**INTRODUCTION:** Osteoarthritis (OA), which affects roughly 15% of the U.S. population [1], is characterized by progressive cartilage destruction and damage to the integrity of bone and other joint tissues. OA pathogenesis is not well understood, nor is there an effective treatment to cure or even slow its progression. Cartilage degradation in OA is mediated primarily by upregulated proteolytic enzymes that include members of the MMP (matrix metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) families [2]. Our recent studies found that transcriptional regulator CITED2 suppression expression of multiple MMPs (-1, -3, -13) in nonarthritic cartilage [3, 4], and that electroporation of CITED2 into joints of rats with inflammatory arthritis can reduce cartilage matrix degradation [5]. These findings led us to hypothesize that CITED2 loss may have therapeutic potential to slow OA. Accordingly, in this study we characterized expression of CITED2 in human OA cartilage and in an established mouse OA model [6]. We also tested whether direct administration of CITED2 shRNA into the articular joint would mimic OA disease progression and upregulate expression of representatives of both the MMP and ADAMTS metalloproteinase families.

**METHODS:** *Human OA samples.* Human specimens were collected from patients undergoing total knee or hip replacement surgery with written consent in accordance to an IRB-approved protocol (n=8, avg. age=70±7.3 yrs). Cartilage was dissected and fixed in 10% formalin. 

**Immunohistochemistry and Safranin O staining.** Formalin-fixed, decalcified histological sections were incubated overnight at 4°C with anti-CITED2, anti-MMP-13, and anti-ADAMTS-5, followed by incubation with anti-rabbit or anti-mouse secondary antibody and visualization with DAB chromagen. Negative control sections were prepared using irrelevant isotype-matched antibodies in place of the primary antibody. Safranin O-Fast green staining was carried out to detect proteoglycans.

**Induction of osteoarthritis in mice.** Destabilization of the medial meniscus (DMM) in 8 to 10-wks-old male C57BL/6 mice was carried out by transecting the medial meniscotibial ligament (MMLT) [6]. (n=5).

**CITED2 suppression in vivo.** Intra-articular injections of CITED2 shRNA (10 mg/10 ul PBS) were performed followed by electroporation every 7 days in a separate set of 8- to 10-wks-old male C57BL/6 mice. Contralateral controls received injections of PBS only followed by electroporation (n=3).

**RESULTS:** Loss of CITED2 correlates with cartilage degradation in human OA and DMM mice. In human OA cartilage samples, “healthy,” regions with strong Safranin O staining were correlated with high levels of CITED2 expression, whereas areas of cartilage destruction, indicated by local loss of Safranin O staining, were correlated with low levels of CITED2 expression (Fig 1A). Similarly, CITED2 was detected in healthy mouse cartilage, but was expressed at low levels in degenerated cartilage 4 weeks after OA induction by DMM (Fig 1B). CITED2 suppression produces OA-like changes in normal mouse cartilage. We next determined the direct effect of CITED2 suppression on cartilage homeostasis after intra-articular injection of CITED2 shRNA in normal mouse knees followed by electroporation. Two weeks following the first injection, a loss of Safranin O staining was detected in the articular cartilage (Fig 2A), along with a loss of CITED2 expression (Fig 2B), compared to the PBS-injected contralateral controls. CITED2 suppression unregulates MMP-13 and ADAMTS-5 expression. We next determined the effect of CITED2 suppression on the expression of MMP-13 and ADAMTS-5. Low levels of MMP-13 and ADAMTS-5 were detected in contralateral control samples, but two weeks of shCITED2 treatment resulted in substantial increases in MMP-13 and ADAMTS-5 in the articular cartilage (Figs 3A, 3B).

**DISCUSSION:** OA is characterized by an imbalance between the anabolic and catabolic activities of chondrocytes, yet the set of regulatory mechanisms governing that balance remain incompletely understood. The finding that CITED2 levels are diminished in human and mouse OA and that experimental suppression of CITED2 expression can produce OA-like changes in mice support the hypothesis that CITED2 deregulation contributes to the pathologic upregulation of proteolytic enzymes specifically implicated in OA, i.e. MMP-13 and ADAMTS-5.

**SIGNIFICANCE:** This study directly implicates loss of CITED2 function as a contributor to both human and mouse OA, and furthermore identifies CITED2 as a potential target for development of therapies designed to slow the progression of OA.


**ACKNOWLEDGEMENTS:** NIH Grants AR47628 and AR52743 (HBS); R01-AG022021 (MBG)