CITED2 prevents cartilage degradation in collagen-induced arthritic rats through suppression of matrix metalloproteinases

INTRODUCTION: Accelerated degradation of cartilage extracellular matrix (ECM) is a hallmark of both rheumatoid arthritis (RA) and osteoarthritis (OA). While RA is an autoimmune disorder and OA arises from wear and tear, ECM degradation in both diseases is mediated primarily by members of the MMP (matrix metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motif) families – e.g. MMPs 1, 3, 13 and ADAMTS 4 and 5 [1]. Thus far, MMP inhibitors have failed to show significant efficacy in human diseases without significant side effects [2]. An alternative strategy for reducing pathologic enzyme activity is to modulate MMP expression directly within chondrocytes. A possible target for such an approach is the transcriptional regulator CITED2, which suppresses expression of multiple MMPs in chondrocytes in vitro, and mediates the protective action of moderate mechanical loading on cartilage integrity in vivo [3-5]. The goal of this study was to test whether CITED2 gene transfer in vivo is able to suppress MMP expression and inhibit pathologic cartilage changes in a rodent collagen-induced arthritis model.

METHODS: Collagen-induced arthritis (CIA). Bovine type II collagen solution (Chondrex) was emulsified in Freud’s complete adjuvant at 4°C. Under an IACUC-approved protocol, male Sprague-Dawley rats (6-8 wk) were immunized by two intradermal injections of 100µl emulsion (1µg collagen/µl) at the base of the tail given one week apart. CITED2 gene transfer. After the booster injection, and at weekly intervals thereafter, “CITED2+” rats received 25µg of pCNA3.1 encoding human wild-type CITED2 cDNA by intra-articular injection followed by electroporation (250V, 0.10 ms pulse length, 4 pulses each polarity). Contralateral limbs received an identically delivered empty vector. Animals were sacrificed 0, 8, 11, 14, 17, 21, and 28 days after the first injection (n=3/timepoint, Fig. 1A). Articular cartilage from distal femur and tibial plateau were harvested, flash frozen, and lysed for Western blot. At Day 28, parallel articular cartilage samples were fixed in formalin, decalcified and processed for histology (n=3/group). Immunohistochemistry and Safranin O staining. Histological sections were immunostained overnight at 4°C with antibodies against CITED2, MMP-1, MMP-2, MMP-3, MMP-13 (all Abcam) or Col2-3/4M (Ibex), immunostained overnight at 4°C. Under an IACUC-approved protocol, male Sprague-Dawley rats (6-8 wk) were immunized by two intradermal injections of 100µl emulsion (1µg collagen/µl) at the base of the tail given one week apart. CITED2 gene transfer. After the booster injection, and at weekly intervals thereafter, “CITED2+” rats received 25µg of pCNA3.1 encoding human wild-type CITED2 cDNA by intra-articular injection followed by electroporation (250V, 0.10 ms pulse length, 4 pulses each polarity). Contralateral limbs received an identically delivered empty vector. Animals were sacrificed 0, 8, 11, 14, 17, 21, and 28 days after the first injection (n=3/timepoint, Fig. 1A). Articular cartilage from distal femur and tibial plateau were harvested, flash frozen, and lysed for Western blot. At Day 28, parallel articular cartilage samples were fixed in formalin, decalcified and processed for histology (n=3/group). Immunohistochemistry and Safranin O staining. Histological sections were immunostained overnight at 4°C with antibodies against CITED2, MMP-1, MMP-2, MMP-3, MMP-13 (all Abcam) or Col2-3/4M (Ibex), followed by a 30 minute incubation with anti-mouse or anti-rabbit secondary antibody and visualization with DAB chromagen for 3 min. Negative controls were stained with irrelevant isotype-matched antibodies. Safranin O-fast green staining was carried out to demonstrate proteoglycan content in the articular cartilage. Chromatin immunoprecipitation (ChIP). C28/I2 human chondrocytes were treated with IL-1β (10ng/ml) to induce MMP expression, with or without CITED2 transfection. ChIP was performed using a commercial kit following manufacturer’s instructions (USB). Briefly, cell lysates were immunoprecipitated with anti-Ets-1 (Santa Cruz), and PCR was performed using primers specific for Ets-1 binding sites (GGAA/T) in MMP promoter regions (Fig 3A).

RESULTS: Electroretroporation was effective in delivering CITED2 to rat articular cartilage. CITED2 levels (Western blot) increased until Day 14 and remained elevated throughout the experiment (Fig 1B).

Collagen-induced arthritis (CIA) (Fig 2A, B middle columns) produced degradative changes in articular cartilage relative to controls (left columns), including reduced Safranin-O staining (Fig 2A, middle row) indicating diminished proteoglycan content, and increased levels of denatured type II collagen (2A, bottom row). These changes were accompanied by increased numbers of cells expressing MMPs 1, 2, 3 and 13 (Fig 2B), but a reduction in cells staining positive for CITED2 (2A, top row). By contrast, CIA animal that received CITED2 gene therapy (2A, B right columns) showed increased CITED2 expression in articular chondrocytes (2A, top row), diminished losses of Safranin O staining, lower levels of denatured collagen (Fig 2A) and reduced expression of all MMPs examined (Fig 2B). These anti-catabolic changes were not observed in contralateral control (data not shown).

DISCUSSION: While CITED2 has been suggested to mediate the protective effects of moderate joint loading in vivo [4], this study demonstrates a possible role of CITED2 in altering the progression of rheumatoid arthritis. CITED2 expression in articular cartilage was downregulated in an established RA model, but CITED2 cDNA transfer suppressed expression of several MMPs and inhibited development of arthritic tissue changes. ChIP analysis indicates that CITED2 blocks Ets-1 interactions with several MMP promoters and supports our previously demonstrated mechanism where CITED2 competes with Ets-1 for binding to the transcriptional co-regulator p300 [6]. Finally, the results suggest that CITED2 gene delivery to joint tissues may be a useful approach for the treatment of joint disorders such as RA and OA.

To explore a potential mechanism for the CITED2 suppression of MMPs, ChIP assays were performed using an antibody against Ets-1, a transcriptional activator of MMPs. IL-1β, which stimulates MMP expression in C28/I2 chondrocytes, increased Ets-1 binding to promoter regions of MMPs 1, 2, 3, and 13; however, these protein-DNA interactions were decreased when CITED2 was overexpressed in IL-1β treated cells. These data indicate that CITED2 may inhibit MMP transactivation by preventing Ets-1 from forming transcriptional complexes.

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