Cartilage-specific Elf3 Knockout Mice are Protected from Cartilage Degradation in a Surgically-induced Osteoarthritis Model.

Elisabeth Wondimu, B.Sc.1,2, Jun Chang, B.Sc.1, Kirsty Culley, PhD1, Cecilia Dragomir, MD1, Darren Plumb, PhD1, Justin Quinn1, Mary B. Goldring, PhD1,2, Miguel Otero, PhD1.
1Hospital for Special Surgery, New York, NY, USA, 2Weill Cornell Medical College, New York, NY, USA.


Introduction: Osteoarthritis (OA) is the leading cause of disability in the adult population and it is responsible for over 500,000 joint replacement surgeries annually in the United States (1). One of the challenges for understanding OA disease relates with its complex and multifactorial etiology, involving both mechanical and inflammatory stresses that lead to abnormal activation of common signaling pathways in the cells resident in joint tissues. In chondrocytes, the unique cell type of articular cartilage, the abnormal signaling in OA disease leads to release from growth arrest, and chondrocyte activation, resulting in aberrant expression of pro-inflammatory and catabolic genes (2). The transcription factor E74-like factor 3 (ELF3) plays a central role in mediating these stress- and inflammatory signals in OA chondrocytes. We showed that ELF3 levels are elevated in OA chondrocytes, where it contributes to the IL1β-induced expression of matrix metalloproteinase 13 (Mmp13), Nos2, and Ptgs2/Cox2 in vitro (3,4). These findings suggested a pivotal role of ELF3 in cartilage degradation. In this study, we aimed to investigate the contribution of Elf3 to cartilage degradation in vivo in a surgical model of OA.

Methods: Generation of cartilage-specific Elf3 knockouts: we generated mice with cartilage-specific deletion of Elf3 using Col2-Cre (Col2a1Cre;Elf3fl/fl) because in situ hybridization (ISH) analysis showed the absence of Elf3 mRNA in the developing cartilage anlagen (not shown). Elf3fl/fl mice were generated, verified for homologous recombination within the 5’ and 3’ targeting arms (not shown), crossed with the Flipase mouse (JAX) to delete extraneous sequences (LacZ, Neo, etc.), and finally crossed with Col2a1Cre mice (JAX) to obtain Col2a1Cre;Elf3fl/+ mice, which were crossed with Elf3fl/+ to produce Col2a1Cre;Elf3fl/fl mice. Surgical Model of OA: 12-weeks-old male Col2a1Cre;Elf3fl/f (KO) and Cre-negative (WT, Elf3f/f) control mice (n=9/each) were subjected to the destabilization of the medial meniscus (DMM) surgical model of OA (5). DMM was performed on the right knees, while the left knees were left as unoperated controls. At 8-wks post-DMM, knees were harvested, fixed, decalcified and paraffin-embedded. Histological assessment of OA was conducted on Safranin-O/Fast green-stained serial coronal sections, as described (6). Osteophyte size and maturity were graded also as described (7). RTqPCR analysis: Total RNA was isolated from the articular cartilage of control and DMM-operated (OA) legs at 8-wks post-DMM. Briefly, articular cartilage was removed from operated or contra-lateral controls mouse knees with a scalpel blade whilst bathed in RNAlater (Ambion) under a dissection microscope. After removal of the RNAlater, cartilage samples were homogenized in TRIzol® (Invitrogen) and total RNA was extracted using the miRVana kit (Ambion). A total of 100 ng of RNA (with 260/280 ratios >1.8 and RNA Integrity Number >7) was reverse-transcribed using the QuantiTect Reverse Transcription kit (Qiagen), and amplifications were carried out using SYBR Green I-based qPCR on the Opticon 2 real time PCR detector system (BioRad), using specific primers for Elf3, Mmp13, Nos2, and
Ptgs2. Data were normalized using Eef1a1 as housekeeping gene, with Gapdh and Hprt1 as additional housekeeping controls.

**Results:** We have successfully generated Col2a1Cre;Elf3fl/fl (KO) mice, and successful breeding, without disruption of the expected Mendelian ratio, has been maintained. No obvious difference in size, weight, or growth plate morphology was observed between Elf3fl/fl control (WT) and KO, or wild-type C57/B6 mice (not shown). Cartilage-specific Elf3-deficiency in KO compared to WT mice was verified (not shown). Histological assessment of OA severity showed attenuation of cartilage loss at 8 wks post-DMM in WT mice compared to KO animals (Fig 1A), without changes in osteophyte size (Fig 1B) or maturity (not shown). We observed reduced MMP13-mediated collagenase activity (assessed using the C1,2C antibody) in the KO mice at 8 wks post-DMM (Fig 2), and decreased expression of Mmp13, Nos2, and Ptgs2/Cox2, assessed by RTqPCR analysis of RNA isolated from DMM-operated mice (Fig 3).

**Discussion:** We here report that cartilage-specific Elf3 deficiency protects against surgically-induced OA in mice. Cartilage-specific Elf3 knockout mice have decreased OA severity, accompanied with decreased MMP13/collagenase activity and decreased expression of several Elf3 target genes, including Mmp13. The latter is consistent with our previous reports in vitro, showing reduced IL-1β-driven Mmp13 expression in murine Elf3-/- chondrocytes and in human OA primary chondrocytes with siRNA-mediated ELF3 knockdown (4), and suggest that Elf3 plays pivotal roles in mediating stress/inflammatory responses in articular chondrocytes. Together, our in vivo an in vitro data represent strong evidence of a central role of Elf3 in the pathogenesis of OA, by controlling MMP13-mediated collagen degradation. Osteophytes developed with no difference between genotypes at 8 weeks post-DMM, indicating that ELF3 does not have impact on osteophyte development and highlighting the fact that Mmp13 deficiency does not alter osteophyte formation post-DMM surgery (7). Altogether, our results show that Elf3 is a critical regulator of Mmp13 in articular chondrocytes, and that Elf3 deficiency attenuates disease progression without impacting normal physiology. Thus, understanding the precise contribution of Elf3 to OA pathogenesis, defining downstream targets and interacting factors, and uncovering its effects in the early and late stages of the disease holds the promise of identifying novel targets with potential therapeutic applicability in OA disease.

**Significance:** Our results show that Elf3 is a critical mediator of Mmp13 expression and activity in a surgical model of OA. Thus, a better understanding of the mechanisms of action of Elf3 in chondrocytes will lead to the identification and development of targeted therapies for OA.
**Figure 1:** Cartilage-specific Eif3 knockout mice (KO) are protected from cartilage degradation post-DMM surgery compared to wild-type littermate controls (WT) (A) but showed no differences in osteophyte size (B). **∗∗** indicates p<0.01.

**Figure 2:** Immunohistochemical analysis showed increased collagenase-driven collagen degradation at 8-wks in WT mice post-DMM (DMM) compared to unoperated controls (control), and reduced collagenase activity (C1,2C staining) in KO mice compared to WT counterparts. Representative images are shown (original magnification 200X).

**Figure 3:** RT-qPCR analyses on RNA isolated from unoperated control (Ctrl) and DMM-operated (DMM) cartilage samples isolated from WT and KO mice at 8-wks post-DMM showed reduced Mmp13 (B), Nos2 (C), and Ptg2 (D) mRNA expression in KO mice. Eif3 mRNA was elevated in WT mice post-DMM and not detectable in KO mice (A).