The Role Of Foxa Factors In The Onset And Development Of Osteoarthritis

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Introduction:

We have recently identified FoxA transcription factors as key regulators of chondrocyte hypertrophy in the developing skeleton (1).

Methods:

Methods are incorporated in the Results Section

Results:

Therefore, we next asked whether the Fox A family of transcription factors is involved in the onset and development of osteoarthritis (OA) in the adult cartilage. To evaluate the expression of FoxA2 in postnatal articular cartilage, we employed a tamoxifen-inducible Cre driver knocked-into the 3' UTR of the FoxA2 gene. After crossing this mouse line into the ROSA26 reporter line, we observed that FoxA2 expression was highest in the knee joint and the femoral cap articular cartilage of one-month old animals, it precipitously declined at 3 months of age and remained at this low level by 6 months of age. By 6 months of age, the number of b-gal stained cells was only 36 % of the number of b-gal stained cells in one month old animals, indicating a significant decrease in the FoxA2 promoter activity. In the adult articular cartilage, FoxA2 expression was associated with the deepest hypertrophic layers of the articular cartilage adjacent to the tidemark. In fact, at all ages (1 month, 3 months, 6 months), 85% of the b-gal stained cells were located on the tidemark, while the remaining 15% were found above the tidemark, in the more superficial layers of the articular cartilage.

Given that the level of FoxA2 is very low in the superficial articular cartilage throughout mouse adult life, I have asked next whether FoxA2 expression is induced in the pathological condition of osteoarthritis (OA). One murine model of OA involves surgical destabilization of the knee joint by cutting the the ligaments that connect the medial meniscus to the tibial articular cartilage. This operation results in knee joint instability which leads to the degradation and loss of articular cartilage (2). To assay whether FoxA2 expression is induced in articular cartilage following joint destabilization, I either cut the meniscal ligaments or performed a sham operation in FoxA2-CreERT2; ROSA26 animals. The mice were administered Tamoxifen for 10 days prior to their sacrifice to assay the relative expression of FoxA2-CreERT2. While beta-galactosidase expression was very low in the superficial articular cartilage of sham operated animals, it was twice elevated in the articular cartilage of mice that had undergone surgical joint destabilization.

A second murine model of osteoarthritis is provided by the cho/+ mouse, which contain haplo-insufficient levels of type XI collagen and display an OA-like pathology (3). Although a hereditary defect in type XI collagen is not responsible for most forms of human OA, disruption of matrix composition in cho/+ mice results in the loss of joint stability and associated biochemical alteration that mimics human OA. In this murine model of OA, the first signs of the disease appear by 3 months of age, when cell clustering becomes visible. Both control and cho/+ mice were exposed to tamoxifen for 10 days, through daily IP injections, and their knees were harvested at sacrifice point. In mice harboring the cho/+ allele, there were 3 times more labeled cells in the superficial articular cartilage than in the control mice. This suggests that the FoxA2 promoter was substantially more active in driving beta-galactosidase expression in mildly-osteoarthritic mice than in healthy 3 month old mice. These findings were also supported by Real Time - qPCR of FoxA family gene expression in dissected knee articular cartilage isolated from either 3 month old cho/+ mice or their wild-type (WT) littermates. Interestingly, I found that both FoxA2 and FoxA3 are specifically induced in the articular cartilage of 3 month old cho/+ mice, which correlates with the induction of MMP13 and decrease in collagen type II in the knee joints of these animals.

To evaluate whether FoxA family members are necessary for cartilage degradation, we conditionally deleted FoxA2 in the articular cartilage of FoxA3-/- mice, using a tamoxifen-inducible CRE recombinase driven by the Prg4 (Lubricin) regulatory sequences. As such, I wanted to test whether combined loss of both FoxA2 and FoxA3 in the superficial articular cartilage can slow the progression of osteoarthritis in murine models for OA. Thus, I generated 1.5 month old Prg4CreERT2-GFP/+; FoxA2fl/fl; FoxA3fl/fl mice, which I injected with tamoxifen for 10 days to induce recombination and conditionally remove FoxA2 from the superficial zone of their articular cartilage. Subsequently, I let the mice reach 2 months of age and I performed unilateral surgical destabilization
of the medial meniscus on either the tamoxifen treated $Prg4^{CreER12/+}; FoxA2^{fl/fl}; FoxA3^{-/-}$ mice or wild-type C57BL/6 animals. 4 months post-surgery, the knee joints of these animals were fixed and processed for Safranin O/Fast green staining. While wild-type C57BL/6 mice that were sham operated had normal cartilage in their knees, the C57BL/6 mice that have undergone surgical destabilization of their meniscus developed osteoarthritis rapidly and by 4 months post-surgery, cartilage degradation was almost complete (OARSI score 5.87±0.12). In striking contrast, $Prg4^{CreER12/+}; FoxA2^{fl/fl}; FoxA3^{-/-}$ mice that had been treated with tamoxifen developed very little sign of articular cartilage degradation and the cartilage in their knee joints was fairly intact (OARSI score 1.45±0.26).

I next asked whether overexpression of FoxA2 in murine articular cartilage cells is sufficient to accelerate cartilage degradation. To drive exogenous FoxA2 expression in articular chondrocytes, I have generated mice containing the FoxA2 transgene driven by a reiterated reverse tetracycline transactivator (rtTA) response element ($TRE_{tight}$-FoxA2). This transgenic animal was in turn mated to a mouse containing rtTA knocked into the ROSA 26 locus downstream of a “floxed” STOP transcription cassette. Tissue specific expression of rtTA was achieved with the tamoxifen-inducible $Prg4^{CreER12/+}$ line. In mice containing these three transgenes, administration of doxycycline in the drinking water induced the expression of ectopic FoxA2 in superficial zone articular chondrocytes. At 8 weeks of age, I performed surgical destabilization of the medial meniscus on either the triple transgenic mice $Prg4^{CRE};rtTA;TgFoxA2$ mice or their control littermates $Prg4^{CRE};rtTA$ littermates. Control mice, lacking the FoxA2 transgene, that have undergone surgery developed mild symptoms of the disease (OARSI score 1.37±0.33). In contrast, triple transgenic mice that have undergone surgery developed far more cartilage damage with more lesions on the articular cartilage (OARSI score 2.63±0.43).

**Discussion:**
Overexpression of FoxA2 in a murine model of osteoarthritis accelerates progression of the disease while conditional deletion of this gene in a murine model of osteoarthritis prevents disease progression and protects against cartilage degradation in the joints.

**Significance:** This raises the interesting possibility that the induced expression of FoxA transcription factors during osteoarthritis leads to cartilage degradation and renders FoxA2 as an interesting target for small-molecule inhibition in hopes of OA-prevention.

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**References:**

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