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E74-like Factor 3 (ELF3) Impacts on Matrix Metalloproteinase 13 (MMP13) Transcriptional Control in Articular Chondrocytes under Proinflammatory Stress*[S]

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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 E26 transformation-specific sequence binding sites 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 E26 transformation-specific sequence binding site at position −78 bp and by cooperating with AP-1. In addition, ELF3 binding to the proximal *MMP13* promoter is enhanced by 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 MMP13 protein and activity in human osteoarthritic cartilage. These studies define a novel role for ELF3 as a procatabolic factor that may contribute to cartilage remodeling and degradation by regulating *MMP13* gene transcription.

The matrix metalloproteinases (MMPs)[3] 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, proinflammatory 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 MMP13 (collagenase 3) plays a very prominent role here. MMP13 preferentially and more potently cleaves type II collagen compared with other collagenases (5–7). Moreover, MMP13 levels and activity are enhanced in OA cartilage, associated with degenerative changes and co-localizing with MMP13-specific type II collagen cleavage products, inflammatory cytokines, and their receptors (4, 8). Further, *in vivo* evidence has shown OA changes in transgenic mice overexpressing constitutively active MMP13 (9), whereas *Mmp13* knock-out 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 MMP13 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-

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[3] The abbreviations used are: MMP, matrix metalloproteinase; OA, osteoarthritis; ETS, E26 transformation-specific sequence; OARSI, Osteoarthritis Research Society International; RT-qPCR, real-time quantitative RT-PCR; EBS, ETS binding site(s); AGRE, AG-rich element.

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 procatabolic/antianabolic 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 proinflammatory mediator in OA disease.

Herein, we provide evidence for a novel role of ELF3 as a regulatory component that drives the abnormal expression of MMP13 under proinflammatory 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 MMP13 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 MMP13 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 and patient consent. Immediately after surgery, the cells were isolated by sequential digestion with Pronase (Promega) and collagenase P (Promega), cultured to confluence in Dulbecco's modified Eagle's medium (DMEM)/F-12 containing 10% fetal bovine serum (FBS), and used for experimental purposes at passage one. Human immortalized T/C-28a2 chondrocytes were cultured in DMEM/Ham's F-12 containing 10% FBS, as described previously (27). *Elf3* knock-out mice (17) were obtained from Dr. Melanie A. Pritchard (Monash University, Clayton, Victoria, Australia). Primary mouse chondrocytes were isolated as described (29) from 5–6-day-old wild type (C57BL/6) or *Elf3* knock-out mouse articular cartilage. Passage 0 chondrocytes were seeded in DMEM/F-12 containing 10% FBS, allowed to reach confluence, trypsinized, and seeded in 6-well plates in DMEM/F-12 containing 10% FBS for experimental purposes. All experiments involving IL-1β and TNFα (R&D Systems) stimulation were performed in serum-free conditions after overnight starvation.

siRNA Transfection—Human primary or immortalized chondrocytes were transfected, as described previously (28). Briefly, 2.5×10^5 cells were seeded 24 h before transfection in 6-well plates in DMEM/F-12 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 RT-PCR (RT-qPCR) Analysis—Total RNA was isolated using the RNeasy Plus minikit (Qiagen), and 150 ng were reverse transcribed, as described (28). Amplifications were carried out using SYBR Green I-based real-time PCR on the Opticon 2 real-time PCR detector system (Bio-Rad), as described (28), using the PCR primers and conditions indicated in supplemental 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× radioimmune precipitation assay buffer (Santa Cruz Biotechnology, Inc.) 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 polyvinylidine fluoride (PVDF) membranes using a semidry transfer system (Bio-Rad). Membranes were blocked with 5% nonfat milk in Tris-buffered saline with 0.1% Tween 20 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 Biosciences).

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

TABLE 1
PCR mutagenesis primers
The mutated EBS sites are indicated in boldface type, with lowercase indicating the mutation site.

Description	Primer sequences
Common forward (XmaI)	5′-TAACATAACCCGGGTGTACTACTCTCTGCT-3′
Common reverse (XhoI)	5′-GCCAAGCTTACTTAGATCTCGAGTCTAGATTG-3′
EBS-A mutant	Forward: 5′- CACACTCGGGAG**atAA**AAAGAAAAAGTCGCC-3′
	Reverse: 5′-GGCGACTTTTTCTTT**TTat**CTCCCGAGTGTG-3′
EBS-B1 mutant	Forward: 5′- TTCAAGTGACTA**atAA**GTGGAAACCTATCC-3′
	Reverse: 5′-GGATAGGTTTCCAC**TTat**TAGTCACTTGAA-3′
EBS-B2 mutant	Forward: 5′-AAGTGACTAGGAAGT**GtcA**ACCTATCCATA-3′
	Reverse: 5′-TATGGATAGGT**TgaC**ACTTCCTAGTCACTT-3′

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 (EBS) (designated 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 XmaI 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 XmaI and XhoI restriction sites utilized for the first PCR. 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 2.5×10^4 cells/cm^2 in DMEM/F-12 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 the 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 IL-1β for 2 h, with or without a 45-min pretreatment with U0126 (2.5 μM; 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 250–1000 bp. Chromatin was precleared by incubation with 25 μl of protein G magnetic beads and 5 μg of nonspecific (control) rabbit IgG (Cell Signaling) for 2 h at 4 °C with rotation. After preclearing 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 precleared 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′-CAATGGTGAGTCATCACTTATGG-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 (Bio-Rad) 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 with Institutional Review Board approval from the medial tibial plateaus of the knee joints of 10 OA patients undergoing total knee replacement. After fixation, decalcification, and paraffin embedding, sections of 6 μm were cut, deparaffinized in xylene, and rehydrated through an ethanol series. Every 10th section was collected for Safranin O/Fast green staining, and the OA stage was graded independently by two blinded observers according to the 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 Biotechnology, Inc.) was used in place of the primary antibodies. The sections were counterstained with methyl green, and the Vectastain ABC

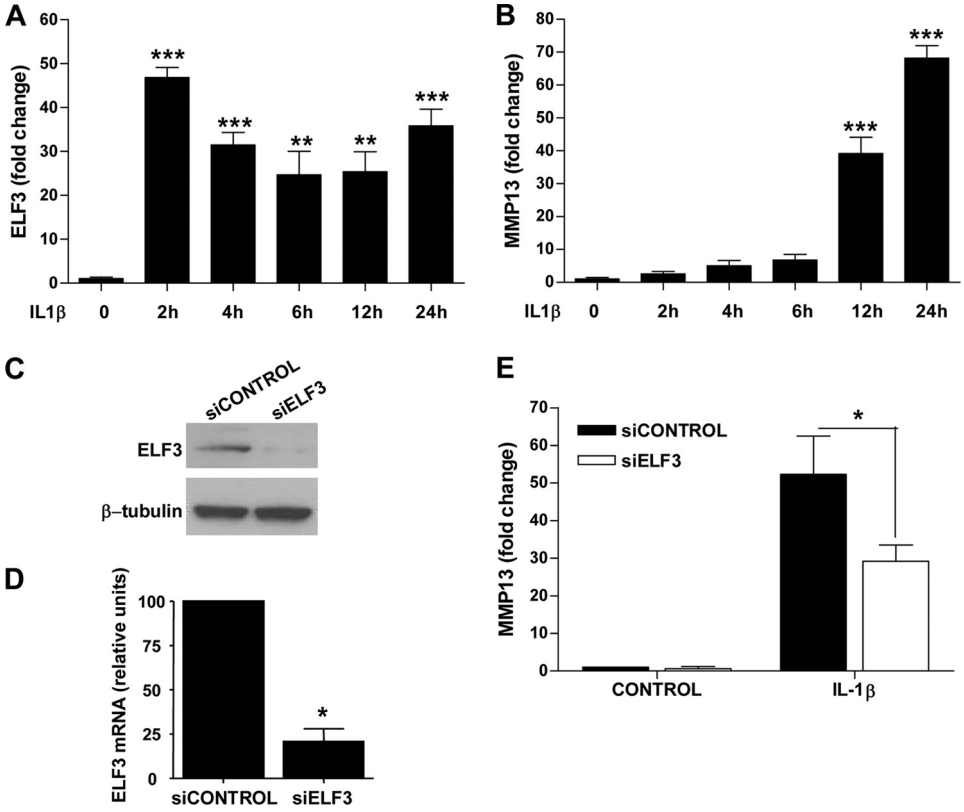

FIGURE 1. **ELF3 regulates IL-1β-induced *MMP13* gene expression in human primary articular chondrocytes.** Chondrocytes were isolated from articular cartilage from the femoral condyles of five OA patients undergoing total knee replacement, and cultures at passage 1 were examined for the relative levels of (*A*) ELF3 and (*B*) MMP13 mRNA in response to IL-1β stimulation for 2–24 h. The same primary chondrocytes were transfected with 50 nM siRNA oligonucleotides against ELF3 (siELF3) or non-targeting siRNA (siCONTROL). Knockdown efficiency was assessed at 72 h by immunoblotting (*C*) and RT-qPCR (*D*) at 72 h post-transfection, the cells were stimulated with 1 ng/ml IL-1β for 24 h, and cellular MMP13 mRNA levels were analyzed by RT-qPCR (*E*). The values were normalized to GAPDH and are shown as mean ± S.E. (*error bars*). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ELF3 Western blots were reprobed for β-tubulin as a loading reference control.

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 ± standard error (S.E.) of at least three independent experiments. Statistical analysis was performed by analysis of variance 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 an NF-κB (p65/p50)-dependent manner (22, 23, 27), and in previous work, we showed that ELF3 mediates the antianabolic 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 screen of RNAs isolated from primary human chondrocytes transfected with siRNA-ELF3 or non-targeting siRNA oligonucleotides, which showed that MMP13 was among 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. 1, *A* and *B*). Like that of MMP13, the IL-1β-dependent expression profiles of MMP1, -3, and -9 were also enhanced at later times, whereas COL2A1 mRNA was suppressed (supplemental Fig. S1). Importantly, IL-1β-induced MMP13 mRNA in primary human OA chondrocytes was significantly down-modulated by ELF3 knockdown (*KD*) (Fig. 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 remaining stable up to 24 h (Fig. 2, *A* and *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 with their wild type counterparts (Fig. 2*C*), 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 (supplemental Fig. S2*A*). TNFα-induced Mmp13 mRNA levels were

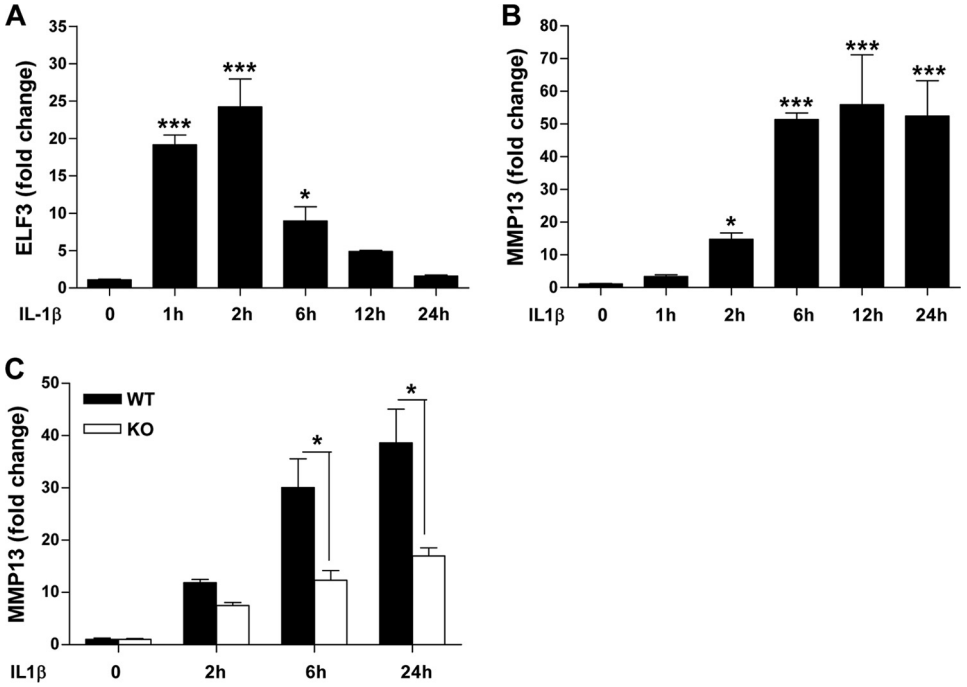

FIGURE 2. **Effects of ELF3 on MMP13 expression in mouse primary chondrocytes.** Mouse primary chondrocytes were isolated from articular cartilage from wild-type 5–6-day-old C57BL/6 mice and incubated with 1 ng/ml IL-1β for the indicated times. IL-1β-induced ELF3 (*A*) and MMP13 (*B*) mRNA levels were analyzed by RT-qPCR. *C*, mouse primary chondrocytes isolated from articular cartilage from wild-type (*WT*) and *Elf3* knock-out (*KO*) mice were incubated with 1 ng/ml IL-1β for the indicated times. Total RNAs were isolated, and MMP13 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E. (*error bars*); *, $p < 0.05$; ***, $p < 0.001$.

suppressed in *Elf3*$^{-/-}$ mouse chondrocytes compared with their wild-type counterparts (supplemental Fig. S2*B*). 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 (supplemental 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 with wild type mouse chondrocytes, whereas ELF3 did not impact the IL-1β-induction of Mmp2, -3, and -9 mRNA levels (supplemental Fig. S3).

ELF3 Protein Levels Are Enhanced in Human OA Cartilage and Co-localize with Increased MMP13 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 MMP13 proteins in different sections of human OA cartilage. Immunohistochemical analysis revealed enhanced ELF3 immunostaining in OA cartilage, in conjunction with increased MMP13 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 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 Fig. 4*A*) 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 (Fig. 4*B*). 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 (Fig. 4*C*).

ETS transcription factors can work as co-factors of other transcriptional regulators (12, 13), and the *MMP13* promoter contains evolutionarily conserved proximal 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 Fig. 4*D*, 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. 5*A*), 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

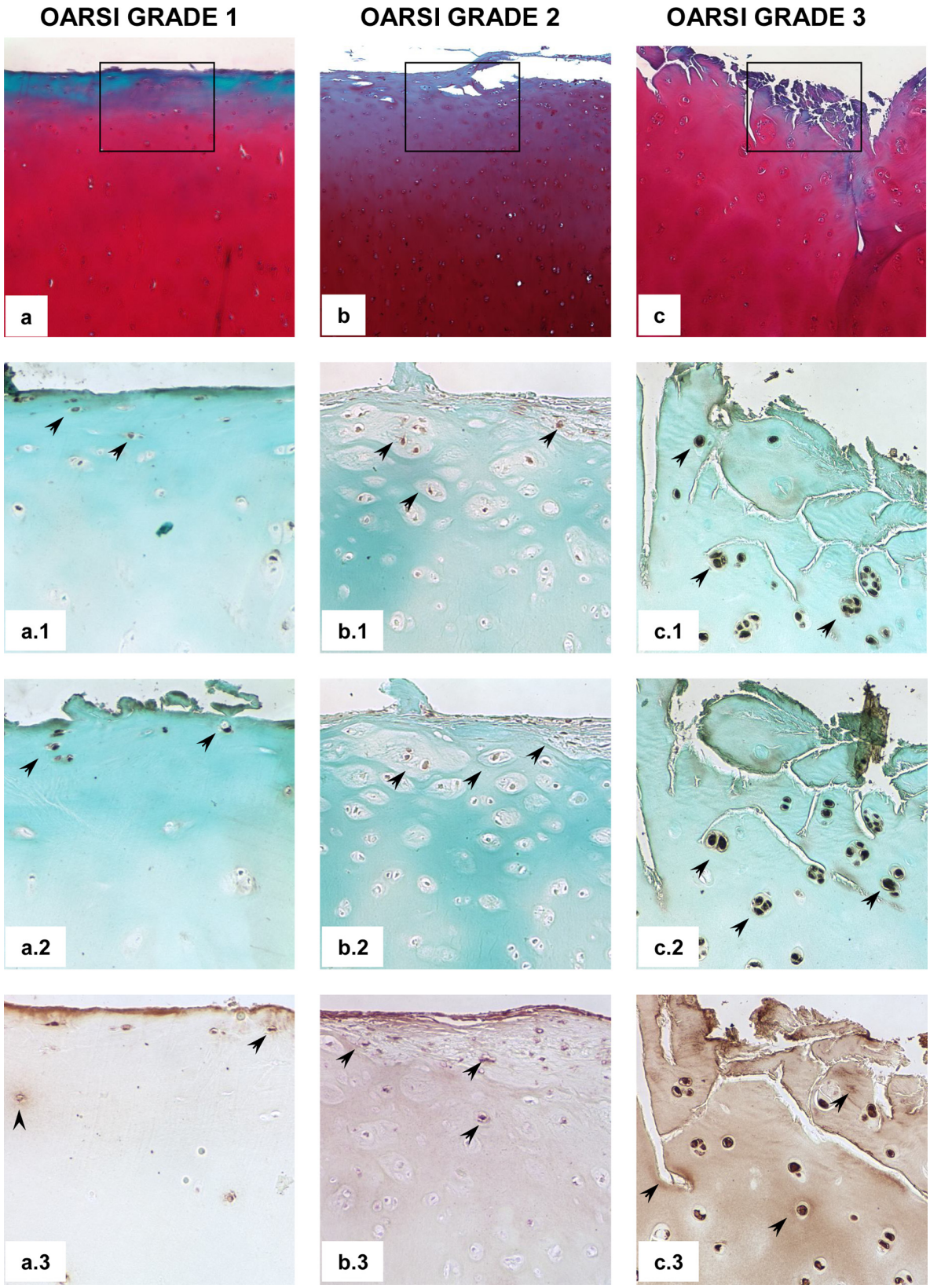

FIGURE 3. **ELF3 protein levels co-localize with increased MMP13 protein levels and activity in human OA cartilage.** Sections of knee articular cartilage obtained from OA patients undergoing total knee replacement ($n = 10$) were Safranin O-stained (*a*, *b*, and *c*) and subjected to evaluation according 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*), MMP13 (*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. The *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 40× (*a*, *b*, and *c*) and 100× (*a.1*, *b.1*, *c.1*, *a.2*, *b.2*, *c.2*, *a.3*, *b.3*, and *c.3*).

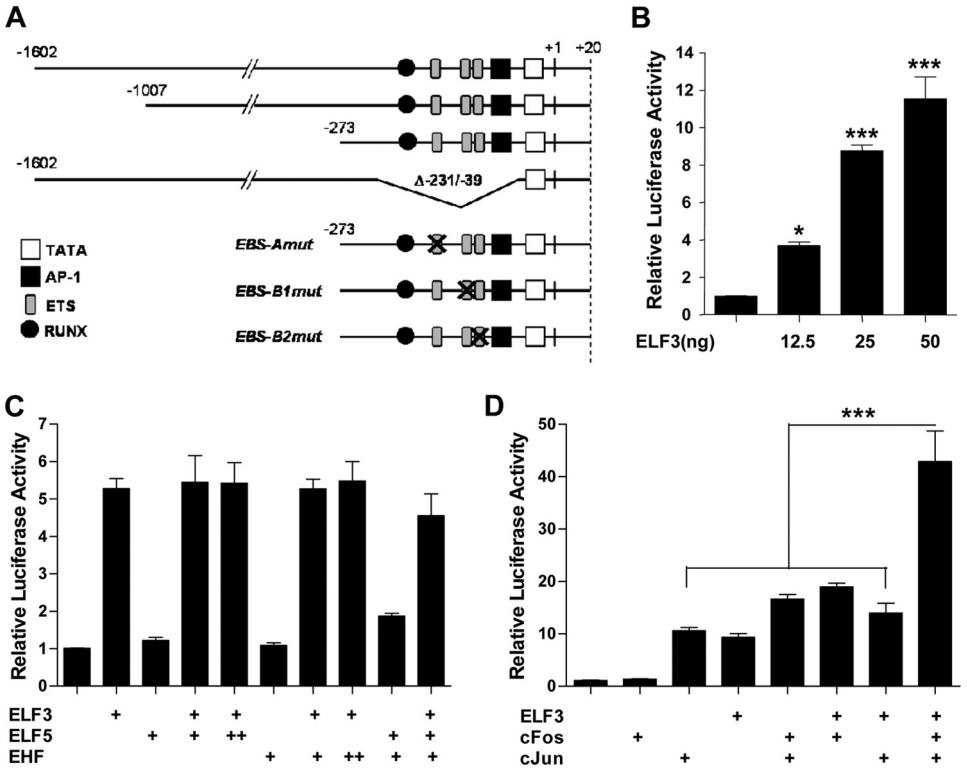

FIGURE 4. **ELF3 transactivates *MMP13* and enhances the AP-1-driven *MMP13* activation.** Schematic representation (not scaled) of the *MMP13*-luciferase reporter constructs utilized in this study (*A*). T/C-28a2 cells were co-transfected with 325 ng of the −1602/+20 bp *MMP13* promoter and 12.5, 25,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

the −231/−39 sequence (denoted as Δ−231/−39-*MMP13* in Fig. 4*A*). 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 (Fig. 5*B*), 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 University of California Santa Cruz 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 supplemental Fig. S4*A* 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 (supplemental Fig. S4*B*). We next assessed 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. 5*C*). 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. 5*D*) indicated that mutation of the A site did not affect transactivation by ELF3. Compared with 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, although 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,

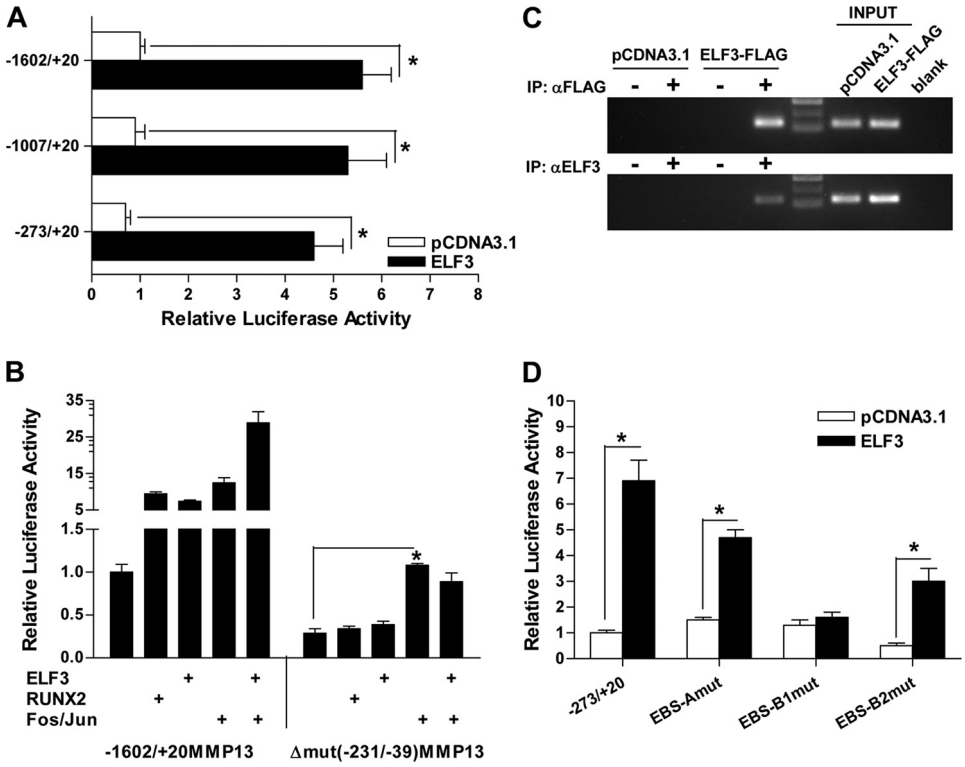

FIGURE 5. **ELF3 binds to the proximal *MMP13* promoter and transactivates *MMP13* via an evolutionarily conserved proximal EBS.** T/C-28a2 cells were co-transfected with 325 ng of luciferase reporter constructs spanning −1602/+20, −1007/+20, or −273/+20 bp of the *MMP13* promoter along with 25 ng of ELF3 expression vector (*A*) or wild type −1602/+20 bp or Δ−231/−39 *MMP13* reporter construct and 25 ng of expression vector encoding ELF3, RUNX2, c-Fos, or c-Jun (*B*). Luciferase activities in *B* 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 precleared lysates were incubated overnight at 4 °C with antibodies against FLAG (+) or normal rabbit IgG (−) (*top*) or against ELF3 (+) or normal rabbit IgG (−) (*bottom*). 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. *, $p < 0.05$. *Error bars*, S.E. *IP*, immunoprecipitation.

the EBS-B1 mutation also disrupted ELF3 enhancement of AP-1-driven *MMP13* transactivation without impeding AP-1-mediated *MMP13* transactivation (supplemental 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 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 MMP13 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. 6*A*, IL-1β treatment of the cells increased endogenous ELF3 binding to the *MMP13* proximal promoter without significantly modifying total ELF3 protein levels (Fig. 6*B*), 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 MMP13 RNA in response to IL-1β in T/C-28a2 cells. In addition, ELF3 knockdown led to decreased MMP13 expression at 6 h after IL-1β stimulation (Fig. 6*C*), implicating ELF3 in the early activation of *MMP13* transcription, in agreement with our results utilizing *Elf3*$^{-/-}$ mouse chondrocytes (Fig. 2*C*).

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. 7*A*, MEK1/ERK1 overexpression significantly enhanced ELF3-driven *MMP13* transactivation, whereas overexpressing MKK7/JNK (Fig. 7*B*) or MKK6/p38 (Fig. 7*C*) did not. Furthermore, enforced, concentration-dependent expression of MAPK phosphatase 1 (MKP1) reduced both ELF3 activation of *MMP13* (Fig. 7*D*) and the MEK1/ERK1 enhancement of ELF3-1-driven *MMP13* promoter activation (Fig. 7*E*). Moreover, U0126, a MEK1/2-specific pharmacological inhibitor, dose-dependently decreased *MMP13* promoter activity induced by ELF3 overexpression (Fig. 7*F*), further indicating that MEK/ERK signaling contributes to ELF3-driven *MMP13* transcription in chondrocytes.

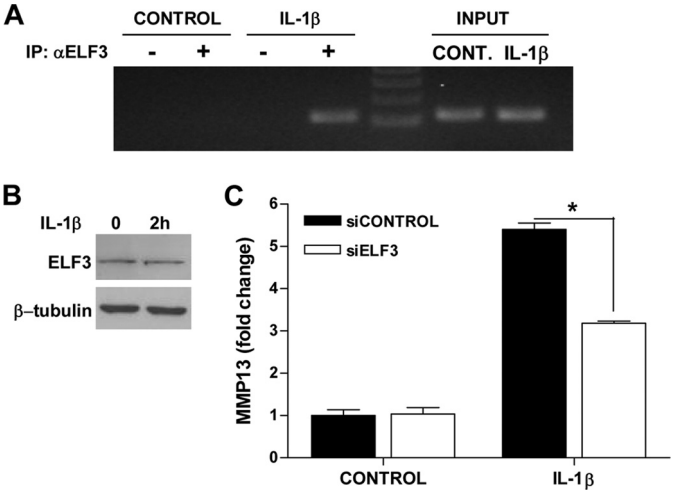

FIGURE 6. **IL-1β enhances the endogenous ELF3 binding to the proximal *MMP13* promoter.** *A*, after overnight incubation in serum-free medium, T/C-28a2 cells were incubated with 1 ng/ml IL-1β for 2 h. After stimulation, the chromatin was cross-linked and enzymatically sheared, and after reverse cross-linking of the DNA-protein complexes, the precleared 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 IL-1β for 2 h. *C*, T/C-28a2 cells were transfected with 50 nM siRNA oligonucleotides against ELF3 (*siELF3*) or non-targeting siRNA (*siCONTROL*). At 72 h post-transfection, cells were stimulated with 1 ng/ml IL-1β for 6 h. Total RNA was isolated, and MMP13 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E. *, $p < 0.05$. *IP*, immunoprecipitation.

Because 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 pretreatment with 2.5 μM U0126 inhibited both basal and IL-1β-induced ERK1/2 phosphorylation without affecting p38 or SAPK/JNK phosphorylation (supplemental Fig. S6). We analyzed IL-1β-induced ELF3 binding to the *MMP13* promoter in ChIP assays performed in T/C-28a2 cells pretreated with vehicle (DMSO) or 2.5 μM 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 MMP13 gene expression. Wild type and *Elf3*$^{-/-}$ mouse primary chondrocytes were stimulated with IL-1β with or without U0126 pretreatment (Fig. 9*A*). 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 overexpression or knockdown in mammary epithelium-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. 9*B*). We conclude from these results that MEK/ERK signaling also acts independently of ELF3 to induce MMP13 gene expression in response to IL-1β.

DISCUSSION

Abnormal Stress-related Induction and Activation of MMP13 in Chondrocytes Fuels OA Disease Phenotype—Among the different MMPs abnormally expressed by OA chondrocytes, special attention has been focused on MMP13 due to its potent ability to cleave type II collagen (5). Evidence has shown increased MMP13 expression and activity in OA cartilage (7), co-localization of MMP13-specific type II collagen cleavage products with cytokines and their receptors (4, 8), OA-like changes associated with overexpression of constitutively active MMP13 (9) and protection of the *Mmp13* knock-out mice against surgically induced OA (10). Consequently, numerous efforts have been directed toward a complete understanding of the mechanisms leading to the dysregulated expression of MMP13 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 MMP13 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 proinflammatory 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 Effects of Multiple DNA Binding Activators—Proinflammatory 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)

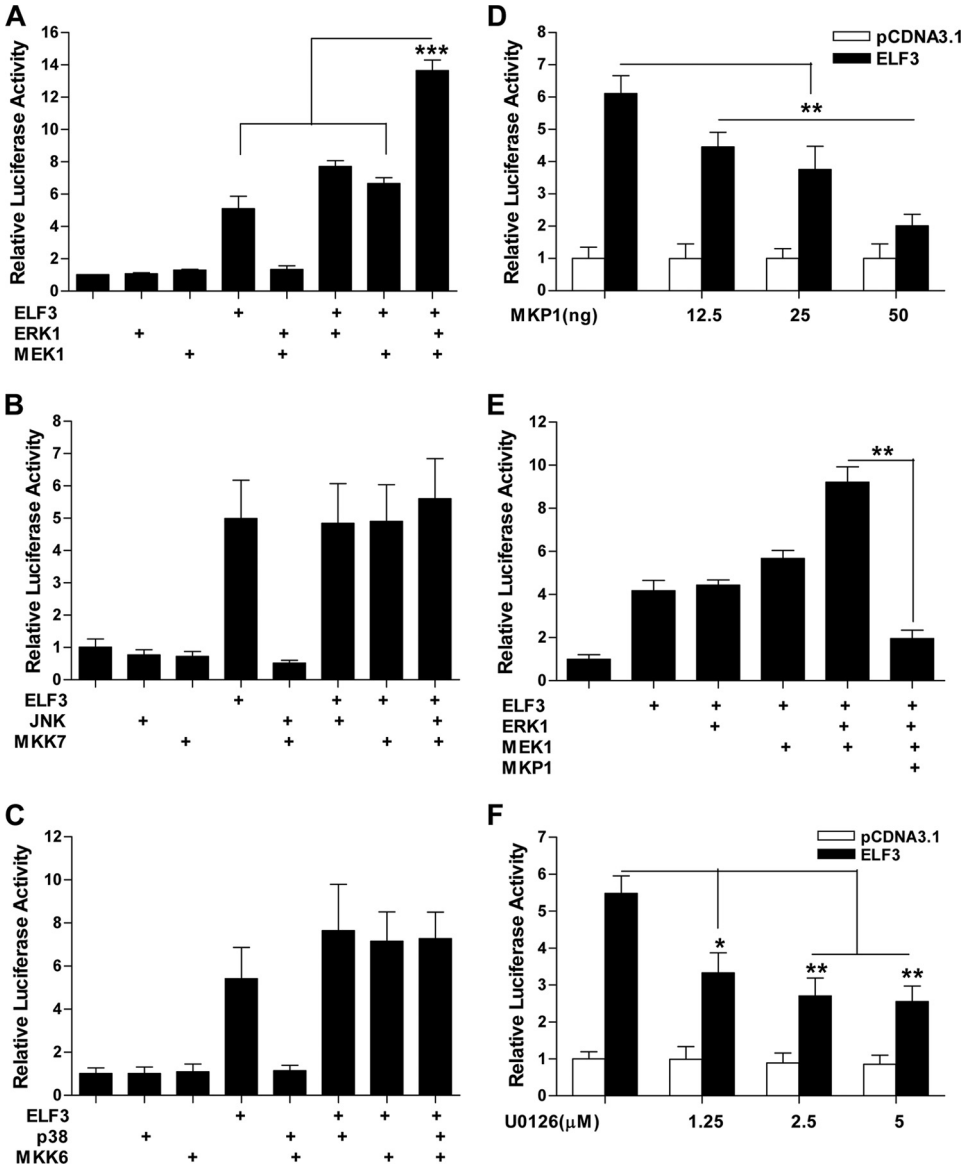

FIGURE 7. **MEK/ERK overexpression enhances the ELF3-driven *MMP13* promoter activation.** T/C-28a2 cells were co-transfected with 325 ng of the −1602/+20 bp *MMP13* promoter, 25 ng of ELF3 expression vector, and (*A*) 25 ng of each expression vector encoding ERK1 and MEK1; (*B*) 25 ng of each expression 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. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$. *Error bars*, S.E.

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 MMP13 expression by enhancing ELK1 transcriptional activation (64). Furthermore, ETS1 overexpression leads to increased MMP13 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 MMP13 expression (66). These findings indicate that the induction of MMP13 by different ETS factors is stimulus- and cell type-dependent.

ELF3 Is 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 MMP13 transcription in response to IL-1β. Importantly, ELF3 deficiency, both by knockdown *in vitro* and by *Elf3* knock-out *in vivo*, resulted in significantly reduced MMP13 expression in response to IL-1β and TNFα. ELF3 overexpression 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 mem-

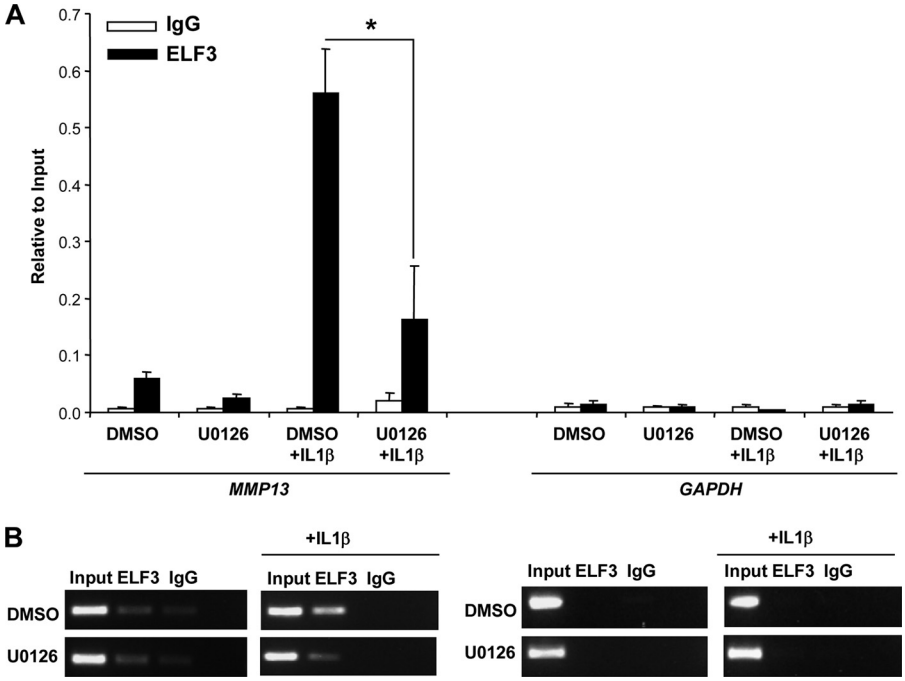

FIGURE 8. **MEK/ERK signaling is required for the IL-1β-induced ELF3 binding to the proximal *MMP13* promoter.** After overnight incubation in serum-free medium, T/C-28a2 cells were pretreated with vehicle (DMSO) or 2.5 μM U0126 for 45 min before incubation with 1 ng/ml IL-1β in the presence of inhibitor. 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 precleared 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 quantitative PCR (*A*) and conventional PCR (*B*), with PCR products resolved on a 2.5% agarose gel. GAPDH gene-specific primers were used as a negative control. *, $p < 0.05$. *Error bars*, S.E.

bers. Moreover, ELF3/AP-1 interaction at the *MMP13* proximal promoter probably 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 proinflammatory 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 Transcriptional Activation of Proinflammatory Mediators in Stressed Chondrocytes—ELF3 is also known to mediate stress-related, proinflammatory 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 proinflammatory 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 overexpression 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 (supplemental 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 overexpression 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 phosphoacceptor sites for p38, JNK, ERK, and dual specificity kinases have been identified in the ELF3 structure (11). Our results in overexpressing the relevant constitutively active MAP2K along with the relevant MAPK show that the ELF3 activity is enhanced by MEK/ERK overexpression. In accord with the latter observation, MKP1 overexpression 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 reinforces the contribution of MEK/ERK to ELF3 activity, in accord with ELF3 being a downstream effector of MEK/ERK-mediated MMP13 expression in chondrocytes. However, the precise mechanisms by which MEK/ERK modulate ELF3 activity, whether by favoring specific

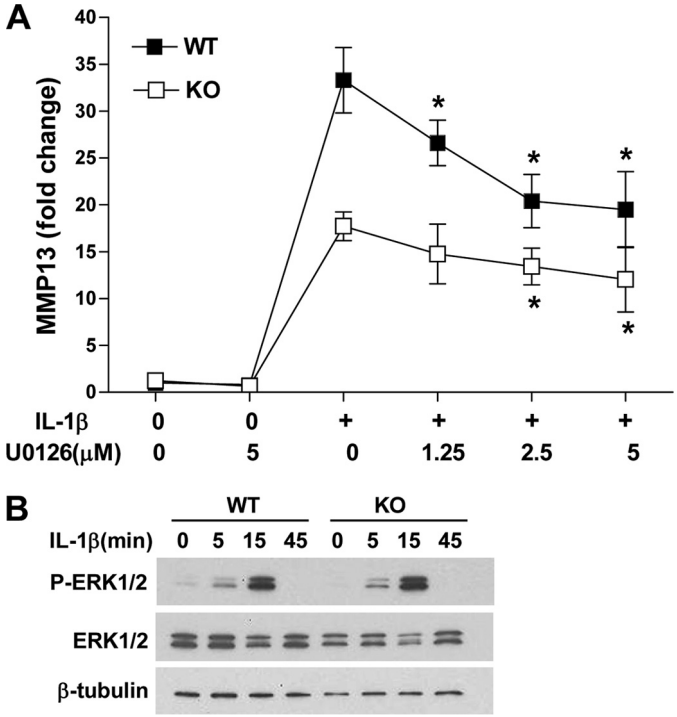

FIGURE 9. **Effects of ELF3 on MEK/ERK-dependent MMP13 expression in mouse primary chondrocytes.** Mouse primary chondrocytes were isolated from articular cartilage from wild type (*WT*) C57BL/6 and *Elf3* knock-out (*KO*) 5–6-day-old mice. *A*, after overnight incubation in serum-free medium, cells were pretreated with vehicle (DMSO) or the indicated concentrations of U0126 for 45 min before incubation with 1 ng/ml IL-1β in the presence of the inhibitor. At 24 h after IL-1β stimulation, total RNAs were isolated, and MMP13 mRNA was analyzed by RT-qPCR. Each value was normalized to GAPDH in the same sample and shown as mean ± S.E. (*error bars*). *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* knock-out primary chondrocytes stimulated with 1 ng/ml IL-1β for the indicated times after overnight incubation in serum-free conditions. Western blots were reprobed for β-tubulin as a loading reference control. *, $p < 0.05$ *versus* the IL-1β-stimulated controls.

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,[4] 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* knock-out 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* knock-out mice, which would be necessary to determine 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 proinflammatory 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 MMP13 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 MMP13 and MMP13 cleavage products in OA cartilage, a functional role for ELF3 in IL-1β-induced MMP13 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 MMP13, 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 MMP13 expression and activity in this and other disease contexts now merits further investigation.

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[4] D. A. Plumb, M. Otero, C. L. Dragomir, and M. B. Goldring, unpublished observations.

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