NFAT1 Deficiency Promotes the Initiation and Progression of Posttraumatic Osteoarthritis Induced by Meniscal Destabilization

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Introduction: The risk of posttraumatic osteoarthritis (PTOA) after joint injuries ranges from 20% to more than 50% even with the best current care of joint injuries, such as anatomic reduction and rigid fixation of intra-articular fractures and reconstruction of ruptured ligaments with successful restoration of joint biomechanics. The time course for the progression of PTOA is highly variable and risk of PTOA increases with age of patients, suggesting that biologic factors may be involved in the progression of PTOA. However, the key biologic factors responsible for PTOA progression remain unclear. NFAT1 (NFATc2) is a member of the nuclear factor of activated T cells (NFAT) family of transcription factors originally identified as a regulator of the expression of cytokine genes during the immune response. Our recent studies revealed that mice lacking NFAT1 exhibit normal skeletal development but display dysfunction of articular chondrocytes and OA-like changes in adults, with early osteoarthritic changes in the knee joints after 6-8 months of age [1, 2]. This study aimed to test our hypothesis that the global NFAT1 expression level affects the time of onset and progression of knee PTOA after surgical destabilization of the medial meniscus (DMM) in mice.

Methods: We generated bilateral DMM by surgical transection of the medial meniscotibial ligament (MMTL) as described by Glasson et al. [1] in 2 to 3-month-old, both sexes, Nfat1 knockout (Nfat1-/-), Nfat1+/-, and wild-type (WT) mice. All animal procedures were approved by the Institutional Animal Care and Use Committee. Animals were anesthetized by intra-peritoneal injection of ketamine and xylazine. Under a surgical microscope, the knee joint was exposed through a medial parapatellar incision under sterile conditions. The MMTL was transected with micro-surgical scissors to destabilize the medial meniscus. The patella and patellar tendon, cruciate ligaments, and other ligaments around the knee joint were preserved to maintain the stability and mobility of the joint after surgery. For sham surgery, the MMTL was visualized but not transected. The joint capsule was closed with 8-0 Vicryl sutures and the skin incision closed with 5-0 non-resorbable sutures. Operated animals were allowed caged mobility after surgery and were monitored to ensure healthy recovery. Animals were euthanized at 4, 8, and 16 weeks after surgery. The knee joints were harvested and processed for histochemical and histomorphometric analyses to evaluate the severity of knee osteoarthritis (OA). Articular cartilage samples harvested from the medial femoral condyle and medial tibial plateau were processed for gene expression analyses by quantitative real-time PCR (qPCR). Statistical analyses were performed with Student t-test and ANOVA.

Results: Tissue sections stained with safranin-O and fast green were used for histochemical and histomorphometric analyses to determine the severity of knee OA. We utilized a semi-quantitative OA grading scale as previously described [1]. Histologic scoring was performed on the four quadrants: medial and lateral femoral condyles and medial and lateral tibial plateaus. Histomorphometric analyses
revealed that after the DMM surgery, Nfat1/- and Nfat1+/ mice displayed significantly more severe osteoarthritic changes in knee joints than WT mice (p < 0.05 at 4 weeks and p < 0.01 at 8 and 16 weeks). The osteoarthritic changes such as cartilage lesions and chondro-osteophyte formation were more apparent on the medial side than the lateral side of the knee joint in all three groups. The histopathological changes in the medial compartment of the Nfat1/-, Nfat1+/-, and WT knee joints at 8 weeks after DMM surgery are presented in Figure 1. Nfat1/-, Nfat1+/-, and WT mice receiving the sham surgery displayed focal loss of safranin-O staining in the articular cartilage of the knee joints at 4 and 8 weeks but recovered at 16 weeks without apparent osteoarthritic changes. The expression levels of mRNAs for specific collagens, matrix-degrading enzymes, and pro-inflammatory cytokines in articular cartilage of the medial femoral condyle and medial tibial plateau at 4 and 16 weeks post-DMM surgery are presented in Figure 2A-B.

Discussion: After DMM surgery, Nfat1/- and Nfat1+/ mice displayed earlier onset of OA and significantly more severe OA in the knee joints than WT mice. The expression levels of Col10a1 (a marker of hypertrophic chondrocytes) and specific pro-inflammatory cytokines and matrix-degrading enzymes in articular cartilage are significantly higher in Nfat1-deficient mice than WT mice, suggesting that Nfat1 is a repressor of these catabolic molecules in adult articular cartilage.

Significance: This study identifies Nfat1 deficiency as a risk factor for progression of PTOA. The results from Nfat1+/- mice are particularly important as they reflect the effect of Nfat1 “gene dosage” on PTOA. This will direct us to explore if a decrease in NFAT1 expression in joint tissues is a risk factor for development of OA in humans.

OR5 2015 Annual Meeting
Poster No: 1202