Characterization of Cartilage-specific IKKβ and IKKα Inducible Knockout Mice in a Surgical Model of Osteoarthritis

Kirsty L. Culley, PhD1, Miguel Otero, PhD1, Eleonora Olivotto, PhD2, Jun Chang, BSc1, Cecilia Dragomir, MD1, Kenneth B. Marcu, PhD3, Mary B. Goldring, PhD1,4.

1Hospital for Special Surgery, New York, NY, USA, 2Istituto Ortopedico Rizzoli, Bologna, Italy, 3Stony Brook University, Stony Brook, NY, USA, 4Weill Cornell Medical College, New York, NY, USA.


Introduction: NF-κB signaling orchestrates most stress- and inflammation-induced pathological responses in a variety of cell types, including chondrocytes. Thus, the NF-κB pathway represents a potential therapeutic target for Osteoarthritis (OA) treatment. IKKβ and IKKα are the essential activating NF-κB kinases, with IKKβ being almost always the canonical activating kinase in response to stress and inflammatory stimuli. We have previously demonstrated that these kinases exert differential effects on chondrocyte differentiation in vitro (1,2), with IKKα playing a predominant role in chondrocyte hypertrophic differentiation and MMP13-driven collagenase activity (2). Here, we aim to investigate the contribution of canonical and non-canonical NF-κB signaling to OA onset and progression in vivo, via surgical induction of OA in novel murine strains with inducible aggrecan (Agc1)-driven (3) cartilage-specific ablation of either IKKβ or IKKα.

Methods: Mouse strains: IKKβf/f;Agc1CreERT2/+;Lzf/f(R26R) (IKKβ-deleter mouse) and IKKαf/f;Agc1CreERT2/+;Lzf/f(R26R) (IKKα-deleter mouse) mice are wild type (WT) in all respects unless treated with tamoxifen via intraperitoneal injection, allowing vehicle-treated littermates to serve as controls for all experiments. Verification of cartilage-specific IKK ablation: Tissues harvested from IKK-deleter mice post tamoxifen (IKK-ablated, KO) or vehicle (WT) treatment were fixed in 4% paraformaldehyde and bathed in X-gal solution overnight to assess specific CRE-recombinase activity (3). IKKα or IKKβ mRNA knockout was assessed by qRT-PCR in articular cartilage dissected from tibial plateaus and femoral condyles of vehicle or tamoxifen-treated mice. Destabilization of the medial meniscus (DMM) surgery: IKK-ablation was induced in 11-wk-old male mice, with littermate control mice injected with vehicle alone. DMM surgery was completed on the right knee at 12-weeks of age, with the contra-lateral non-operated leg serving as control (4). Mice were sacrificed at 8 and 12 weeks post-DMM, and knees processed for histology or RNA isolation. Histology: Paraffin sections were Safranin O-stained and graded for OA severity by the OARSI system, as described (4).

Results: The IKK-deleter strains carry a Cre-inducible LacZ reporter gene in the Rosa26 locus (5), allowing the specificity of tamoxifen-induced Cre-recombinase activity and IKK knockout to be assessed via X-gal staining. Staining of tissues isolated from mice at three days post tamoxifen or vehicle injection revealed specific Cre-recombinase activity in aggrecan-expressing tissues in response to tamoxifen, with no staining observed in tissues isolated from vehicle-treated littermates (Fig 1A). Real-time PCR analysis of total RNA isolated from articular cartilage of tamoxifen- or oil-treated mice indicated efficient ablation of IKKβ and IKKα mRNA expression in response to tamoxifen (Fig 1B). Unexpectedly, IKKβ-ablated mice showed only a marginal protection against cartilage degradation at both 8 and 12 weeks post-DMM.
compared to WT controls (Fig 2A). Interestingly, no difference was observed in OA severity between IKKα-ablated and control mice at 8 weeks post DMM, but cartilage degradation was significantly reduced in IKKα-ablated mice at 12 weeks post-DMM (Fig 2B). Importantly, the scoring of the contra-lateral non-operated knees of both IKKβ- or IKKα-ablated mice showed no histological change when compared to vehicle-treated controls (not shown).

**Discussion:** We have successfully generated and characterized two novel murine strains that allow for tamoxifen-induced aggrecan (Agc1)-driven cartilage specific ablation of IKKβ or IKKα, enabling the investigation of the contribution of both canonical and non-canonical NF-κB pathways to OA initiation/progression in vivo. Surprisingly, while the canonical NF-κB pathway has been described as essential for chondrocytes to express stress-and inflammation-related genes associated with cartilage damage, the IKKβ KO mice showed only a marginal protection against cartilage degradation. The lack of significant protection against OA severity in IKKβ-ablated mice post DMM could be related with the contribution of canonical NF-κB signaling in deep zone chondrocytes, since tamoxifen-induced knockout is only expected to occur in the aggrecan-expressing chondrocytes which are primarily located in the superficial zone of articular cartilage (3). The contribution of deep zone chondrocytes to the canonical NF-κB-driven cartilage erosion; along with potential compensatory mechanisms and confounding factors, including the possibility of increased cell death of superficial zone chondrocytes due to IKKβ ablation, merit further investigation in the IKKβ-deleter mouse strain. The significant protection against OA severity observed in the IKKα-ablated mice compared to control at 12 weeks post surgery was accompanied by reduced collagenase activity (assessed by C1,2C immunostaining) (not shown). The latter is in agreement with our other recent work, showing that IKKα acts independently of its kinase activity to drive chondrocyte hypertrophic differentiation and MMP13-mediated collagen degradation in vitro (2). Thus, ablation of IKKα in vivo may prevent the hypertrophic-like phenotypic shift associated with OA disease. We are currently further investigating whether IKKα contributes to chondrocyte hypertrophy in vivo. In order to further explore our findings, we have isolated total RNA from the articular cartilage of both IKK-ablated and WT mice post-DMM, and we are currently performing whole genome transcriptome analysis and microRNA expression arrays, which will allow us to identify and dissect molecular signatures that are disease and IKK-dependent. Importantly, we will conduct comparative analysis with human OA specimens in order to establish parallels and identify common targets between murine and human specimens. We are confident that this approach, coupled with more in-depth histology, will uncover the roles of NF-κB signaling in OA initiation and progression, and will uncover future therapeutic targets for the treatment of OA disease.

**Significance:** Our results show that IKKα and IKKβ have differential contributions to OA progression in vivo, utilizing a surgical model of OA. Our ongoing studies will provide a better understanding of the contribution of NF-κB signalling in chondrocytes, and will lead to the identification and development of targeted therapies for OA.
Figure 1. (A) X-gal-stained tissues showed cartilage-specific Cre-recombinase activity in tamoxifen-treated mice compared to oil-treated controls. (B,C) RT-qPCR analysis of RNA isolated from IKK-deleter mice showed efficient cartilage-specific IKK mRNA ablation upon tamoxifen injection (KO).

Figure 2. (A) At 8 and 12 wks post-DMM surgery, the IKKβ-deleted (KO) mice showed a trend to reduced OA severity compared to oil-treated (WT) controls. (B) At 8 wks post-DMM surgery, the IKKα-deleted (KO), mice showed no change in OA severity compared to oil-treated (WT) controls, but significant protection was detected at 12 wks post surgery in response to IKKα KO (D).

ORS 2015 Annual Meeting
Poster No: 0335