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ORIGINAL ARTICLE

TGFβ1-Induced Transglutaminase-2 Triggers Catabolic Response in Osteoarthritic Chondrocytes by Modulating MMP-13

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Abstract

BACKGROUND: Transforming growth factor beta 1 (TGFβ1) plays an essential role in maintaining cartilage homeostasis. TGFβ1 is known to upregulate anabolic processes in articular cartilage, but the role of TGFβ1 in chondrocyte catabolism remains unclear. Thus, we examined whether TGFβ1 increases catabolic processes in the osteoarthritic joint via transglutaminase 2 (TG2). In this study, we investigated whether interplay between TGFβ1 and TG2 mediates chondrocyte catabolism and cartilage degeneration in osteoarthritis.

METHODS: To investigate the role of TGFβ1 and TG2 in osteoarthritis, we performed immunostaining to measure the levels of TGFβ1 and TG2 in 6 human non-osteoarthritic and 16 osteoarthritic joints. We conducted quantitative reverse transcription polymerase chain reaction and western blot analysis to investigate the relationship between TGFβ1 and TG2 in chondrocytes and determined whether TG2 regulates the expressions of matrix metalloproteinase (MMP)-13, type II, and type X collagen. We also examined the extent of cartilage degradation after performing anterior cruciate ligament transection (ACLT) and destabilization of the medial meniscus (DMM) surgery in TG2 knock-out mice.

RESULTS: We confirmed the overexpression of TGFβ1 and TG2 in human osteoarthritic cartilage compared with non-osteoarthritic cartilage. TGFβ1 treatment significantly increased the expression of TG2 via p38 and ERK activation. TGFβ1-induced TG2 also elevated the level of MMP-13 and type X collagen via NF-κB activation in chondrocytes. Cartilage damage after ACLT and DMM surgery was less severe in TG2 knock-out mice compared with wild-type mice.

CONCLUSION: TGFβ1 modulated catabolic processes in chondrocytes in a TG2-dependent manner. TGFβ1-induced TG2 might be the therapeutic target for treating cartilage degeneration and osteoarthritis.

Keywords TGF-β · Transglutaminase-2 · Articular chondrocyte · Cartilage · Catabolism

1 Introduction

Osteoarthritis (OA) is a whole joint disease characterized by synovial fibrosis, subchondral bone changes, osteophyte formation, and articular cartilage degradation. The progressive destruction of cartilage contributes to physical disability and discomfort which is a primary feature of OA. Cartilage degeneration is indicative of altered metabolism of chondrocytes. In osteoarthritic cartilage, chondrocytes upregulate a variety of catabolic enzymes including matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), which are capable of degrading the extracellular matrix and are proposed to be important mediators of cartilage destruction [1, 2]. However, the signaling mechanism behind upregulation of catabolic factors in OA is not completely understood.

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Transforming growth factor-β (TGF-β) is a pleiotropic cytokine that plays an important role in regulating joint homeostasis. TGFβ1 is a critical factor in modulating chondrocyte metabolism and maintaining the structural integrity of cartilage [3]. TGFβ1 plays a role in chondrogenesis and terminal differentiation of chondrocytes. Altered TGFβ1 signaling has been reported in OA patients [4, 5]. The ratio of TGF-β receptors or activin receptor-like kinases (ALKs) is changed in the osteoarthritic joint, showing an increase in ALK5 receptor expression over ALK1 expression [4]. Activation of ALK5 phosphorylates Smad2/3, which inhibits hypertrophy in chondrocytes, while ALK1 phosphorylates Smad1/5/8, promoting chondrocyte hypertrophy, a characteristic observed in OA [6, 7]. However, the underlying mechanism behind how TGFβ1 contributes to the progression of OA remains unclear.

Transglutaminase 2 (TG2) is a multifunctional protein that catalyzes transamidation reactions by crosslinking glutamine and lysine residues [8]. Elevated level of TG2 in the synovial fluid is proposed to correlate with OA severity [9]. Accumulation of TG2 in articular cartilage is associated with calcification and chondrocyte maturation [10]. Inhibition of endogenous TG2 increased glycosaminoglycan content of chondrocyte [11]. These findings suggest TG2 may contribute to OA, but the role of TG2 in the development of OA is yet to be elucidated.

In this study, we aimed to determine whether TGFβ1 and TG2 modulate catabolic activity in the osteoarthritic joint and contribute to the disease progression.

2 Materials and methods

2.1 Cell isolation and culture from human osteoarthritic joint

Human articular cartilage, synovium, fat pad and meniscus from patients undergoing total knee arthroplasty were obtained. This study followed the guidelines of the 1975 Helsinki Declaration, revised in 2000. This research has been approved by the IRB of Seoul national university hospital. (IRB No. 1510-077-711) Patients gave informed consent for donation of tissue for research purposes. Osteoarthritic cartilage tissues were obtained from patients who underwent total knee replacement for medial compartment dominant knee. Non-osteoarthritic cartilage tissues were separated from the patients who underwent tumor removal in knee joint, and were used as a control group.

Tissue was finely minced and digested with 0.025% collagenase (Sigma-Aldrich, St. Loise, MO, USA) in high glucose DMEM (Gibco, Grand Island, NY, USA) with 1% antibiotic–antimycotic solution (Gibco) and incubated on an orbital shaker overnight at 37 °C with 5% CO_2. Digested material was filtered using 70-μm strainer. 1×10^6 cells were seeded in each 150 mm dish and medium was changed after 3 days. Cells were cultured in high glucose DMEM containing 10% FBS (Gibco) and 1% antibiotic–antimycotic solution at 37 °C with 5% CO_2.

2.2 Histology and immunohistochemistry

Human and murine knee joint tissues were fixed in 4% paraformaldehyde for 16 h, dehydrated with graded concentrations of ethanol solutions and embedded in paraffin. Sections (5-mm) were stained with Safranin-O/Fast Green and hematoxylin and eosin (H&E). For immunostaining experiments, paraffin-embedded sections were deparaffinized with xylene and dehydrated. Sections were then reacted with 3% H_2O_2, processed with hyaluronidase and incubated with 10% FBS to block non-specific binding. Sections were incubated with primary antibody TG2 (Abcam, Cambridge, UK) diluted in 4% BSA for 1 h at 37 °C.

2.3 Immunofluorescence and image acquisition

Human chondrocytes grown on glass coverslips were fixed in 4% paraformaldehyde, permeabilized with 0.5% Triton X-100 and incubated with 2.5% BSA to block non-specific binding. Cells were incubated overnight at 4 °C with primary antibodies to TG2 and p65 (Cell Signaling Technology, Danvers, MA, USA), and with secondary antibodies conjugated to peroxidase diluted in 1% BSA for 1 h at room temperature. Nuclei were counterstained with DAPI (Thermo Fisher Scientific, Waltham, MA, USA). Coverslips were mounted and visualized with a Leica TCS SP8 confocal microscope.

2.4 Enzyme-linked immunosorbent assay (ELISA)

The concentration of TGFβ1 in synovial fluid was measured using ELISA kit (R&D system, Minneapolis, MN, USA). Total TGFβ1 levels were measured by activating all latent TGFβ1 in each group by acid activation method involving 15 min acidification with 1 N HCl followed by adding equal volume of 1.2 N NaOH/0.5 M HEPES for neutralization. Activated sample was diluted and read from standard curve. Each test group was added to TGFβ1 microplate, conjugated to TGFβ1, and incubated with substrate solution containing color reagent. Each sample was read at 450-nm using a microplate reader.

2.5 SiRNA transfection

Chondrocytes were transfected with TG2 siRNA [5′-CAGTTCGAGGATGGAATCCTGGATA-3′ for TG2 siRNA (#1); 5′-TCACACAGTGCCTAGACTA(dTdT)-3′ for TG2 siRNA (#2)] by Lipofectamine 2000 according to manufacturer's instructions (Invitrogen, Carlsbad, CA, USA). In short, chondrocytes were seeded in 6-well plate, grown to be 80% confluent, and transfected with TG2 siRNA and lipofectamine complex for 6 h. Cells were harvested after 48 h and used for analysis.

2.6 Reverse transcription-polymerase chain reaction

Total RNA was extracted from chondrocytes and synovial cells using RNeasy Mini kit (Qiagen, Hilden, Germany) and reverse transcription was conducted using cDNA synthesis kit (Fermentas Life Sciences). Real-time polymerase chain reaction was performed for amplification of TG2 (forward: 5′-TCAACTGCAACGATGACCAGG-3′ and reverse: 5′-TGTTCTGGTCATGGGCCGAG-3′) and GAPDH (forward: 5′-ATTGTTGCCATCAATGACCC-3′ and reverse: 5′-AGTAGAGGCAGGGATGATGTT-3′) for 30 cycles using GoTaq® qPCR Master Mix (Promega, Madison, WI, USA). Gene expression was normalized to GAPDH and determined using $\Delta\Delta C_T$ method.

2.7 Western blot analysis

Chondrocytes were lysed with RIPA buffer (Thermo Fisher Scientific). Protein concentration was measured using BCA assay (Pierce Manufacturing, Appleton, WI, USA), separated by SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked with 5% skim milk in TBST for 1 h at room temperature and probed with primary antibodies phosphorylated-ERK, ERK, phosphorylated-p38, p38, phosphorylated-JNK, JNK, MMP-13 (Cell Signaling Technology, Danvers, MA, USA), and TG2 (Abcam) overnight. Proteins were visualized using secondary antibodies conjugated with horseradish peroxidase.

2.8 Mice

We used male C57BL/6J mice and TG2 knock-out (TG2−/−) mice. Generation of TG2−/− mice has been previously described [12]. TG2−/− mice were backcrossed 12 times on a C57BL/6J background [13]. All animal experiments were performed in line with protocol approved by Institutional Animal Care and Use Committee.

2.9 Surgical OA induction

Both anterior cruciate ligament transection (ACLT) and destabilization of the medial meniscus (DMM) were performed on wild-type (WT) C57BL/6J mice and TG2−/− mice aged 10 weeks. All surgeries were carried out on the same day. Mice were anesthetized before surgery and maintained under anesthesia with 2% isoflurane. Incision was made over the medial patella with a #11 blade. After exposing the joint capsule, anterior cruciate ligament and medial meniscus were transected. Knee joint was rinsed with saline before closing the incision with vicryl suture. At 4 weeks after surgery, mice were euthanized by inhalation of CO_2 and murine knee joints were fixed in 4% paraformaldehyde for 16 h on a shaker at 4 °C. Collected knee joints were used for western blot analysis and Safranin-O/Fast Green staining. The OA research society international (OARSI) cartilage histopathology grading system was used to compare the groups [14].

2.10 Statistical analysis

Statistical analysis was conducted using one-way ANOVA or Student's *t*-test. Findings were considered to be statistically significant when p value < 0.05.

3 Results

3.1 Elevated levels of TGFβ1 and TG2 in OA human articular cartilage

First, we examined the levels of TGFβ1 and TG2 in 16 human osteoarthritic knee joints as compared to 6 non-osteoarthritic joints. The baseline characteristics of included subjects are shown in Table 1. Mean age of OA patients was 70.0 (standard deviation [SD], 9.1) years, 87.5% were female, and a mean BMI was 28.6 (SD, 3.0) kg/m^2. Patients with OA were significantly older, and had higher BMI as compared to subjects without OA.

The level of TGFβ1 was significantly higher approximately four-fold in the synovial fluid of OA patient compared to that of non-OA subjects (Fig. 1A; $p < 0.05$). Immunohistochemistry staining revealed that TG2 expressions were significantly enhanced especially in the superficial zone of osteoarthritic cartilage where the erosive damages are prominent (Fig. 1B). Further, the elevated level of TG2 was found in the synovium and fat pad of OA patient, suggesting TG2 is abundantly expressed in the osteoarthritic joint (Fig. 1C).

Table 1 Baseline characteristics of study subjects

Category	Patients with osteoarthritis joint (n = 16)	Patients with non-osteoarthritis joint (n = 6)	*p* value
Age (years)	70.0 (± 9.1)	46.2 (± 18.0)	0.021
Female	14 (87.5%)	3 (50%)	0.062
BMI (kg/m^2)	28.6 (± 3.0)	25.3 (± 3.6)	0.040

BMI, body mass index

Values are in mean (± standard deviation) or number of patients (%)

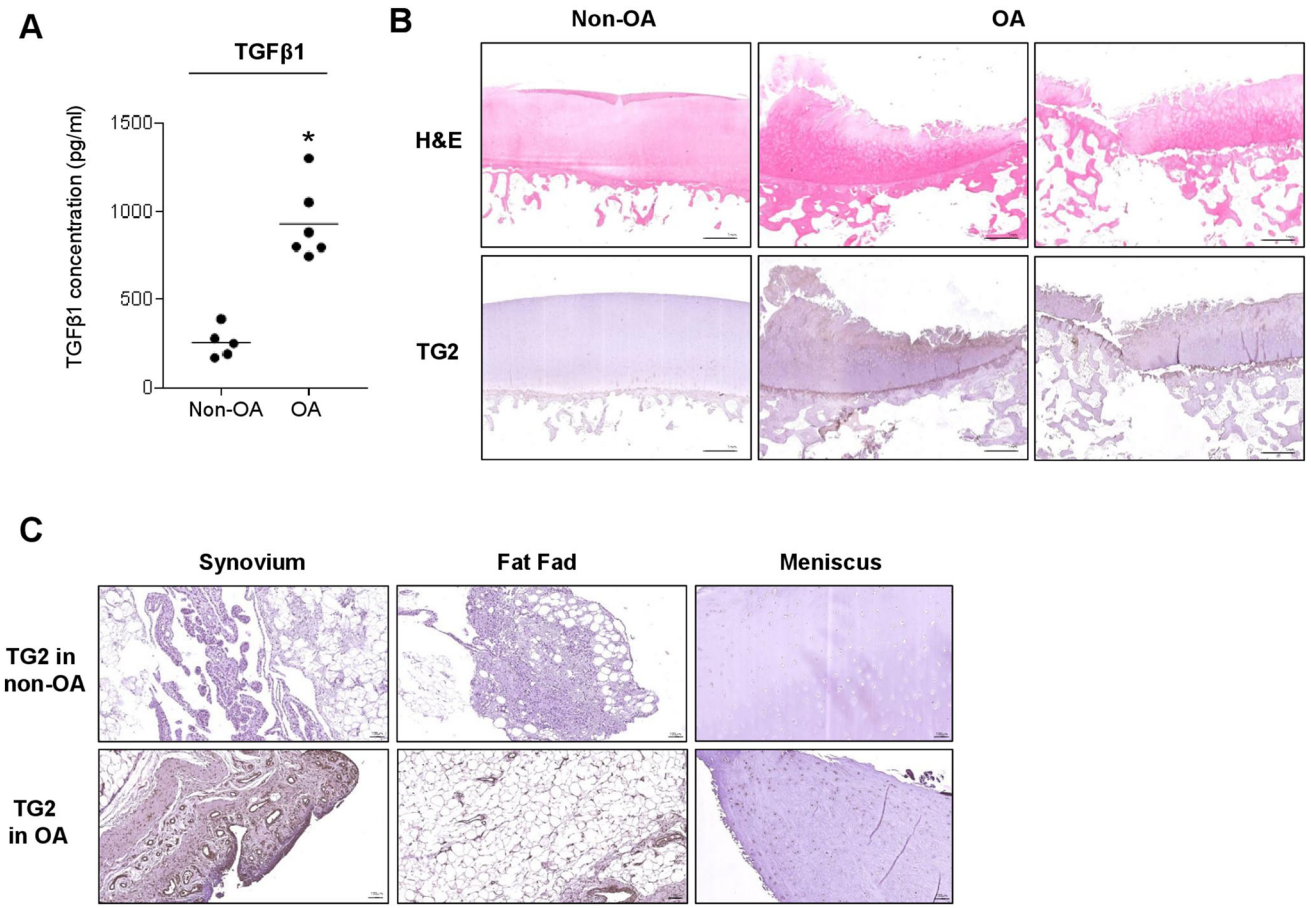

Fig. 1 Expression of TGFβ1 and TG2 were upregulated in the articular joint of OA patient. **A** Circulating level of TGFβ1 in synovial fluid of non-OA and OA patient. The horizontal line indicates the mean level of TGFβ1. The error bars represent the SD. **B** Top, H&E staining of articular cartilage of non-OA and OA patient. Bottom, TG2 immunostaining of articular cartilage of non-OA and OA patient. *$p < 0.05$ **C** TG2 immunostaining of synovium, fat pad and meniscus of non-OA and OA patient

3.2 TGFβ1-induced TG2 expression was specific to chondrocytes

Having confirmed the involvement of elevated TGFβ1 and TG2 activities in the osteoarthritic joint, we next tested whether TGFβ1 treatment alters TG2 expression in chondrocytes and synovial cells. Time- and dose-dependent treatment of TGFβ1 resulted in changes in the amount of TG2 expressed in chondrocytes as demonstrated by quantitative reverse transcription polymerase chain reaction (RT-PCR) (Fig. 2A) and western blot analysis (Fig. 2B). Upregulation of TG2 mRNA was apparent 24 h after TGFβ1 treatment (Fig. 2C; $p < 0.05$). The expression of TG2 mRNA increased up to around 200-fold after 48 h of TGFβ1 treatment at 10 ng/mL (Fig. 2C; $p < 0.05$). The protein level of TG2 also increased in a dose-and time-dependent manner, confirming TGFβ1-induced TG2 upregulation in chondrocytes (Fig. 2D). In synovial cells,

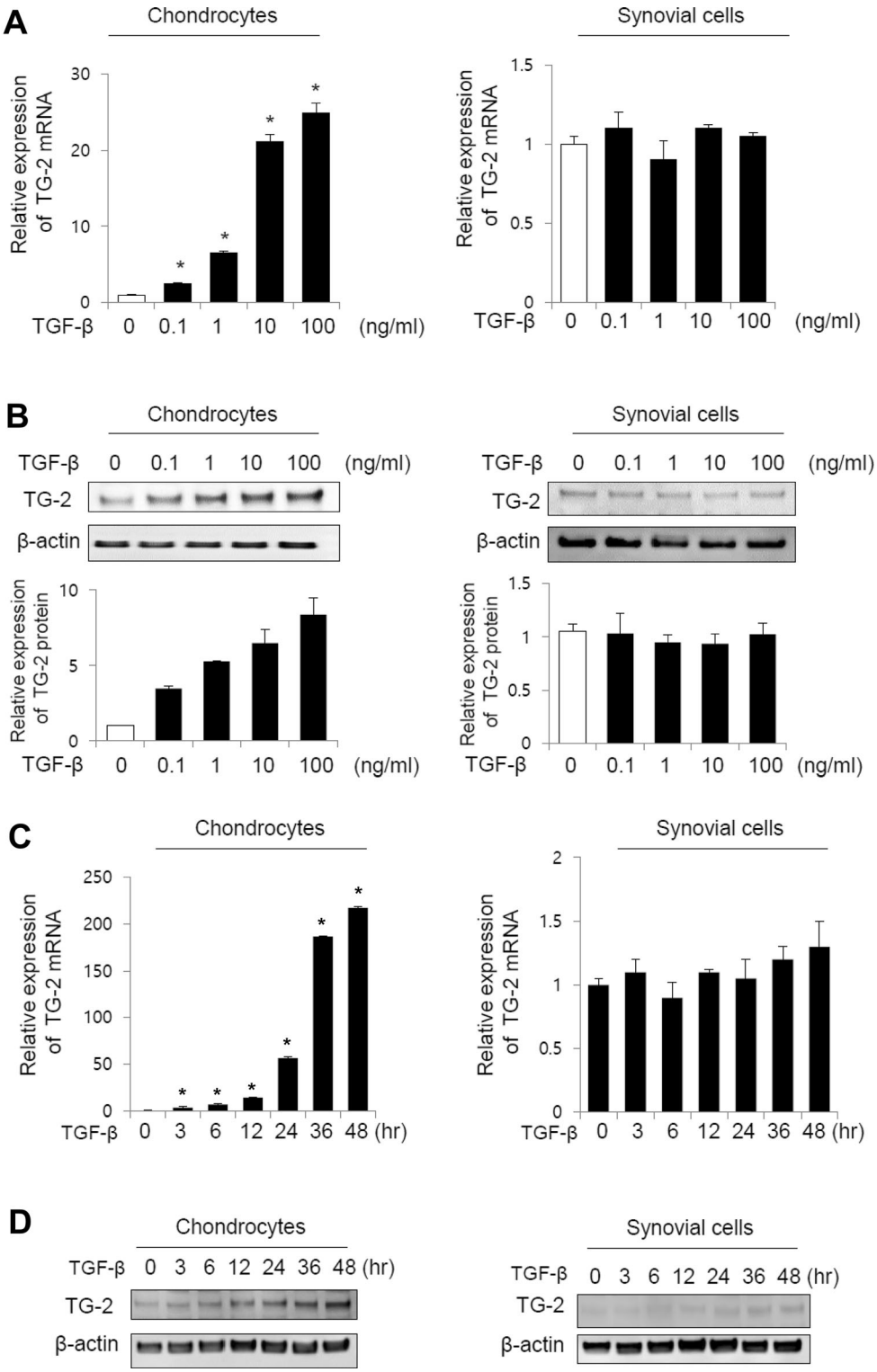

Fig. 2 Treatment of TGFβ1 induced the expression of TG2 in chondrocytes by ERK and p38 phosphorylation. **A**, **B** TG2 mRNA and protein expression was measured by quantitative RT-PCR and western blot analysis in chondrocytes and synovial cells treated with increasing concentrations of TGFβ1 (0.1 to 100 ng/mL) for 24 h. The experiment was repeated three times. The data are expressed as the mean ± standard deviation. $^{*}p < 0.05$ **C**, **D** The mRNA and protein levels of TG2 were measured by qRT-PCR and western blot analysis in chondrocytes and synovial cells treated with 10 ng/mL of TGF-β at the indicated times (0 to 48 h). The experiment was repeated three times. The data are expressed as the mean ± standard deviation. $^{*}p < 0.05$

the mRNA and protein levels of TG2 remained stable despite increasing doses of TGFβ1 and increasing time of TGFβ1 treatment (Fig. 2). TGFβ1-regulated TG2 induction was specific to chondrocytes.

3.3 TGFβ1 upregulated TG2 expression by ERK/p38 MAPK activation

Next, we investigated the associated signaling pathway of TGFβ1-induced TG2 enhancement in chondrocytes.

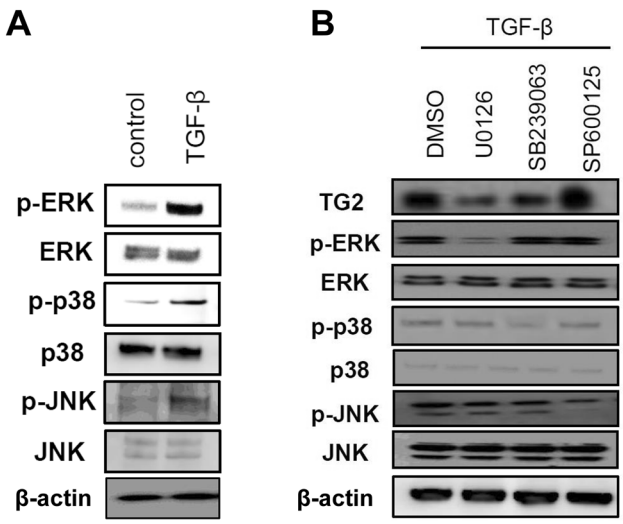

Fig. 3 TGFβ1 upregulated TG2 expression by ERK/p38 MAPK activation. **A** Phosphorylated and total protein level of ERK, p38 and JNK was measured by western blot analysis in chondrocytes treated with TGFβ1 (10 ng/mL). The experiment was repeated three times. **B** The protein level of TG2 and phosphorylated and total protein levels of ERK, p38 and JNK were measured by western blot analysis in chondrocytes treated with TGFβ1 (10 ng/mL) and respective MAPK inhibitors. The experiment was repeated three times

Western blot analysis showed that the phosphorylated levels of ERK, p38, and JNK were enhanced in chondrocytes by TGFβ1 treatment for 24 h (Fig. 3A). We also pretreated chondrocytes with U0126, SB239063, and SP600125, which inhibits ERK, p38, and JNK, respectively, followed by TGFβ1 treatment for 24 h. Inhibition of ERK phosphorylation significantly reduced the TG2 expressions, and inhibition of p38 phosphorylation showed the similar effect but to a lesser extent (Fig. 3B). Collectively, inhibition of phosphorylated ERK and p38, but not JNK, significantly reduced the protein level of TG2 in chondrocytes, suggesting ERK and p38 mediates TGFβ1-induced TG2 expressions.

3.4 TGFβ1-induced TG2 modulated type X collagen and MMP-13 levels by activating NF-κB signaling

We investigated whether TG2 expression modulates the collagen content of articular cartilage. Increased protein expressions of type II and type X collagen were observed in chondrocytes of TGFβ1 treatment for 24 h (Fig. 4A). To determine the role of TG2 in mediating this effect, we assessed whether TG2 inhibition affects TGFβ1-induced expressions of type II and type X collagen. TG2 siRNA transfection significantly reduced the induction of type X collagen, suggesting that the upregulation of type X collagen by TGFβ1 is dependent to TG2 (Fig. 4B). The inhibition of TG2 showed no significant change in the level of type II collagen in chondrocytes (Fig. 4B).

To test whether TGFβ1-induced TG2 expression contributes to degeneration of cartilage, we assessed the level of MMP-13 and type X collagen in chondrocytes. The protein level of MMP-13 and type X collagen increased in response to TGFβ1 treatment. TG2 inhibitor (cystamine) also suppressed the induction of MMP-13 and type X collagen in chondrocytes (Fig. 4C).

Further, we performed *in vivo* murine study inducing OA performing combining ACLT and DMM. We found that the level of MMP-13 and type X collagen in chondrocytes was suppressed in TG2−/− mice as compared to that in WT mice, indicating that the absence of TG2 is associated with an attenuated expression of MMP-13 and type X collagen (Fig. 4D).

To identify the signaling mechanism involved in TG2-mediated induction of MMP-13, we examined the role of NF-κB, a major catabolic pathway in OA. Inhibition of NF-κB by Bay11-7082 reduced the level of MMP-13 and type X collagen in a dose-dependent manner (0 to 25 μM) of TGFβ1 treatment in chondrocytes (Fig. 4E). Next, we tested the involvement of TG2 in TGFβ1-induced activation of NF-κB pathway in chondrocytes by observing the localization of p65, a subunit of NF-κB. We observed nuclear localization of p65 in chondrocytes treated with 10 ng/ml of TGFβ1 for 24 h, indicating NF-κB pathway activates upon treatment of TGFβ1 (Fig. 4F). Treatment of cystamine, TG2 inhibitor, led to cytosolic localization of p65, suggesting that the activation of TGFβ1-induced NF-κB pathway is dependent toTG2 (Fig. 4F). Together, our results suggest that TGFβ1-induced TG2 increases MMP-13 and type X collagen levels in chondrocytes by activating NF-κB pathway.

3.5 The absence of TG2 attenuated cartilage degeneration in mice

Our data suggests that TG2 contributes to loss of cartilage and induces hypertrophy in chondrocytes. To confirm this hypothesis, we examined the extent of cartilage degradation in TG2 −/− (n = 4) and WT mice (n = 4) after OA induction by ACLT and DMM. Before surgical induction of OA, we confirmed gross appearance of TG2−/− mice showed no difference from WT mice, similar to a previous report[13]. Safranin-O staining revealed minor observed differences between cartilage of TG2−/− mice and WT mice, and the extent of cartilage damage was significantly lower in TG2−/− mice compared with WT mice 4 weeks after OA induction (Fig. 5A). Moreover, OARSI score of TG2−/− mice was significantly lower than that of WT mice (Fig. 5B; $p < 0.05$).

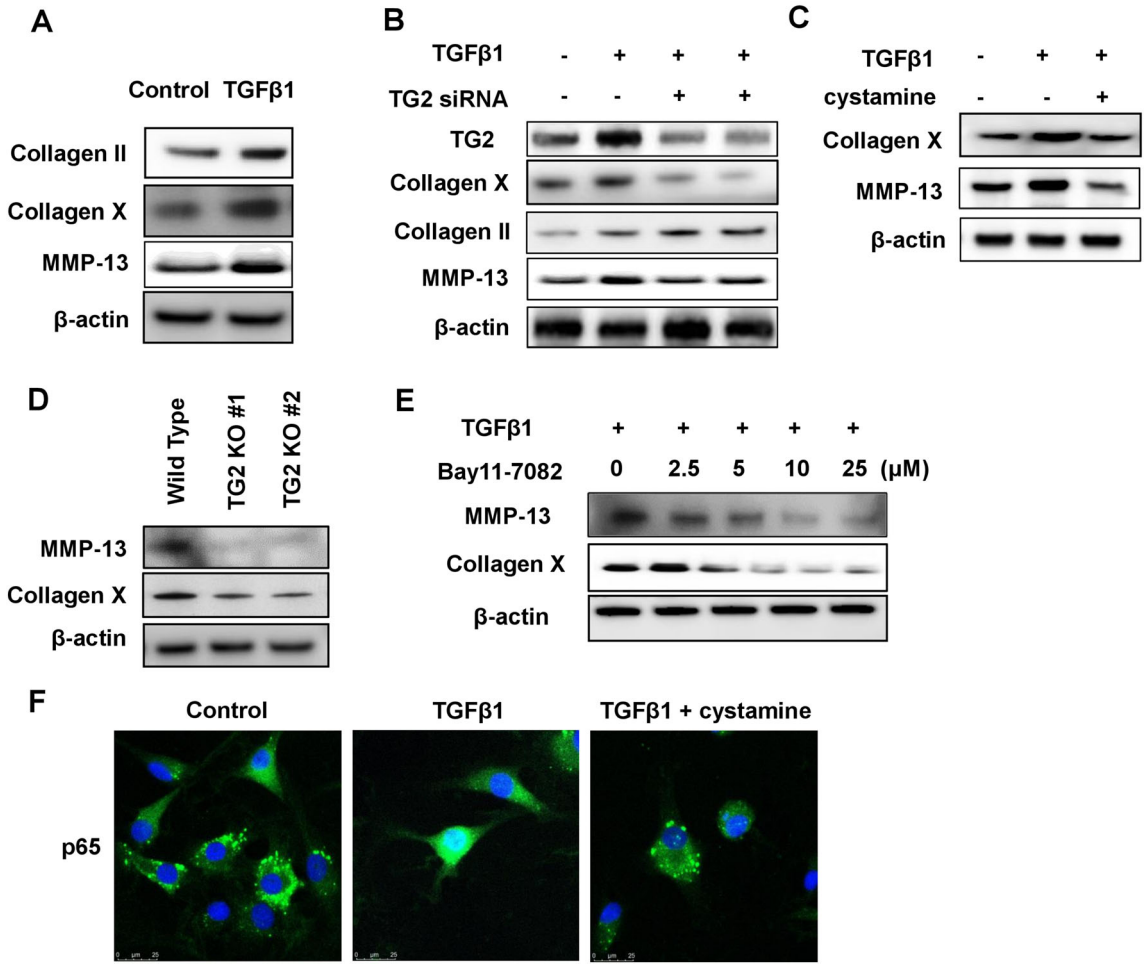

Fig. 4 TGFβ1-induced TG2 modulated the levels of type II and type X collagen, and elevated the MMP-13 expression by activating NF-κB signaling in chondrocytes. **A** Protein level of MMP-13, type II and type X collagen was measured by western blot analysis in chondrocytes treated with TGFβ1 (10 ng/mL). The experiment was repeated three times. **B** Chondrocytes treated with 10 ng/mL of TGFβ1 were transfected with TG2 siRNA. The protein levels of TG2, MMP-13, type II and type X collagen were measured by western blot analysis. The experiment was repeated three times. **C** The protein level of type X collagen and MMP-13 was measured by western blot analysis in chondrocytes pretreated with TGFβ1 and/or cystamine (TG2 inhibitor). The experiment was repeated three times. **D** The protein level of type X collagen and MMP-13 was measured by western blot analysis in chondrocytes isolated from wild-type and TG2 knock-out (KO) mice. The experiment was repeated three times. **E** The protein level of type X collagen and MMP-13 was measured by western blot analysis in chondrocytes pretreated with TGFβ1 and/or Bay11-7082 (NF-κB inhibitor) before treatment of TGFβ1. The experiment was repeated three times. **F** Chondrocytes were treated with 10 ng/mL of TGF-β for 24 h. Confocal analysis showed the localization of p65 in chondrocytes treated with or without TGFβ1 and/or cystamine. The experiment was repeated three times

4 Discussion

This study found the relationship between TGFβ1 and TG2, and their significant relationship in OA pathogenesis in knee joint. TGFβ1 plays a critical role in modulating maintenance and development of articular cartilage, and in the development of OA. Mutation in Smad3, signaling molecule of the canonical TGFβ1 pathway, is associated with higher incidence of knee OA [15], and Smad3−/− mice exhibit progressive joint degradation characterized by loss of cartilage and reduced proteoglycan production [16]. Further, inhibition of ALK1 increases expression of anabolic factors collagen II and aggrecan while ALK5 inhibition elevates the level of MMP-13 in chondrocytes [17], suggesting a dual role for TGFβ1 in modulating anabolic and catabolic balance in articular cartilage. Here, we hypothesized TGFβ1 contributes to the development of OA by modulating chondrocyte catabolism in a TG2-dependent manner.

First, we confirmed that the levels of TGFβ1 and TG2 were elevated in the osteoarthritic joint. The level of TGFβ1 was upregulated in the synovial fluid of OA patient Further, TG2 was abundantly expressed in articular cartilage, especially in the eroded region, suggesting an association between TG2 and OA severity. TG2 expression changed in response to treatment of TGFβ1 in

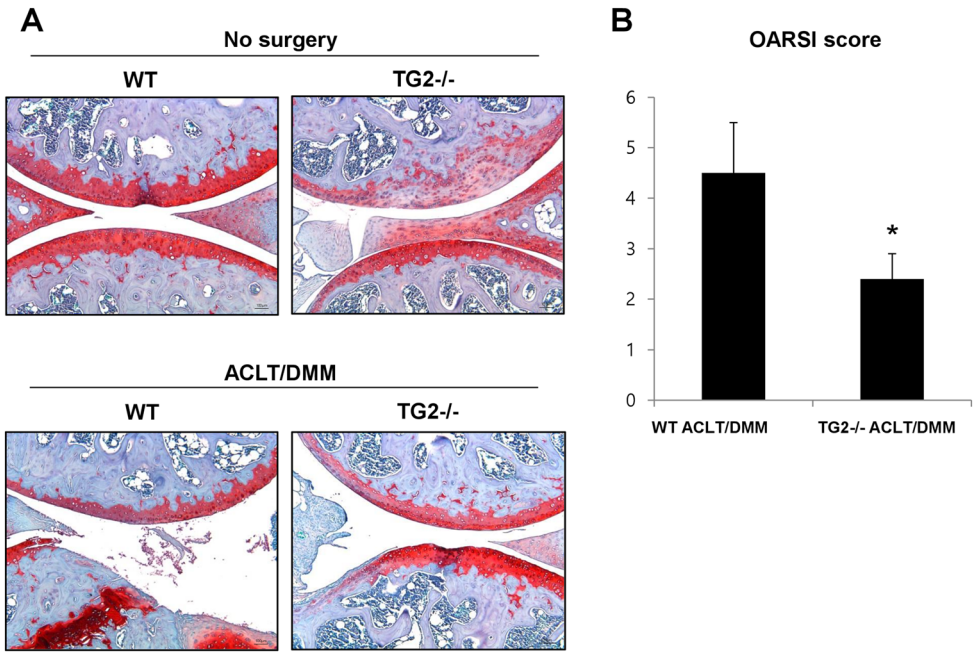

Fig. 5 Absence of TG2 attenuated the cartilage degradation in mice. **A** Safranin-O and Fast Green staining of sagittal sections of cartilage in ACLT/DMM and no surgery WT and TG2−/− mice (n = 4 for each group). **B** OARSI score was significantly lower in TG2−/− mice compared to WT mice that underwent ACLT/DMM surgery (*$p < 0.05$). The data are expressed as the mean ± SD

chondrocytes, not in synovial cells. TGFβ1-induced TG2 expression occurred in a cell-specific manner, which may attest to the context-dependent role of TGFβ1. TGFβ1 upregulated the expression of TG2 in chondrocytes by ERK and p38 activation, suggesting non-canonical pathway activation is involved. Inhibition of ERK and p38 reduced the protein level of TG2 in chondrocytes, suggesting that ERK and p38 mediates TGFβ1-induced TG2 expressions. Although the exact mechanism of the mediators has not been identified, we have confirmed that there exists an axis of TGFβ1- ERK/p38-TG2.

There is still a controversy whether TGFβ1 is the precursor that regulates TG2. Zsuzsa et al. reported that the activation of apoptosis-specific form of macrophage involves a TG2-dependent step in the production of active TGFβ1 [18]. Tang et al. also reported that tissue TG regulated TGF-β in the parasite infection with liver fibrosis [19]. There is a possibility of crosstalk between TGFβ1 and TG2, which might act reciprocally.

We also found that NF-κB signaling pathway was involved in modulating TGFβ1-mediated TG2 increased expression of MMP-13 and type X collagen in chondrocytes, which upregulates an array of catabolic enzymes and inflammatory factors in OA chondrocytes [20]. It was reported that TG2 degrades IκBα inhibitor, liberating p65/p50 and binds to the NF-κB complex, which subsequently translocates to the nucleus and binds to the NF-κB binding site [21]. Further, a previous study found that p65 binds to the MMP-13 promoter *in vivo* [22], showing the activation of NF-κB signaling directly upregulates MMP-13 expression. In our *in vivo* study of TG2−/− mice also suggests that TG2 mediates MMP-13 and type X collagen induction. In sum, TGFβ1-mediated TG2 may contribute to OA pathogenesis by upregulating MMP-13 level in chondrocytes.

TGFβ1-mediated TG2 increased the expression of MMP-13 and type X collagen in chondrocytes, suggesting TG2 may contribute to OA by promoting terminal differentiation of chondrocytes. A previous study showed that the TG2 levels in the synovial fluid correlate with OA severity. The TG2 mRNA expression increased together with the type X collagen and MMP-13 mRNA expressions in medial tibial cartilage of Hartley guinea pigs that spontaneously developed OA [9]. Other studies also showed that NF-κB-mediated HIF-2α regulation increases of type X collagen and MMP-13 expressions. HIF-2α, which is mediated by NF-κB, is involved in the development of OA [23, 24]. HIF-2α was identified as a transcription factor that greatly enhances type X collagen promoter activity in hypertrophic cartilage and enhances the promoter activity of MMP-13. These reports identified the role of NF-κB, a major catabolic pathway in OA by showing that inhibition of NF-κB reduced the level of MMP-13 and collagen X. Although TGFβ1-mediated TG2 increased the expression of MMP-13 and type X collagen in chondrocytes, further studies are necessary to determine the signaling mechanism of TG2-mediated expression of MMP-13 and type X collagen in chondrocytes.

Finally, we investigated the deleterious effect of TG2 in cartilage repair by examining the cartilage of TG2−/− mice compared with WT mice in ACLT/DMM model. Our results indicate that the extent of proteoglycan loss was less

severe in cartilage of TG2−/− mice compared to that in WT mice, suggesting TG2 induction may stimulate cartilage damage. This finding is consistent with a previous study that found TG2−/− mice showed reduced extent of cartilage damage after surgical OA induction [25].

In this study, we found that TG2 might be a therapeutic target of OA. Actually, previous studies tried to develop TG2 inhibitor as a novel drug for OA. Interestingly, treatment of arthritic joints with cystamine to block FXIIIA activity significantly attenuated the severity of arthritis in mice [26]. However, cystamine is generally nonspecific for TG2 and therefore not practical for drug development [27]. It is necessary to develop a novel drug for intraarticular injection which also has little effect on systematic circulation with little adverse effect.

Some limitations of this study should be mentioned. One limitation is the lack of performance testing in mice after surgery to evaluate qualities such as pain and motor function. Second, examination of knee joint from mice overexpressing TG2 would have aided in understanding the role of TG2 in the development of OA. We also did not examine whether inhibition of TGFβ1 alleviates pain and attenuates OA in mice after surgical OA induction, and more studies are needed to elucidate the role of TGFβ1/TG2 axis in OA.

The present study suggests that TGFβ1 and TG2 are relevant to the development of OA. TGFβ1-induced TG2 expression increases the level of MMP-13 via NF-κB activation. TGF-β-mediated TG2 induction may increase chondrocyte catabolism and facilitate degeneration of articular cartilage, suggesting TG2 might be the therapeutic target for OA.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical statement This research has been approved by the IRB of Seoul National University Hospital. (IRB No. 1510-077-711).

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