E74-like factor 3 (ELF3) impacts on matrix metalloproteinase 13 (MMP13) transcriptional control in articular chondrocytes under pro-inflammatory stress

Miguel Otero,1 Darren A. Plumb,1 Kaneyuki Tsuchimochi,1,§ Cecilia L. Dragomir,1 Ko Hashimoto,1 Haibing Peng,2 Eleonora Olivotto,3 Michael Bevilacqua,1 Lujian Tan,2 Zhiyong Yang,4 Yumei Zhan,2 Peter Oettgen,2 Yefu Li,5 Kenneth B. Marcu,3,6 and Mary B. Goldring1*

1Laboratory for Cartilage Biology, Research Division, The Hospital for Special Surgery, Weill Cornell Medical College, New York, NY 10021, 2Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA 02115, 3SC Laboratorio di Immunoreumatologia e Rigenerazione Tissutale, Centro di Ricerca Codivilla-Putti, Istituto Ortopedico Rizzoli, 40136 Bologna, Italy 4Inflammation and Remodeling Research Unit, Pfizer Biotherapeutics Research, Cambridge, MA 02140, 5Harvard School of Dental Medicine, Boston, MA 02115, and 6Department of Biochemistry and Cell Biology, Stony Brook University, Stony Brook, NY 11794-5215

Running title: MMP-13 activation by ELF3 in articular chondrocytes

*Address correspondence to: Mary B. Goldring, PhD, Hospital for Special Surgery, Caspary Research Building, 5th Floor, 535 East 70th Street, New York, NY 10021. Phone: 212-774-7564. Fax: 212-249-2373. E-mail: goldringm@hss.edu.
§Present address: Department of Orthopaedic Surgery, Graduate School of Medicine and Dentistry, Kagoshima University, Japan

Matrix metalloproteinase (MMP)-13 has a pivotal, rate-limiting function in cartilage remodeling and degradation due to its specificity for cleaving type II collagen. The proximal MMP13 promoter contains evolutionarily conserved ETS binding sites (EBS) that are closely flanked by AP-1 and Runx2 binding motifs and interplay among these and other factors has been implicated in regulation by stress and inflammatory signals. Here we report that ELF3 directly controls MMP13 promoter activity by targeting an EBS at position −78 bp and by cooperating with AP-1. In addition, ELF3 binding to the proximal MMP13 promoter is enhanced by interleukin (IL)-1β stimulation in chondrocytes, and the IL-1β-induced MMP13 expression is inhibited in primary human chondrocytes by siRNA-ELF3 knockdown and in chondrocytes from Elf3−/− mice. Further, we found that MEK/ERK signaling enhances ELF3-driven MMP13 transactivation and is required for IL-1β-induced ELF3 binding to the MMP13 promoter, as assessed by chromatin immunoprecipitation. Finally, we show that enhanced levels of ELF3 co-localize with MMP-13 protein and activity in human osteoarthritic cartilage. These studies define a novel role for ELF3 as a pro-catabolic factor that may contribute to cartilage remodeling and degradation by regulating MMP13 gene transcription.

The matrix metalloproteinases (MMPs) are a family of enzymes that coordinately degrade components of the extracellular matrix in physiological/normal matrix remodeling processes (1), and in disease states wherein their aberrant and enhanced expression contributes to exacerbated matrix degradation (2,3). Type II collagen is a major constituent of articular cartilage that contributes to its structural and functional properties by conferring tensile strength; and its degradation is the pivotal event that determines the irreversible progression of osteoarthritis (OA), in which articular cartilage is slowly and
progressively destroyed (2). OA occurs in conjunction with changes in the synovium and subchondral bone that are associated with dysregulated chondrocyte physiology exemplified in part by the abnormal expression of catabolic and anabolic gene products (2). In this context, pro-inflammatory cytokines have been shown to trigger a diverse array of intracellular signaling pathways leading to the overexpression of a variety of matrix-degrading enzymes, including MMPs (2).

Because collagen degradation is mediated almost exclusively by MMPs, those with higher collagenolytic activity (collagenases) are the rate-limiting, major players in irreversible cartilage destruction (4); and MMP-13 (collagenase 3) plays a very prominent role here. MMP-13 preferentially and more potently cleaves type II collagen compared to other collagenases (5-7). Moreover, MMP-13 levels and activity are enhanced in OA cartilage, associated with degenerative changes and co-localizing with MMP-13-specific type II collagen cleavage products, inflammatory cytokines, and their receptors (4,8). Further, in vivo evidence has shown OA changes in transgenic mice over-expressing constitutively active MMP-13 (9), while Mmp13 knockout mice are refractory to surgically-induced OA (10). Therefore, a thorough understanding of how MMP13 is transcriptionally regulated by intracellular regulatory factors and how their activities are modulated by extracellular cues leading to aberrant MMP-13 expression in OA cartilage is essential.

The E74-like factor 3 (ELF3), also known as ESE1, ESX, ERT, and JEN, is an epithelium-specific member of the E26 transformation-specific sequence (ETS) family of transcription factors (11). ETS transcription factors are trans-acting phosphoproteins that share a highly conserved DNA-binding winged helix-turn-helix domain (ETS domain), which specifically binds to ETS sequence motifs in the transcriptional control elements of target genes (12). ETS factors induce or repress transcription depending on their activation by mitogen-activated protein kinases (MAPKs) and by their association with other cofactors in a promoter context-specific manner (12,13). ETS proteins mediate a variety of biological processes, including cell proliferation, differentiation, transformation and tumor invasion, with the latter involving ECM remodeling by concerted modulation of MMPs and their inhibitors (14). ELF3 plays essential roles during epithelial cell differentiation (11,15,16), gut development (17), apoptosis (18,19), and physiology of normal breast and breast cancer epithelial cells (20,21). ELF3 activity within a given cell may depend on its expression levels and involve both nuclear transcriptional mechanisms and events independent of the nucleus (19). In addition, ELF3 is strongly induced in a variety of cell types by stress or inflammatory conditions dependent, at least in part, on canonical NF-κB (p65/p50) binding to a high affinity NF-κB site in the proximal ELF3 promoter (22,23). ELF3 mediates inflammatory responses by regulating genes such as nitric oxide synthase 2 (NOS2), cyclooxygenase 2 (PTGS2/COX2), and angiopoietin-1 (22,24-26), and thus may contribute as a pro-catabolic/anti-anabolic regulatory factor in inflammatory disease states such as airway inflammation, OA, and rheumatoid arthritis (22,23). In chondrocytes, ELF3 expression is induced in vitro by IL-1β; and we have previously reported that it accounts for the NF-κB-dependent and partially for the IL-1β-mediated repression of the COL2A1 promoter (27), thus making ELF3 a potential pro-inflammatory mediator in OA disease.

Herein, we provide evidence for a novel role for ELF3 as a regulatory component that drives the abnormal expression of MMP-13 under pro-inflammatory conditions in chondrocytes. We show that ELF3 activates MMP13 transcription by binding to a conserved ETS site in its proximal promoter region and that ELF3 up-regulates MMP-13 expression by acting in conjunction with MEK/ERK signaling and enhancing AP-1-driven MMP13 promoter activity in response to IL-1β stimulation. Importantly, dysregulated MMP-13 expression and activity in OA articular cartilage is associated with enhanced levels of ELF3.

**Experimental procedures**

**Cell Culture**—Human primary chondrocytes were isolated, as described (28), from articular cartilage obtained from intact regions of femoral condyles of OA patients undergoing total knee replacement, with approval by the Institutional Review Board (IRB) and patient consent. Immediately after surgery, the cells were isolated by sequential digestion with pronase (Promega) and collagenase P (Promega), cultured to confluence in DMEM/F12 containing 10% FBS, and used for experimental purposes at passage one. Human immortalized T/C-28a2 chondrocytes were
cultured in Dulbecco’s Modified Eagle’s medium (DMEM)/Ham's F12 containing 10% fetal bovine serum (FBS), as described previously (27). Elf3 knockout mice (17) were obtained from Dr. Melanie A. Pritchard, Monash University, Clayton, Victoria, Australia. Primary mouse chondrocytes were isolated as described (29) from 5- to 6-day-old wild type (C57BL/6) or Elf3 knockout mouse articular cartilage. Passage 0 chondrocytes were seeded in DMEM/F12 containing 10% FBS, allowed to reach confluence, trypsinized, and seeded in 6-well plates in DMEM/F12/10% FBS for experimental purposes. All experiments involving IL-1β and TNFα (R&D) stimulation were performed in serum-free conditions after overnight starvation.

siRNA transfection—Human primary or immortalized chondrocytes were transfected, as described previously (28). Briefly, 2.5x10^5 cells were seeded 24 h before transfection in 6-well plates in DMEM/F12 containing 10% FBS. The non-targeting control siRNA (Dharmacon) or siRNAs against ELF3 (Dharmacon) were transfected at a final concentration of 50 nM using LipofectAMINE PLUS reagents in serum-free medium. Transfection efficiency was assessed using non-targeting siRNA conjugated with rhodamine (Dharmacon), and knockdown efficacy was assessed by real-time PCR and Western blotting. At 72 h after transfection, cells were stimulated with IL-1β or vehicle for the indicated times.

Real time quantitative PCR (RT-qPCR) analysis—Total RNA was isolated using the RNeasy Plus Mini Kit (QIAGEN) and 150 ng were reverse transcribed, as described (28). Amplifications were carried out using SYBR Green I-based RT-PCR on the Opticon 2 Real Time PCR Detector System (BioRad), as described (28), using the PCR primers and conditions indicated in Table S1. The data were calculated as the ratio of each gene to GAPDH, and HPRT1 was utilized as an additional housekeeping control.

Western blot analysis—Human and mouse primary chondrocytes, and human immortalized chondrocytes were maintained in DMEM/F-12 medium containing 10% FBS. Before treatments with IL-1β, cells were incubated in serum-free medium overnight and challenged with the cytokine for the indicated times. To collect total cell lysates, cells were rinsed with cold PBS and lysed with 1× RIPA buffer (Santa Cruz) containing PMSF, proteinase inhibitors, and sodium orthovanadate, as indicated by the supplier. Protein content was determined using Coomassie Plus Protein Assay Reagent (Pierce), and equal amounts of protein lysates were resolved in 10% Tris-HCl polyacrylamide gels under reducing conditions. Proteins were then transferred to polyvinylidene fluoride (PVDF) membranes using a semi-dry transfer system (Biorad). Membranes were blocked with 5% non-fat milk in Tris-Buffered Saline with 0.1% Tween-20 (TBS-T 0.1%) for 1 h at room temperature and then incubated with primary antibodies against ELF3 (Abcam), phospho-p38, phospho-SAPK/JNK, phospho-ERK1/2, total p38, total SAPK/JNK or total ERK1/2 (Cell Signaling); β-tubulin (Abcam) was used as loading control. After washing, the membrane was incubated with the corresponding secondary antibody conjugated with horseradish peroxidase. Signals were detected using enhanced chemiluminescence (Amersham).

Plasmid construction—The –1602/+20-MMP13-Luc promoter construct was described previously (30). The MMP13 promoter sequences spanning –1007/+20 and –273/+20 bp were prepared by PCR using the pCAT-MMP13 promoter construct as template as described (30). The sense primer for each construct included an artificial SacI site and the antisense primer included an artificial XhoI site. The constructs were cloned into the pGL2-Basic luciferase reporter gene vector (Promega).

The deletion mutant lacking the proximal –231/+39 bp sequence was generated by digestion of the –1602/+20 bp promoter construct with BbvCI and StuI (New England Biolabs) followed by blunt-ended ligation (Takara). Point mutants of the proximal ETS binding sites (labeled as EBS-A, B1 and B2mut) within the MMP13 promoter were generated by two-step PCR mutagenesis using the wild type –273/+20-MMP13 construct as template. For the first PCR, common forward or reverse primers, containing Xmal and XhoI sites, respectively, were used in combination with the primers indicated in Table 1. The resulting PCR products were purified and utilized in the second PCR, performed with the common forward and reverse primers containing Xmal and XhoI restriction sites utilized for the first PCR reaction. The resultant amplification products were purified and digested with SacI and XhoI (New England Biolabs) and transferred into the pGL2-Basic backbone treated with the same enzymes.
Expression vectors encoding ELF3, ELF5, EHF, RUNX2, c-Fos and c-Jun have been described (27,30). Vectors encoding JNK, ERK1/2, p38, MKP1 and the constitutively active MAP2K MKK7, MKK6 and MEK1 were purchased from Addgene and described elsewhere (30). All constructs were confirmed by DNA sequencing, performed at the Cornell University Life Sciences Core Laboratories Center.

Transfection and reporter assays—Transient transfection experiments were carried out in T/C-28a2 cells using LipofectAMINE PLUS™ Reagents (Invitrogen). Cells were seeded 24 h before transfection in 24-well tissue culture plates at 2.5 x 10^4 cells/cm² in DMEM/F12 containing 10% FBS. Transfections were carried out in serum-free and antibiotic-free medium using no more than 450 ng of plasmid DNA, including 350 ng of reporter constructs. Cell lysates were prepared by extraction with 100 µl of Reporter Lysis Buffer (Promega) and the protein content was determined using the Coomassie Plus Protein Assay Reagent. Unless specified otherwise, luciferase activities were normalized to the pRL-SV40 Renilla luciferase control vector (Promega).

Chromatin immunoprecipitation (ChIP) assay—The ChIP-IT Express Enzymatic Kit (Active Motif) was used to perform ChIP assays, according to manufacturer’s instructions with minor modifications. Briefly, T/C-28a2 cells were plated on 150-mm dishes and either transfected with FLAG-tagged ELF3 expression vectors or incubated with 1 ng/ml of IL-1β for 2 h, with or without a 45-min pre-treatment with U0126 (2.5µM; Sigma). Cross-linking was performed with 1% formaldehyde for 10 min at room temperature; nuclei were isolated and chromatin was enzymatically sheared for 8 min at 37°C, resulting in chromatin fragments of 250 to 1000 bps. Chromatin was pre-cleared by incubation with 25 µl of protein G magnetic beads and 5 µg of non-specific (control) rabbit IgG (Cell Signaling) for 2 h at 4°C with rotation. After pre-clearing and removal of the protein G magnetic beads, the lysates were incubated at 4°C for 16 h with 5 µg of rabbit anti-ELF3 antibody (Orbigen), rabbit anti-FLAG antibody (Sigma), or normal rabbit IgG (Cell Signaling), and 10 µl of the pre-cleared chromatin was stored to be used as assay input. After reverse cross-linking of the DNA-protein complexes, the DNA was subjected to PCR analysis using 5 µl of the eluted DNA and the following set of primers: 5’-CCCTCAAATTCTACCACAAACC-3’ (forward) and 5’-CAATGATGGTACCATCATTATGG-3’ (reverse), spanning from –157 to –38 bp of the proximal human MMP13 promoter. The PCR products were resolved on a 2.5% agarose gel. For real time PCR analysis, the precipitated DNA was purified using DNA minicolumns (QIAGEN) and the final DNA preparations were PCR amplified using 2 µl of the purified DNA and the Opticon 2 Real Time PCR Detector System (BioRad) utilizing the aforementioned primers. Primer efficiency was calculated utilizing serial dilutions of the pooled input DNA samples. For real time PCR analysis, the C_T of each sample was normalized to the C_T of the input sample (10%). Specific GAPDH primers provided by the manufacturer (Active Motif) were used for assessing the ChIP quality of the digested chromatin and as negative controls for the precipitated DNA.

Histological and immunohistochemical analysis—Full thickness human articular cartilage samples, including cartilage and bone, were obtained from the medial tibial plateaus of the knee joints of 10 OA patients undergoing total knee replacement with IRB approval. After fixation, decalcification and paraffin-embedding, sections of 6 µm were cut, deparaffinized in xylene and rehydrated through an ethanol series. Every tenth section was collected for Safranin O/Fast green staining and the OA stage was graded independently by two blinded observers according to OARSI grading scale (31). For immunostaining, successive adjacent sections within each patient were deparaffinized and quenched for endogenous peroxidase activity. The sections were then blocked and incubated overnight with antibodies against ELF3 (Abcam), MMP13 (Chemicon) and C1,2C (IBEX). For negative controls, isotype-matched IgG (Santa Cruz) was used in place of the primary antibodies. The sections were counterstained with methyl green and the Vectastain ABC Elite kit (Vector Laboratories) was used as described by the manufacturer. Immunostainings were performed in at least three different donors per OARSI grade and repeated at least three times per donor.

Statistical analysis—Data are reported as means ± SEM of at least three independent experiments. Statistical analysis was performed by ANOVA followed by Student’s t-test with p values of <0.05 considered significant.
RESULTS

ELF3 is a novel regulator of MMP13 expression in human and murine primary chondrocytes—ELF3 expression is induced by inflammatory cytokines, including IL-1β, in different cell types in a NF-κB (p65/p50)-dependent manner (22,23,27); and in previous work, we showed that ELF3 mediates the anti-anabolic actions of IL-1β in chondrocytes by binding to the COL2A1 promoter and suppressing its activity (27). We initially screened for genes whose expression depended on ELF3 for their response to IL-1β by performing a TaqMan Low Density Array (TLDA) screen of RNAs isolated from primary human chondrocytes transfected with siRNA-ELF3 or non-targeting siRNA oligos, which showed that MMP13 was amongst the genes that were differentially regulated by ELF3 in response to IL-1β stimulation (data not shown). Indeed, subsequent RT-qPCR analyses revealed that IL-1β rapidly induced the sustained expression of ELF3 in human primary OA articular chondrocytes, which was followed by the strong up-regulation of MMP13 mRNA (Fig. 1A & B). Like MMP13, the IL-1β-dependent expression profiles of MMPs 1, 3 and 9 were also enhanced at later times, while COL2A1 mRNA was suppressed (Fig. S1). Importantly, IL-1β-induced MMP13 mRNA in primary human OA chondrocytes was significantly down-modulated by ELF3 knockdown (KD) (Figure 1 C-E). In murine chondrocytes, IL-1β-induced Elf3 mRNA peaked at 2 h (declining thereafter), followed by the induction of Mmp13 mRNA peaking at 6 h and remainingstable up to 24 h (Fig. 2A & B). Similar to our findings in human primary chondrocytes, IL-1β-induced Mmp13 mRNA was strongly although not completely reduced in Elf3−/− mouse chondrocytes compared to their wild type counterparts (Fig. 2C), clearly indicating that ELF3 is an important contributing factor for IL-1β-induced MMP13 expression in chondrocytes and also that other regulatory factors contribute to MMP13 expression independent of ELF3.

We also investigated the contribution of ELF3 in induction of Mmp13 mRNA by TNFα in murine wild-type and Elf3−/− chondrocytes. Consistent with previous reports using chondrocyte cell lines and other cell types (22,23), RT-qPCR analysis showed that stimulation with TNFα leads to increased Elf3 mRNA expression but not with the potency of IL-1β (Fig. S2A). TNFα-induced Mmp13 mRNA levels were suppressed in Elf3−/− mouse chondrocytes compared to their wild-type counterparts (Fig. S2B). This latter decrease in Mmp13 mRNA only reached statistical significance after 24 h of TNFα stimulation, indicating that other mediators are also involved in the induction of Mmp13 by TNFα in chondrocytes (32,33).

Next, we explored the relative contribution of ELF3 to the IL-1β-induced expression of other important factors involved in cartilage degradative processes (Fig. S3). Consistent with reports showing that ELF3 acts as a transactivator of NOS2 and COX2/PTGS2 (25,26), RT-qPCR analyses revealed that the IL-1β-induced Nos2 and Ptgs2 mRNA levels were significantly reduced in Elf3−/− compared to wild type mouse chondrocytes, whereas ELF3 did not impact on the IL-1β-induction of Mmp2, 3 and 9 mRNA levels (Fig. S3).

ELF3 protein levels are enhanced in human OA cartilage and co-localize with increased MMP-13 protein levels and activity—To further investigate their regulatory and spatial relationship in the context of OA disease, we analyzed the relative in situ levels of ELF3 and MMP-13 proteins in different sections of human OA cartilage. Immunohistochemical analysis revealed enhanced ELF3 immunostaining in OA cartilage, in conjunction with increased MMP-13 protein levels and activity, as judged by positive immunostaining for collagen type II cleavage epitopes with the C1,2C antibody, residing within areas of cartilage degradation (Fig. 3).

ELF3 activates the MMP13 promoter and enhances AP-1-driven MMP13 transactivation via a highly conserved proximal ETS binding site—To determine if ELF3 activates MMP13 transcription, we used human MMP13 promoter-driven luciferase reporter constructs (depicted in Figure 4A) in co-transfection experiments in T/C-28a2 human immortalized chondrocytes. Indeed, enforced ELF3 expression transactivated the -1602/+20 bp-MMP13 reporter construct in these cells (Figure 4B). However, the overexpression of two other ETS family members, ELF5 (ESE2) and EHF (ESE3), which share a high degree of homology with the ELF3 DNA binding domain (34,35), did not transactivate the MMP13 promoter, nor did...
they enhance or interfere with ELF3-driven MMP13 transactivation (Figure 4C).

ETS transcription factors can work as cofactors of other transcriptional regulators (12,13) and the MMP13 promoter contains evolutionarily conserved proximal ETS binding sites (EBS) that are adjacent to an AP-1 motif, which may then interact in promoter transactivation (36). We therefore investigated if ELF3 can act in a collaborative fashion with c-Fos and c-Jun (AP-1) family members in the context of the human MMP13 promoter. As shown in Figure 4D, c-Fos/c-Jun overexpression not only leads to the transactivation of the −1602/+20 bp MMP13 promoter, but also co-enforced ELF3-driven MMP13 promoter activation. These results appear to explain the functional EBS-AP-1 interaction in transactivating the MMP13 promoter.

To more precisely define the ETS binding site(s) responsible for ELF3-driven MMP13 activation, we first used luciferase reporter constructs containing different sequences of the proximal human MMP13 promoter. ELF3 overexpression transactivated each of three MMP13 reporter constructs spanning −1602/+20, −1007/+20 and −273/+20 bp of the MMP13 promoter (Fig. 5A), suggesting that ELF3-driven MMP13 transactivation depends upon the EBS located within the −273/+20 bp proximal promoter region. We generated, therefore, a deletion construct of the −1602/+20 bp MMP13 promoter lacking the −231/+39 bp sequence (denoted as Δ−231/−39-MMP13 in Fig. 4A). Note that the Δ−231/−39-MMP13 construct, in addition to missing the EBS, also lacks the adjacent AP-1 and RUNX2 binding sites. As expected due to the absence of the proximal AP-1 site (37), the Δ−231/−39-MMP13 construct had reduced basal activity. Importantly, whereas overexpression of either c-Fos/c-Jun, RUNX2 or ELF3 transactivated the wild type −1602/+20-MMP13 promoter construct, deletion of the −231/+39 bp sequence completely abolished RUNX2- and ELF3-driven MMP13 promoter transactivation. Due to other remaining upstream AP-1 binding sites, the Δ−231/−39-MMP13 construct modestly responded to AP-1 (c-Fos/c-Jun) overexpression, but the positive effect of ELF3 on AP-1-induced MMP13 activation was absent (Figure 5B), demonstrating that this proximal promoter region is responsible for ELF3-dependent EBS-AP-1 enhancement.

Next, we investigated ELF3 binding in vitro and in vivo to the ETS binding sites contained within the −231/+39 bp proximal promoter sequence. Comparative analyses of DNA sequences from multiple species using the UCSC Genome Browser (Human Assembly, March 2006) confirmed the presence of the previously described evolutionarily conserved and functional RUNX2, AP-1 and −78-bp ETS/PEA3 (labeled as B1) binding sites in the proximal MMP13 promoter (37,38). This analysis also identified a putative ETS site (B2) in tandem with the −78-bp EBS, and another upstream EBS (A), which overlaps with a negative regulatory AG-Rich Element (AGRE) (39) that is less well conserved in different species (see Fig. S4A for further details). EMSA and antibody-mediated supershift analysis showed binding of in vitro-translated ELF3 to the EBS sequences present in the proximal MMP13 promoter region (Fig. S4B). We next assessed chromatin immunoprecipitation (ChIP) in T/C-28a2 cells, in which we overexpressed a FLAG-tagged ELF3 construct. Utilizing anti-FLAG or anti-ELF3 specific antibodies and PCR primers spanning the −157/−38 bp-MMP13 promoter region, we detected the binding of ELF3 to the MMP13 proximal promoter in situ (Fig. 5C). These results are in accord with our interpretation that the EBS in the MMP13 proximal promoter region is responsible for its ELF3-driven transactivation.

To precisely identify the ELF3-responsive proximal site or sites, we generated point mutations of all three proximal EBS contained within the −231/+39 bp sequence. Reporter assays performed with the wild-type −273/+20 bp-MMP13 promoter sequence and the EBS-MMP13 mutant constructs (Fig. 5D) indicated that mutation of the A site did not affect transactivation by ELF3. Compared to the wild-type construct, the EBS-A mutant had significantly (p=0.007) increased basal promoter activity, consistent with previous reports showing inhibitory actions of the MMP13 proximal AGRE site (39,40), which overlaps with the A site. Mutation of the B2 site, though decreasing the basal promoter activity, also did not affect the ELF3-driven MMP13 promoter transactivation. In contrast, the EBS-B1 mutation, corresponding to the evolutionarily conserved ETS binding site located at −78 bp, completely abolished ELF3-driven MMP13 promoter activity. In addition, the EBS-B1 mutation also disrupted ELF3 enhancement of AP-1-driven MMP13 activity.

Importantly, whereas overexpression of either c-Fos/c-Jun, RUNX2 or ELF3 transactivated the wild type −1602/+20-MMP13 promoter construct, deletion of the −231/+39 bp sequence completely abolished RUNX2- and ELF3-driven MMP13 promoter transactivation. Due to other remaining upstream AP-1 binding sites, the Δ−231/−39-MMP13 construct modestly responded to AP-1 (c-Fos/c-Jun) overexpression, but the positive effect of ELF3 on AP-1-induced MMP13 activation was absent (Figure 5B), demonstrating that this proximal promoter region is responsible for ELF3-dependent EBS-AP-1 enhancement.

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transactivation without impeding AP-1-mediated MMP13 transactivation (Fig. S5). Taken together, these results show that ELF3 binds to and activates the proximal MMP13 promoter via a highly conserved proximal ETS binding site in a manner that may involve direct or indirect interactions with AP-1 family members.

**Contribution of MEK/ERK signaling to IL-1β-induced ELF3 binding and transactivation of the MMP13 promoter**—ETS factors can be regulated by MAPK-mediated phosphorylation (12,13), and the IL-1β induction of MMPs in chondrocytes relies, at least in part, on MAPK activation of different downstream transcription factors (2,41). Consequently, the mechanism whereby ELF3 regulates MMP-13 expression in response to IL-1β stimulation may involve not only increased ELF3 gene expression, but also modulation of its activity. To begin to address this question, we first investigated whether IL-1β stimulation induced changes in ELF3 activity by analyzing ELF3 binding to the MMP13 promoter in ChIP assays performed with immortalized chondrocytes challenged with the cytokine for 2 h. As shown in Fig. 6A, IL-1β treatment of the cells increased endogenous ELF3 binding to the MMP13 proximal promoter without significantly modifying total ELF3 protein levels (Fig. 6B), indicating that IL-1β indeed modulates ELF3 activity in chondrocytes. IL-1β-induced ELF3 binding to the endogenous MMP13 promoter correlated with the enhanced level of MMP-13 RNA in response to IL-1β in T/C-28a2 cells. In addition, ELF3 KD lead to decreased MMP-13 expression at 6 h after IL-1β stimulation (Fig. 6C), implicating ELF3 in the early activation of MMP13 transcription, in agreement with our results utilizing Elf3−/− mouse chondrocytes (Fig. 2C).

We next analyzed whether ELF3-driven MMP13 transactivation was modulated by MAPK signaling. We first performed luciferase reporter assays using cell extracts of immortalized chondrocytes co-transfected with the −1602/+20-MMP13 (Fig. 7) and −273/+20-MMP13 (not shown) reporter constructs and vector(s) expressing ELF3, p38, JNK, ERK-1, MKK-6, MKK-7 and MEK-1. As shown in Fig. 7A, MEK1/ERK1 overexpression significantly enhanced ELF3-driven MMP13 transactivation, whereas over-expressing MKK7/JNK (Fig. 7B) or MKK6/p38 (Fig. 7C) did not. Furthermore, enforced, concentration-dependent expression of MAPK phosphatase 1 (MKP1) reduced both ELF3 activation of MMP13 (Fig. 7D) and the MEK1/ERK1 enhancement of ELF3-1-driven MMP13 promoter activation (Fig. 7E). Moreover, U0126, a MEK1/2 specific pharmacological inhibitor, dose-dependently decreased MMP13 promoter activity induced by ELF3 over-expression (Fig. 7F), further indicating that MEK/ERK signaling contributes to ELF3-driven MMP13 transcription in chondrocytes.

Since IL-1β treatment induced ELF3 binding to the proximal MMP13 promoter, and MEK/ERK signaling enhanced ELF3-driven MMP13 transactivation, we next investigated if IL-1β-induced ELF3 binding to the MMP13 promoter required MEK/ERK signaling. Depending upon the structural features of each ETS factor, along with cellular and DNA sequence-specific effects, modulation of ETS transcriptional activity in response to MAPKs can involve changes in subcellular localization, unmasking of their DNA binding domains, or differential interactions with transcriptional co-activators/repressors (12,13). To determine the effect of IL-1β-dependent phosphorylation of ERK1/2, p38 and SAPK/JNK on ELF3 function, we employed the MEK1/2 specific inhibitor U0126 in T/C-28a2 cells. In agreement with previous reports in human primary (42) and immortalized chondrocytes (43), p38, SAPK/JNK or ERK1/2 phosphorylation was induced by IL-1β treatment, and pre-treatment with 2.5 µM U0126 inhibited both basal and IL-1β-induced ERK1/2 phosphorylation without affecting p38 or SAPK/JNK phosphorylation (Fig. S6). We analyzed IL-1β-induced ELF3 binding to the MMP13 promoter in ChIP assays performed in T/C-28a2 cells pre-treated with vehicle (DMSO) or 2.5 µM of U0126 for 45 min and stimulated for 2 h with 1 ng/ml of IL-1β. ChIP analysis revealed that the IL-1β-induced ELF3 binding to the proximal MMP13 promoter was significantly reduced by U0126-mediated inhibition of MEK/ERK signaling in human chondrocytes (Fig. 8). Finally, we explored whether MEK/ERK signaling requires ELF3 to drive MMP-13 gene expression. Wild type and Elf3−/− mouse primary chondrocytes were stimulated with IL-1β with or without U0126 pre-treatment (Fig. 9A). RT-qPCR analysis revealed that blocking MEK/ERK signaling significantly suppressed IL-1β-induced levels of Mmp13 mRNA in both wild type and Elf3−/− chondrocytes. Previous reports (44,45) have
shown that ELF3 over-expression or knockdown in mammary epithelial-derived cells leads to enhanced or reduced MAPK activity, respectively. However, wild-type and Elf3−/− chondrocytes did not show a significant change in IL-1β-induced ERK1/2 phosphorylation (Fig. 9B). We conclude from these results that MEK/ERK signaling also acts independently of ELF3 to induce MMP-13 gene expression in response to IL-1β.

DISCUSSION

The abnormal stress-related induction and activation of MMP13 in chondrocytes fuels the OA disease phenotype—Among the different MMPs abnormally expressed by OA chondrocytes, special attention has been focused on MMP-13 due to its potent ability to cleave type II collagen (5). Evidence has shown increased MMP-13 expression and activity in OA cartilage (7), co-localization of MMP-13-specific type II collagen cleavage products with cytokines and their receptors (4,8), OA-like changes associated with over-expression of constitutively active MMP-13 (9), and protection of the Mmp13 knockout mice against surgically induced OA (10). Consequently, numerous efforts have been directed towards a complete understanding of the mechanisms leading to the dysregulated expression of MMP-13 in OA chondrocytes. In response to a variety of stress and inflammatory insults, an array of signaling pathways becomes abnormally activated in metabolically dormant articular chondrocytes, For instance, RAS/RAF/MEK/ERK signaling has been shown to control DDR2 receptor-driven up-regulation of MMP-13 in OA disease (46). Moreover, stress-mediated activation of the NF-κB and MAPK pathways drives the induction of downstream transcriptional effectors with direct impact on chondrocyte physiology (2,41), including the inhibition of anabolic genes (27,47,48) and the exacerbated expression of matrix degradative enzymes as found in OA disease (49-52). In addition, because OA chondrocytes themselves produce pro-inflammatory factors including IL-1β, a positive feedback loop is generated, which contributes to the dysregulation of chondrocyte functions and exacerbation of cartilage erosion and loss of function (8,53).

MMP13 transcription is subject to the effects of multiple DNA binding activators—Pro-inflammatory stimuli, including IL-1β and TNFα, transactivate MMP promoters depending on the presence and availability of AP-1 and ETS elements (2,41). Similar to other MMP genes, the MMP13 promoter contains conserved ETS factor binding sites, which are adjacent to AP-1 binding sites (38). Different studies have shown that the proximal AP-1 site is crucial for both basal and cytokine-induced MMP13 transcription in response to c-Fos/c-Jun heterodimers or c-Jun/c-Jun homodimers, which can cooperate with ETS factors to activate transcription (36,38,54). In chondrocytes, the NF-κB (p65/p50) members (55,56), RUNX2 (37), HIF2α (57), C/EBPβ (58) and c-Fos/c-Jun (59) have each been implicated in the mechanisms of cytokine-induced MMP13 transcription (37,54,56,57,59-63). Activation of JNK, p38 and ERK1/2 signaling mediates MMP transcription by activating AP-1 (56) and RUNX2 (63) in addition to ETS factors, including ELK1 (64). ERK1/2 phosphorylation is increased in vivo in OA cartilage, and the MEK/ERK-induced phosphorylation of ELK1 mediates FGF-2-induced MMP-13 expression by enhancing ELK1 transcriptional activation (64). Furthermore, ETS1 over-expression leads to increased MMP-13 expression both in vitro and in vivo (65), and IFNα stimulation of primary cultures of hepatic stellate cells induces PEA3 engagement with Jak1 and Stat1 and subsequent MMP-13 expression (66). These findings indicate that the induction of MMP13 by different ETS factors is stimulus- and cell type-dependent.

ELF3 is a novel activator of MMP13 transcription in stressed chondrocytes—Our results show for the first time that ELF3 is among the primary transcription factors that activate MMP-13 transcription in response to IL-1β. Importantly, ELF3 deficiency, both by knock down in vitro and by Elf3 knockout in vivo, resulted in significantly reduced MMP-13 expression in response to IL-1β and TNFα. ELF3 over-expression transactivated the MMP13 promoter, dependent on its −78-bp (B1) evolutionarily conserved ETS site; and ELF3 acted in conjunction with AP-1 (c-Fos/c-Jun) to drive MMP13 transcription, which helps to explain the previously described molecular interplay of these cis-acting elements and their trans-acting binding factors (14,36). In addition, IL-1β stimulation enhanced ELF3 binding to the MMP13 proximal promoter and MEK/ERK signaling both enhanced ELF3-driven activation of MMP13 transcription and
participated in IL-1β-induced ELF3 binding to the promoter. However, it remains to be determined if the ELF3/AP-1 functional interplay uncovered here involves either direct or indirect interactions with AP-1 family members. Moreover, ELF3/AP-1 interaction at the MMP13 proximal promoter likely represents an important nexus for the recruitment of other critical transcriptional co-activators and chromatin-modifying proteins, which now also warrant further investigation. Although our results collectively show that ELF3 is an important new contributing factor for the control of MMP13 transcription in response to pro-inflammatory stimuli in chondrocytes, the delay in MMP13 mRNA induction in response to IL-1β or TNFα stimulation indicates that other factors, which are activated after ELF3, must work in conjunction with ELF3 to drive MMP13 transcription in response to stress-related stimuli.

ELF3 also contributes to the transcriptional activation of pro-inflammatory mediators in stressed chondrocytes—ELF3 is also known to mediate stress-related, pro-inflammatory reactions involving the repression of anabolic genes (27) and the activation of inflammatory response genes such as NOS2 and PTGS2/COX2 (25,26). ELF3 has also been reported to modulate the transcription of pro-inflammatory cytokines such as IL-6 (23). ELF3 may also activate (20,67) or repress (68) the transcription of MMPs. However, previous reports have shown no transactivating effect of ELF3 over-expression on a rat MMP3 promoter construct whereas it strongly activated a reporter construct with a multimerized stromelysin EBS (20). Indeed, our experiments confirmed that ELF3 contributes to the IL-1β-induced expression of NOS2 and PTGS2/COX2 in human chondrocytes (Fig. S3). Thus, ELF3 could mediate stress/inflammatory responses that drive MMP transcription via the direct transcriptional activation of the relevant promoters and also by other indirect mechanisms.

**ELF3 activity is post-translationally modulated by MEK/ERK signaling**— An array of post-translational modifications, including phosphorylation, glycosylation and sumoylation, can regulate ETS activity, resulting in loss of repression, increased activation, increased or decreased stability, alterations in protein-protein interaction, nuclear translocation, or enhanced DNA binding (12,13). Previous reports have demonstrated that ELF3 stability and activity are modulated by phosphorylation (44), and that stable ELF3 over-expression leads to MAPK activation (45). However, to the best of our knowledge our results provide evidence of the first functional link between MEK/ERK signaling and ELF3 activation. Initial comparison of the ELF3 structure with that of ETS1 revealed little conservation within the PNT domain (11), which contains ERK2 docking sites in both ETS1 and 2 (69), and removal of the PNT domain did not affect the ability of ELF3 to transactivate the type II TGF-β-receptor promoter (67). However, potential phospho-acceptor sites for p38, JNK, ERK and dual-specificity kinases have been identified in the ELF3 structure (11). Our results in over-expressing the relevant constitutively active MAP2K along with the relevant MAPK show that the ELF3 activity is enhanced by MEK/ERK over-expression. In accord with the latter observation, MKP1 over-expression shows that the potency of ELF3 in transactivating MMP13 also depends upon phosphorylation events. Moreover, the pharmacological inhibition of MEK/ERK in both reporter and ChIP assays reinforce the contribution of MEK/ERK to ELF3 activity, in accord with ELF3 being a downstream effector of MEK/ERK-mediated MMP-13 expression in chondrocytes. However, the precise mechanisms by which MEK/ERK modulate ELF3 activity, whether by favoring specific protein-protein interactions, increasing DNA binding affinity, or modifying protein stability, remain unclear and require further investigation.

**Linkage of abnormal levels of ELF3 with MMP13 activity in OA disease**—Because ELF3 expression is low in most non-epithelial tissues, including cartilage, and we do not detect Elf3 mRNA in the developing cartilage of mouse embryos by in situ hybridization analysis (unpublished), ELF3 is unlikely to participate in normal cartilage homeostasis. Moreover, little is known regarding in vivo contributions of ELF3 to the development and function of different tissues due to the severe defects in the gastric epithelium of Elf3 knockout mice that compromise their survival (17), and thus impede a detailed analysis over time. In that regard, the in vivo analysis of the intrinsic roles of ELF3 in normal cartilage homeostasis or in driving MMP13 expression during chondrocyte hypertrophy and endochondral ossification processes would require the use of cartilage-targeted, conditional Elf3 knockout mice, which would be necessary to dissect if the roles of ELF3 in controlling MMP13 expression are cell- or
context-specific processes. However, in both OA and RA, ELF3 expression is up-regulated in synovial tissues (22). Our results suggest that ELF3 may serve as a novel sensor in cartilage pathology, responding to and mediating mechanical stress and pro-inflammatory insults and acting as a contributing factor to OA development and/or progression by inducing *MMP13* transcription, among other downstream targets. Indeed, our results showing increased ELF3 expression in response to inflammatory stimuli *in vitro*, as well as increased ELF3 levels correlating with increased MMP-13 protein and activity in areas of cartilage degradation in human OA, are indicative of a detrimental role of ELF3. The latter fits nicely with its *in vitro* contribution to IL-1β-induced *MMP13* enhancement and *COL2A1* repression (27) in articular chondrocytes. This concept is also consistent with reports showing that abnormal ELF3 expression alters normal physiology in different scenarios, resulting in breast tumorigenesis (19,20), contributing to airway inflammation (23), or mediating angiogenesis in the setting of inflammation (24).

In summary, we have shown co-localization of ELF3 protein with MMP-13 and MMP13-cleavage products in OA cartilage, a functional role for ELF3 in IL-1β-induced MMP-13 expression in chondrocytes, and involvement of the MEK/ERK signaling in both enhancing ELF3-driven *MMP13* transactivation and ELF3 binding to the *MMP13* proximal promoter in response to IL-1β. Given the aforementioned results, we speculate that ELF3 is one of the MEK/ERK downstream effectors that induces aberrant expression and activity of MMP-13, when chondrocytes and other cell types respond to inflammation and stress. Modulation of the MEK/ERK/ELF3 axis may be a contributing factor to OA development and/or progression, and its role in determining MMP-13 expression and activity in this and other disease contexts now merits further investigation.
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**FOOTNOTES**

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FIGURE LEGENDS

FIGURE 1. ELF3 regulates IL-1β-induced MMP-13 gene expression in human primary articular chondrocytes. Chondrocytes were isolated from articular cartilage from the femoral condyles of 5 OA patients undergoing total knee replacement, and cultures at passage 1 were examined for the relative levels of (A) ELF3 and (B) MMP-13 mRNA in response to IL-1β stimulation for 2 to 24 h. The same primary chondrocytes were transfected with 50 nM of siRNA oligonucleotides against ELF3 (siELF3) or non-targeting siRNA (siCONTROL). Knock-down efficiency was assessed by (C) immunoblotting and (D) RT-qPCR at 72 h. (E) At 72 h post-transfection, the cells were stimulated with 1 ng/ml of IL-1β for 24 h and cellular MMP-13 mRNA levels were analyzed by RT-qPCR. The values were normalized to GAPDH and are shown as mean ± S.E.M. * indicates p<0.05, ** indicates p<0.01, ***indicates p<0.001; ELF3 Western blots were re-probed for β-tubulin as a loading reference control.

FIGURE 2. Effects of ELF3 on MMP-13 expression in mouse primary chondrocytes. Mouse primary chondrocytes were isolated from articular cartilage from wild type 5- to 6-day-old C57BL/6 mice and incubated with 1 ng/ml of IL-1β for the indicated times. IL-1β-induced ELF3 (A) and MMP-13 (B) mRNA levels were analyzed by RT-qPCR. (C) Mouse primary chondrocytes isolated from articular cartilage from wild type (WT) and Elf3 knockout (KO) mice were incubated with 1 ng/ml of IL-1β for the indicated times. Total RNAs were isolated and MMP-13 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E.M; * indicates p<0.05, *** indicates p<0.001

FIGURE 3. ELF3 protein levels co-localize with increased MMP-13 protein levels and activity in human OA cartilage. Sections of knee articular cartilage obtained from OA patients undergoing total knee replacement (n=10) were Safranin O stained (a, b, c) and subjected to evaluation according to the OARSI cartilage OA histopathology grading system. Representative sections are shown. Successive sections were subjected to immunohistochemical staining using antibodies against ELF3 (a.1, b.1 and c.1), MMP-13 (a.2, b.2 and c.2) and C1,2C (a.3, b.3 and c.3). Isotype-matched IgG was used as negative control (not shown). Squares indicate areas selected for higher magnification photomicrographs. Arrows indicate some of the areas with positive immunostaining. Note the more diffuse, matrix C1,2C positive immunostaining with increased OARSI score. Original magnifications were 40X (a, b, c) and 100X (a.1, b.1, c.1, a.2, b.2, c.2, a.3, b.3, and c.3).

FIGURE 4. ELF3 transactivates MMP13 and enhances the AP-1-driven MMP13 activation. (A) Schematic representation of the MMP13-Luciferase reporter constructs utilized in this study (not scaled). T/C-28a2 cells were co-transfected with 325 ng of the -1602/+20 bp-MMP13 promoter and (B) 12.5, 25 or 50 ng of expression vector encoding human ELF3; (C) 25 ng of expression vector encoding human ELF3, alone or in co-transfection with 25 (+) or 50 (++) ng of ELF5 or EHF expression vector; (D) 25 ng of each expression vector encoding ELF3, cFos and cJun alone or together. Luciferase activities are shown as fold-change; protein input was used to normalize luciferase activities in Fig. 4D. *indicates p<0.05, *** indicates p<0.001

FIGURE 5. ELF3 binds to the proximal MMP13 promoter and transactivates MMP13 via an evolutionarily conserved proximal ETS binding site (EBS). T/C-28a2 cells were co-transfected with 325 ng of luciferase reporter constructs spanning (A) –1602/+20, –1007/+20 or –273/+20 bp of the MMP13 promoter along with 25 ng of ELF3 expression vector or (B) wild type –1602/+20 bp or Δ–231/–39 MMP13 reporter construct and 25 ng of expression vector encoding ELF3, RUNX2, cFos, or cJun. Luciferase activities in Fig. 5B were normalized to the protein
input. (C) T/C-28a2 cells were transfected with ELF3-FLAG or the empty pCDNA3.1 expression vectors for 18 h. Chromatin was cross-linked and enzymatically sheared, and after reverse cross-linking of the DNA-protein complexes, the pre-cleared lysates were incubated overnight at 4°C with antibodies (upper panel) against FLAG (+) or normal rabbit IgG (-), or (lower panel) against ELF3 (+) or normal rabbit IgG (-). The human MMP13 promoter region was PCR-amplified using primers spanning from −157 to −38 bp, and the PCR products were resolved on a 2.5% agarose gel. Data are representative of two independent experiments performed in duplicate. (D) T/C-28a2 cells were co-transfected with 25 ng of the ELF3 expression vector and 325 ng of the wild-type −267/+27 bp-MMP13 promoter sequence or sequences containing point mutations of the proximal A, B1 or B2 ETS binding sites. * indicates p<0.05

FIGURE 6. IL-1β enhances the endogenous ELF3 binding to the proximal MMP13 promoter. (A) After overnight incubation in serum-free medium, T/C-28a2 cells were incubated with 1 ng/ml of IL-1β for 2 h. After stimulation, the chromatin was crosslinked and enzymatically sheared, and after reverse cross-linking of the DNA-protein complexes, the pre-cleared lysates were incubated with antibodies against ELF3 (+) or normal IgG (-) overnight at 4°C. The human MMP13 promoter region was PCR-amplified using primers spanning from −157 to −38 bp, and the PCR products were resolved on a 2.5% agarose gel. (B) ELF3 protein levels were analyzed by Western blotting using cell lysates prepared from T/C-28a2 cells stimulated with vehicle or 1 ng/ml of IL-1β for 2 h. (C) T/C-28a2 cells were transfected with 50 nM of siRNA oligonucleotides against ELF3 (siELF3) or non-targeting siRNA (siCONTROL). At 72 h post-transfection, cells were stimulated with 1 ng/ml of IL-1β for 6 h. Total RNA was isolated and MMP-13 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E.M * indicates p<0.05

FIGURE 7. MEK/ERK overexpression enhances the ELF3-driven MMP13 promoter activation. T/C-28a2 cells were co-transfected with 325 ng of the -1602/+20 bp MMP13 promoter, 25 ng of ELF3 expression vector and (A) 25 ng of each expression vector encoding ERK1 and MEK1; (B) 25 ng of each expression vector encoding JNK and MKK7; (C) 25 ng of each expression vector encoding p38 and MKK6; (D) increasing amounts (0, 12.5 ng, 25 ng, and 50 ng) of expression vector encoding MKP1 and (E) 25 ng of expression vector encoding MEK1 or ERK1 and 50 ng of expression vector encoding MKP1; or (C) treated with vehicle (DMSO) or the indicated concentrations of U0126 at 4 h after transfection, followed by 20 h of incubation after addition of the inhibitor. Luciferase activities are shown as fold-change with untreated controls set at 1.0. * indicates p<0.05, ** indicates p<0.01, *** indicates p<0.001

FIGURE 8. MEK/ERK signaling is required for the IL-1β-induced ELF3 binding to the proximal MMP13 promoter. After overnight incubation in serum-free medium, T/C-28a2 cells were pre-treated with vehicle (DMSO) or 2.5 µM of U0126 for 45 min before incubation with 1 ng/ml of IL-1β in the presence of inhibitor. At 2 h after IL-1β stimulation, chromatin was cross-linked and enzymatically sheared, and after reverse cross-linking of the DNA-protein complexes, the pre-cleared lysates were incubated with antibodies against ELF3 or normal IgG overnight at 4°C. The ELF3 binding to the human MMP13 promoter region was analyzed using primers spanning from −157 to −38 bp of the promoter region by (A) qPCR and (B) conventional PCR, with PCR products resolved on a 2.5% agarose gel. GAPDH gene specific primers were used as a negative control. * indicates p<0.05

FIGURE 9. Effects of ELF3 on MEK/ERK-dependent MMP-13 expression in mouse primary chondrocytes. Mouse primary chondrocytes were isolated from articular cartilage from wild type (WT) C57BL/6 and Elf3 knockout (KO) 5- to 6-day-old mice. (A) After overnight
incubation in serum-free medium, cells were pre-treated with vehicle (DMSO) or the indicated concentrations of U0126 for 45 min before incubation with 1 ng/ml of IL-1β in the presence of the inhibitor. At 24 h after IL-1β stimulation, total RNAs were isolated and MMP-13 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E.M. (B) Phospho-ERK1/2 (P-ERK1/2) and total ERK1/2 (ERK1/2) protein levels were analyzed by Western blotting using cell lysates prepared from wild type and Elf3 knockout primary chondrocytes stimulated with 1 ng/ml of IL-1β for the indicated times after overnight incubation in serum-free conditions. Western blots were re-probed for β-tubulin as a loading reference control. * indicates p<0.05 vs. the IL-1β-stimulated controls
| Description         | Primer sequences                                                                 |
|---------------------|----------------------------------------------------------------------------------|
| Common Forward (XmaI) | 5'-TAACATAACCCGGGTGTACTACTCTCTGCT-3’                                            |
| Common Reverse (Xhol)| 5'-GCCAAGCTTTAGATCTCGAGTCTAGATTG-3’                                            |
| EBS-A mutant        | Forward: 5'-CACACTCGGGGAtAAAAGAAAAAGTCGCC-3’                                    |
|                     | Reverse: 5'-GGCGACTTTTTCTTTTTTatatCTCCCCGAGTGTG-3’                              |
| EBS-B1 mutant       | Forward: 5'-TTCAAGTGACTAtAAGTGAAACCTATCC-3’                                     |
|                     | Reverse: 5'-GGATAGGTTTCCACTTTatatTAGTCACTTGAA-3’                                |
| EBS-B2 mutant       | Forward: 5'-AAGTGACTAGGATGtACACCTATCCATA-3’                                     |
|                     | Reverse: 5'-TATGGATAGGTGtaCACCTCCTAGTCACTT-3’                                   |
Figure 1

A

ELF3 (fold change)

IL1β 0 2h 4h 6h 12h 24h

***

B

MMP13 (fold change)

IL1β 0 2h 4h 6h 12h 24h

***

C

ELF3

β-tubulin

siCONTROL siELF3

D

ELF3 mRNA (relative units)

siCONTROL siELF3

* 

E

MMP13 (fold change)

CONTROL IL-1β

*
Figure 2

A

ELF3 (fold change)

IL-1β 0 1h 2h 6h 12h 24h

B

MMP13 (fold change)

IL1β 0 1h 2h 6h 12h 24h

C

MMP13 (fold change)

IL1β 0 2h 6h 24h

WT KO

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Figure 5

A

**Figure Legend:**
- **A:** Bar graphs showing the relative luciferase activity for different promoter regions (-1602/+20, -1007/+20, -273/+20) of the ELF3 promoter. The graphs compare the activity with and without pCDNA3.1 Empty and ELF3-FLAG constructs. 

B

**Figure Legend:**
- **B:** A bar graph depicting the relative luciferase activity for different promoter regions with various transcription factors (ELF3, RUNX2, Fos/Jun) and deletions (-1602/+20MMP13, Δmut(-231/-39)MMP13).

C

**Figure Legend:**
- **C:** Western blot showing the protein levels of pCDNA3.1 Empty and ELF3-FLAG constructs with specific antibodies (αFLAG, αELF3). The blot includes an input control (INPUT).

D

**Figure Legend:**
- **D:** Bar graphs illustrating the relative luciferase activity for different regions and mutations (Δmut(-231/-39)MMP13, EBS-A, EBS-B1mut, EBS-B2mut).
Figure 6

A

| IP: αELF3 | CONTROL | IL-1β | INPUT |
|-----------|---------|-------|-------|
|           | -       | -     | CONT. IL-1β |

B

C

![MMP13 (fold change)](http://www.jbc.org/)

- siCONTROL
- siELF3

MMP13 (fold change)

CONTROL | IL-1β
---|---
1 | 5

* 

beta-tubulin

ELF3
Figure 7

A

B

C

D

E

F

Relative Luciferase Activity

ELF3
ERK1
MEK1

Relative Luciferase Activity

ELF3
JNK
MKK7

Relative Luciferase Activity

ELF3
p38
MKK6

Relative Luciferase Activity

MKP1(ng) 12.5 25 50

Relative Luciferase Activity

pCDNA3.1
ELF3
MKP1

Relative Luciferase Activity

pCDNA3.1
ELF3
MKP1

Relative Luciferase Activity

U0126(µM) 1.25 2.5 5

***

**

*

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**Figure 8**

**A**

![Bar graph showing relative expression of MMP13 and GAPDH with and without IL-1β treatment.]

**B**

![Western blots showing expression of ELF3 and IgG with and without IL-1β treatment.]

*Note: Images are not rendered in the text format.*
Figure 9

A

MMP13 (fold change)

WT
KO

IL-1β
U0126 (µM)

B

WT
KO

IL-1β (min)

0 5 15 45 0 5 15 45

P-ERK1/2
ERK1/2
β-tubulin

* * *
E74-like factor 3 (ELF3) impacts on matrix metalloproteinase 13 (MMP13) transcrip
tional control in articular chondrocytes under pro-inflammatory stress
Miguel Otero, Darren A. Plumb, Kaneyuki Tsuchimochi, Cecilia L. Dragomir, Ko Hashimoto, Haibing Peng, Eleonora Olivotto, Michael Bevilacqua, Lujian Tan, Zhiyong Yang, Yumei Zhan, Peter Oettgen, Yefu Li, Kenneth B. Marcu and Mary B. Goldring

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