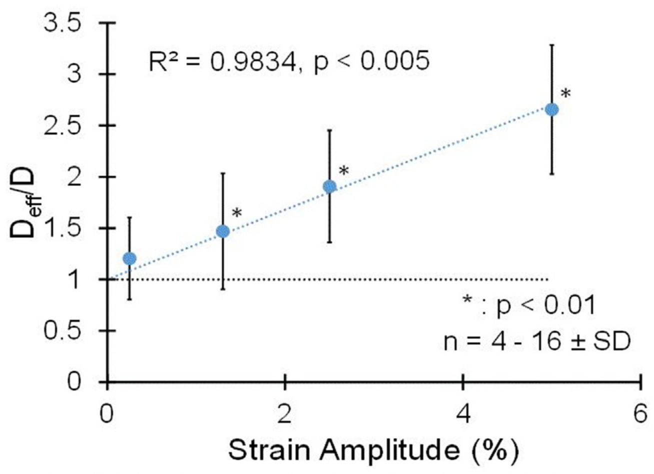

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THE E74-LIKE FACTOR 3 (ELF3) IS A CENTRAL MEDIATOR OF CARTILAGE DEGRADATION IN A SURGICALLY-INDUCED OSTEOARTHRITIS MODEL IN MICE

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Purpose: The transcription factor E74-like factor 3 (ELF3) plays a central role in mediating aberrant stress- and inflammatory signals in osteoarthritic (OA) chondrocytes. ELF3 mRNA levels are elevated in OA chondrocytes and contribute to the IL1β-induced expression of matrix metalloproteinase 13 (Mmp13), Nos2, and Ptgs2/Cox2 in vitro, indicating a pivotal role of ELF3 in cartilage degradation. Here, we aimed to investigate the contribution of gain- and loss-of-function of Elf3 to cartilage degradation in vivo.

Methods: CARTILAGE-SPECIFIC ELF3 KNOCKOUTS: We generated Col2a1Cre-driven cartilage-specific Elf3 knockout (KO) mice. We subjected 12-weeks-old male KO or control Elf3f/f (WT) littermates to the destabilization of the medial meniscus (DMM) surgical model of OA. TET-OFF INDUCIBLE ELF3-OVEREXPRESSING MICE: We generated TRE-Elf3:Comp-tTA (Tg) mice by crossing transgenic mice that express Elf3 under the control of the tetracycline-responsive element (TRE) with Comp-tTA mice expressing tTA under the control of the Cartilage Oligomeric Matrix Protein (Comp) promoter. Inducible, post-natal overexpression of Elf3 was assessed at 3, 6 and 9 months of age in Tg or Ctrl (Comp-tTA) mice. The contribution of Elf3 overexpression to spontaneously-induced OA was assessed in 6 and 9 month-old Tg and Ctrl mice. The contribution of Elf3 to the DMM-induced cartilage degradation was evaluated using 6 months-old Ctrl and Tg mice at 8-wks post-DMM surgery. SURGICAL MODEL OF OA: 12-weeks-old (WT or KO) and 6 months-old (Tg or Ctrl) male mice were subjected to the DMM surgical model of OA. DMM was performed on the right knees, while the left knees were left as unoperated controls. At 4, 8 and 12-wks (WT or KO mice) or at 8-wks (Tg and Ctrl mice) post-DMM, knees were processed for histological assessment of OA, conducted on Safranin-O/Fast green-stained serial coronal sections following OARSI guidelines. RTqPCR ANALYSIS: Total RNA was isolated from the articular cartilage of control and DMM-operated WT and KO mice at 8-wks post-DMM. The total RNA was reverse-transcribed and amplified using SYBR Green I-based qPCR and specific primers for Elf3, Mmp13, Nos2, and Ptgs2. Data were normalized using Eef1a1, Gapdh and Hprt1 as housekeeping genes.

Results: Histological assessment of OA severity showed attenuation of cartilage loss at 8 and 12 wks post-DMM surgery in KO mice compared to WT animals. We observed reduced MMP13-mediated collagenase activity, and decreased expression of Mmp13, Nos2, and Ptgs2/Cox2, assessed by RTqPCR analysis, in KO mice at 8-wks post-DMM. Importantly, while the TRE-Elf3:Comp-tTA (Tg) mice showed a trend towards increased cartilage damage at 6 and 9 months of age, the DMM-operated Tg mice exhibit significantly increased cartilage loss compared to Ctrl counterparts, suggesting that biomechanical challenge may be required to fully activate Elf3.

Conclusions: Here, we provide evidence that Elf3 is a central contributing factor for cartilage degradation in vivo, in OA disease. Cartilage-specific Elf3 deficiency protects against surgically-induced OA and Elf3 overexpression results in exacerbated cartilage degradation in mice subjected to the DMM surgery. Cartilage-specific Elf3 knockout mice have decreased MMP13/collagenase activity and decreased expression of several Elf3 target genes, including Mmp13. Our results are consistent with 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. 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. 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.

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ACUTE MITOCHONDRIAL DYSFUNCTION IN CARTILAGE FOLLOWING MECHANICAL INJURY

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Purpose: Mitochondria (MT) mediate the pathogenesis of many complex and unrelated diseases. MT dysfunction occurs secondary to mechanical injury in syndromes such as fluid shear-induced atherosclerosis and intraocular pressure-induced retinopathy in glaucoma. Trauma to cartilage can initiate post-traumatic osteoarthritis (PTOA) however the mechanisms are not fully understood. Evidence supports MT dysfunction in established OA, however the role of MT dysfunction in the early pathogenesis of PTOA is not clear. The goal of this study was to evaluate MT dysfunction the peracute (within hours) response of cartilage to traumatic injury, and to determine the relationship between MT function and early PTOA. A novel adaptation of the technique of real-time microscale respirometry to evaluate early post-traumatic chondrocyte MT function in situ is described.

Methods: Cartilage was harvested from knees of neonatal bovids. Explants were subjected to unconfined compression (4-8 MPa peak stress; 5-10 GPa/s peak stress rate) using a validated sub-critical damage model. Explants were then divided for use in 3 assays (Fig 1); MT function was assessed in real time by microscale respirometry, MT membrane potential was measured by polarity-sensitive fluorescent staining, and chondrocyte viability was evaluated on confocal microscopy. Microscale respirometry was performed on explants loaded into a 24-well tissue capture microplate and analyzed in a Seahorse XF 24 analyzer. Glycolysis and oxidative phosphorylation were quantified every 8 minutes for a total of 245 minutes by measuring extracellular acidification (ECAR) and oxygen consumption rates (OCR), respectively. To measure specific indices of MT function, a MT stress test was performed by sequentially adding 1) oligomycin, an ATP synthase inhibitor 2) FCCP, a proton circuit uncoupler 3) rotenone + antimycin A (inhibitors of MT complexes I and III) to determine ATP turnover, spare respiratory capacity and proton leak across the inner MT membrane, respectively. Relative MT membrane potential was measured by the fluorescent intensity ratio of a polarity-insensitive MT fluorescent probe (MitoTracker Green) to a polarity sensitive MT marker (TMRM) on confocal microscopy.

Results: Baseline OCRs were higher in control samples than impacted samples and higher in cartilage from the femoral condyle than the patellofemoral groove (Fig 2). Within two hour of injury, explants displayed impaired respiratory control in response to respiratory inhibitors (Fig 2). Most notably, injured samples demonstrated an attenuated response to FCCP with a 61% (range 43-71) decrease in spare respiratory capacity. Significant differences in ECAR between groups were not detected. Cell viability was decreased in impacted samples by an average of 20% (range 5-38) versus non-impacted controls (Fig 2A). MT membrane potential was decreased in impacted samples versus control (Fig 3), indicated by a 34% (range 3-54) decrease in red:green (polarized MT:all MT) fluorescent intensity ratio after injury.

Conclusions: This study revealed that MT dysfunction is a peracute response of chondrocytes to mechanical injury. Over the described range of impact magnitudes, cartilage compression resulted in decreased basal respiration, compromised ATP turnover and reduced maximal respiratory capacity, indicating likely inhibition of electron transport in the mitochondrial respiratory chain. This is the first report of adapting microrespirometry to study the subcellular mechanisms of impact-induced MT dysfunction. This model allows real time