Transcription Factor Nfat1 Deficiency: A Risk Factor for Progression of Posttraumatic Osteoarthritis in Mice

1Kramer, W C; 1Lu, Q; +1Wang, J
+Orthopaedic Surgery, University of Kansas Medical Center, Kansas City, KS
jwang@kumc.edu

Introduction
Osteoarthritis (OA) is the most common form of joint disease and the major cause of chronic disability in middle-aged and older populations. Posttraumatic OA (PTOA) arises from joint trauma, which accounts for a fraction of all patients with OA. In the United States, nearly 6 million patients have disabling PTOA of the hip, knee, or ankle, and the majority of which involve the knee joints. Even with the best current care of joint injuries, such as anatomic reduction and rigid fixation of intra-articular fractures and reconstruction of ruptured anterior cruciate ligament (ACL) with successful restoration of joint biomechanics, the risk of PTOA after joint injuries ranges from 20% to more than 50%. The time course for the progression of PTOA is highly variable and risk of PTOA increases with age of patients at the time of joint injury, suggesting that biologic factors may be involved in the progression of PTOA. However, the key biologic factors responsible for PTOA progression remain unclear. Nfat1/NFAT1 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 [1, 2]. This study was designed to test our hypothesis that Nfat1 deficiency is a risk factor for development of PTOA because loss of Nfat1 causes dysfunction of articular chondrocytes, which predisposes joints to PTOA after joint injury.

Methods
We generated bilateral meniscus instability by surgical transection of the medial meniscotibial ligament (MMTL) as described by Glasson et al. [3] in 2-3 months old Nfat1 knockout (Nfat1−/−) and wild-type (WT) control mice. All animal procedures were approved by the Institutional Animal Care and Use Committee at the University of Kansas Medical Center. Animals were anesthetized by intra-peritoneal injection of ketamine (40-60 mg/kg) and xylazine (5-10 mg/kg). Under a surgical microscope, the knee joint was exposed through a medial parapatellar incision under sterile conditions. The MMTL was visualized and 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 absorbable sutures. Buprenorphine (0.1 mg/kg) was injected subcutaneously for analgesia every 10-12 hours for 2-3 days after surgery. Operated animals were allowed caged mobility with access to food and water after surgery and were monitored to ensure healthy recovery. Animals were euthanized at 4 and 8 weeks after surgery, and the knee joints were harvested and processed for immunohistochemical, histochemical, and histomorphometric analyses to evaluate the severity of PTOA. Statistical analyses were performed with Student t-test and ANOVA.

Results
Six animals per group/time interval were examined in this study. Tissue sections stained with Safranin-O and fast green were used for histochemical and histomorphometric analyses to determine the severity of articular cartilage lesions during the development of knee OA. We utilized a modified semi-quantitative grading scale [3] as follows: 0 = normal cartilage; 0.5 = focal loss of Safranin-O staining with no structural lesions; 1 = roughened articular surface and small fibrillation limited to the superficial zone; 2 = fibrillation involving both superficial and middle zones; 3 = fibrillation involving the calcified cartilage across < 20% of the cartilage width; 4 = articular lesions extending to subchondral bone; 5 = fibrillation and cartilage erosions extending from 20% to 80% of the cartilage width; 6 = cartilage lesions extending beyond 80% of the cartilage width. Histologic scoring was performed on the four quadrants: medial and lateral femoral condyles and medial and lateral tibial plateaus. Histomorphometric analyses revealed that after destabilization of medial meniscus (DMM) by surgical transection of the MMTL, Nfat1−/− mice displayed significantly more severe OA-like changes in knee joints than WT mice (p < 0.05 at 4 weeks and p < 0.01 at 8 weeks). The OA-like 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 both Nfat1−/− and WT mice. The histopathological changes in the medial compartment of the Nfat1−/− and WT knee joints at 4 and 8 weeks after surgery are presented in Figure 1.

Discussion
Surgical DMM produces reproducible PTOA with slow initiation and progression of articular lesions in mice, which is a suitable mouse model of PTOA to challenge mice with gene deletion of a specific target. The present study revealed that after surgical transection of the MMTL, Nfat1−/− mice displayed significantly more severe OA changes in the knee joints than WT mice. The expression of IL-1β and MMP-13 is substantially higher in Nfat1−/− than WT articular cartilage, suggesting that Nfat1 normally suppresses the expression of specific proinflammatory cytokines and matrix-degrading enzymes in articular cartilage. Therefore, Nfat1 deficiency may be a risk factor for progression of PTOA after joint injury.

Significance
This study identifies Nfat1 deficiency as a risk factor for progression of PTOA, which may provide new insights into the pathogenetic mechanisms and development of effective strategies for prevention and treatment of PTOA.

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References
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