-7 rescued the OA-associated reduced Bapx1/Nkx3.2 levels above healthy HAC levels. To determine whether the BMP-7-induced phenotypic rescue was indeed mediated via a Bapx1/Nkx3.2-dependent mechanism, we exposed OA HACs to BMP-7 while interfering with Bapx1/Nkx3.2 expression via RNAi. Indeed, the BMP-7-induced reduction of hypertrophic and katabolic gene expression was ameliorated by knockdown of Bapx1/Nkx3.2. Together these data show that Bapx1/Nkx3.2 levels in OA chondrocytes can be extracellularly rescued by BMP-7, leading to a hypertrophy-suppressive change in the OA chondrocyte phenotype.

**Discussion:** We show that Bapx1/Nkx3.2 is an important, yet unrecognized, molecular switch that is centrally involved in controlling the hypertrophic phenotype of the post-developmental articular chondrocyte. Our data suggest that due to the OA environment functional Bapx1/Nkx3.2 levels drop in OA chondrocytes, leading to destabilization of the Sox9-Runx2 balance and causing a phenotypic shift towards a hypertrophic and katabolic differentiation state. Current experimental OA treatment options are intervening with OA symptoms only. Although central in the development of OA, there is a lack of treatment options that address the chondrocyte phenotype and important effort is needed to identify targetable pathways or individual factors that alter the chondrocyte’s phenotype in OA. A chondrocyte phenotype-centered OA treatment would ideally consist of active suppression of chondrocyte hypertrophy as well as inhibition of the chondrocyte’s inflammatory/katabolic status, while provoking an anabolic cartilage metabolism. We expect that Bapx1/Nkx3.2 provides an interesting candidate to pursue such an approach.

**Significance:** The characterization of Bapx1/Nkx3.2 as a novel major phenotype-determining switch in OA chondrocytes holds the promise to gain a deeper understanding of the origin of the OA-associated shift in the phenotypically metastable articular chondrocyte.
**Figure 1:** Overexpression of BAXP1/NKX3.2 counteracts the OA-associated chondrocyte phenotype. OA HACs (n=3 donors) were transfected with a FLAG-empty or FLAG-BAXP1/NKX3.2 vector and mRNA expression of depicted genes was determined by RT-qPCR.

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