European Review for Medical and Pharmacological Sciences 2021; 25: 1233-1241

Interrupting TGF-β1/CCN2/integrin-α5β1 signaling alleviates high mechanical-stress caused chondrocyte fibrosis

Y.-Z. HUANG, L. ZHAO, Y. ZHU, S.-J. TIAN, W. ZHANG, S. LIU, J.-F. GE

Department of Orthopedics, The Affiliated Zhangjiagang Hospital of Soochow University, Zhangjiagang, China

Abstract. – OBJECTIVE: Mechanical-stress has been reported to trigger cartilage fibrosis, in which transforming growth factor (TGF)-β and connective tissue growth factor (CCN2) are involved. However, the function of integrin-α5β1, a cytomembrane receptor, on mechanical-stress related fibrosis has not yet been elucidated. This study aims to reveal the interaction of TGF-β1/CCN2/integrin-α5β1 in the mechanical-stress induced chondrocyte (CH) fibrosis.

PATIENTS AND METHODS: We used different levels (5% and 10%) of cyclic tension simulation (CTS) to stretch CHs and observed the gene expression of TGF-β1/CCN2/integrin-α5β1 as well as the fibrous related genes containing collagen I/II/III, Runx2, MMP13, and ADAMTS-5 by real-time polymerase chain reaction (RT-PCR) or immunofluorescence. We used the siRNA or the corresponding antagonist of TGF-β1, CCN2, integrin-α5β1 during the CTS to clear the effect of them in the fibrosis progress. In addition, to verify the crosstalk between TGF-β1, CCN2, and integrin-α5β1, we used the recombinant human (rh)-TGF-β1 and CCN2 to culture CHs without CST.

RESULTS: 24 hours-10% CTS was sufficient to induce a decrease of collagen II and increase the collagen I/III, Runx2, MMP13, and ADAMTS-5 gene expression. Under CTS, TGF-β1 silencing resulted in a decline of CCN2, integrin-α5β1, and alleviated the CHs fibrosis. Apart from this, blocking CCN2 or integrin-α5β1 expression also contributed to the suppression of 10% CTS induced CHs fibrosis. Meanwhile, the exogenic protein supplement raised the cellular TGF-β1 or CCN2 expression and increased the integrin-α5β1 mRNA level. However, the downregulation of TGF-β1 or CCN2 did not affect integrin-α5β1 expression, whether the CTS exited or not.

CONCLUSIONS: High mechanical-stress induces CHs fibrosis via the activation of TGF-β1/CCN2/integrin-α5β1 signaling, and interrupting the TGF-β1, CCN2, or integrin-α5β1 expression can alleviate the fibrous process.

Key Words:
Mechanical stress, Chondrocyte fibrosis, TGF-β1, CCN2, Integrin-α5β1.

Introduction

Increased mechanical load, caused by obesity, joint instability, and biomechanical changes, are all common factors that trigger the degeneration of articular cartilage[1]. Abnormal stress stimulation leads to a series of pathologies, like apoptosis of chondrocytes (CHs) and degradation of extracellular matrix (ECM). As a force-sensitive cell, CH receives stress stimulation and generates mechanical signals to regulate gene expression[2]. Proper mechanical stimulation contributes to the maintenance of the physiological function of CHs. On the other hand, abnormal stress stimulation starts and aggravates the degeneration of CHs[3,4].

Integrin is a kind of transmembrane glycoproteins widely existing on the cell membrane, the level of which is closely related to the cellular viability, adhesion, and phenotype. As a mechanically sensitive receptor on the surface of articular cartilage, integrin acts as a converter from external mechanical signals to intracellular chemical signals, and it senses various mechanical stress stimuli and eventually changes the relevant cell phenotype[5,6]. Different subtypes of integrins play different roles in the process of mechanochemical signal transduction. Among them, the β1 subunit and all types of α subunits are highly expressed in osteoarthritis (OA) CHs and participate in the pathology of OA process[7-9]. CHs are highly differentiated cell types after maturity. However, CH gradually loses the chondrogenic morphology and dedifferentiates into the fibroblast-like cells during the OA process. Meanwhile, its metabolism significantly increased as one of the indicators,

Corresponding Author: Jianfei Ge, BM; e-mail: gejianfei0271@tom.com

among which growth factors such as transforming growth factor (TGF)-β and connective tissue growth factor (CCN2, also knowns as CTGF) highly express[10].

The effect of TGF-β and CCN2 in cartilage metabolism is complex and dynamically changing. Although they can promote CH proliferation and ECM synthesis, they are also potential inducers of CH fibrosis. With the fibrous progress of CH, the cellular expression of type II collagen gradually decreased instead of type I and type III collagen. Whereas, most tissue fibrotic diseases based on the excessive deposition of type I and III collagen, such as liver fibrosis, systemic sclerosis, and scleroderma, are closely related to the overexpression of TGF-β and CCN2[11-13]. Apart from this, due to containing GTCT and its complement AGAC motifs on the CCN2 promoter, TGF-β is considered to be an essential inducer of CCN2 expression[14]. Xu et al[15] found TGF-β increases CCN2 expression and exerts a favorable effect on rabbit epidural scar-derived fibroblasts proliferation and transdifferentiation. Furumatsu et al[16] also reported cyclic tensile strain (CTS) increases CCN2 expression by the activation of the TGF-β pathway in chondrocytic cells.

Since integrin-α5β1 accumulates when OA aggravates[17] partly relating to the mechanical stress[18], and it is a CCN-binding receptor that regulates CCN functions in CHs[19]. We wondered whether it existed crosstalk between TGF-β, CCN2, and integrin-α5β1 in the mechanical-stress related CHs fibrosis. Therefore, we isolated the CHs from human knee cartilage and explored the TGF-β, CCN2, integrin-α5β1, and type I/II/III collagen gene expression response to the, and further investigated the effect of TGF-β/CCN2/integrin-α5β1 signaling on the mechanical-stress triggered CHs fibrosis.

Patients and Methods

CHs Source

This study was approved by the Ethics Committee of The Affiliated Zhangjiagang Hospital of Soochow University. We collected the human knee cartilage from five patients (every age 39 years) undergoing traumatic fracture for CHs isolation. We have evaluated the patient's knee joint to exclude who has arthritis and evident cartilage degeneration. Informed consent was obtained from the patient before the operation. We isolated the CHs from the cartilage as the following protocol. The surface layer of the cartilage was cut into small pieces and digested with the solution (0.2% dispase and 0.25% type I collagenase; Invitrogen, Carlsbad, CA, USA) overnight. The digested solution was filtered and centrifuged to get the CHs pellets. CHs were cultured in Dulbecco's modified Eagle medium/F12 (DMEM/F12) medium with 10% fetal bovine serum (FBS; Gibco, Dun Laoghaire, Co Dublin, Ireland) and 1% penicillin-streptomycin.

Drug Treatments

To regulate the TGF-β1, CCN2, and integrin-α5β1 expression in the cultured CHs. We used the recombinant human TGF-β1 protein (rh-TGF-β1, 50 μg/mL) (ab50036; Abcam, Cambridge, UK), recombinant human CCN2 protein (rh-CCN2, 50 μg/mL) (ab269222; Abcam, Cambridge, UK) to upregulated the TGF-β1 and CCN2 expression, respectively. Pamrevlumab (FG-3019, 100 μg/mL) (FibroGen, San Francisco, CA, USA), a human monoclonal antibody interfering with CCN2, was used for blocking CCN2 expression as the previous description[20]. Furthermore, ATN-161 (1 μM; Selleck, Houston, TX, USA), a peptide antagonist of integrin-α5β1, was used to suppress integrin-α5β1 expression.

Cyclic Tension Simulation (CTS)

CHs were subjected to a CTS (5% or 10% linear stretch) at a frequency of 1 Hz using the Mechanical Cell Stretch System (Menicon Life Science, Nagoya, Aichi, Japan) as the previous description[21]. CHs with a density of 1.5×10^4 per ml were seeded on the stretch chamber and allowed to achieve 70 % confluence for one day culture. We set the strain stimulation up to 24 h and assessed the collagen I/II mRNA expression to determine the significantly optimized stretch time, thus resulting in virtually 10% 24 h stretch. The cells seeded on the stretch chamber without CTS were classified as control.

Immunofluorescence

We used immunofluorescence (IF) staining to determine the protein expression of collagen I/II visibly. After the appointed treatment, the cells were washed with PBS and fixed using 4% paraformaldehyde for 15 min, and then incubated with 0.1% Triton-X for membranes permeabilization. Following, CHs were produced with the anti-collagen II or anti-collagen I primary antibody (Abcam, Cambridge, UK) overnight at 4°C. After washing, the cells were incubated with Alexa

Fluor 488 conjugated secondary antibody (Invitrogen, Carlsbad, CA, USA) for 1 h in the dark at room temperature.

Real Time-Polymerase Chain Reaction (RT-PCR) Analysis

We determined the gene expression in the mRNA level by real-time PCR analysis (RT-PCR). Briefly, after the appointed treatment, we isolated the mRNA using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. Then, the RNA was reverse-transcribed into complementary deoxyribose nucleic acid (cDNA) and applied to the RT-PCR analysis of TGF-β1, CCN2, integrin-α5β1, collagen I, collagen II, collagen III, Runx2, MMP13, and ADAMTS-5 expressions performed using SYBR Green Master (Boehringer, Mannheim, Germany). The primers are listed in Table I. The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA level was used to normalize the relative gene expression according to the $2^{-\Delta\Delta Ct}$ method.

siRNA Transfection

We silenced the TGF-β1 expression *via* siRNA transfection. Briefly, after seeding on the stretch chamber until the density reached 70 %. Then, Opti-MEM (Sigma-Aldrich, St. Louis, MO, USA) and siRNA targeting TGF-β1 (Catalog #AM16708, Thermo Fisher Scientific, Waltham, MA, USA) were added to Lipo2000 reagent (Invitrogen, Carlsbad, CA, USA). CHs were transfected with the mixture of Lipo2000 and siRNA reagent for 24 h, and the cells could be used for subsequent experiments.

Statistical Analysis

All the values were expressed as mean ± standard deviation (SD) by analysis using the Statistical Product and Service Solutions (SPSS) Version 21.0 (IBM Corp, Armonk, NY, USA). Differences between two groups were analyzed by using the Student's *t*-test. A comparison between multiple groups was made using a one-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). $p<0.05$ indicated a significant difference.

Results

High Level of CTS Disorders Collagen Components of CHs

To clear how CTS affects the fibrosis process of CHs, we stressed CHs with two degrees of axial tension (5% and 10%) at a frequency of 1 Hz from 12 h to 24 h. The cells seeded on the same stretch chamber were grouped as control. After 12 h CTS, a 5% tensile rate increased the collagen mRNA expression, and a 10% tensile rate increased the collagen I expression. However, 24 h later, 5% tension kept the CHs a higher level of collagen II mRNA expression compared to the control, and the collagen I mRNA expression was not significantly changed. On the contrary, 10% tension decreased the collagen II mRNA expression and further increased the collagen I level after 24 h CTS (Figure 1A and 1B). To our knowledge, the CHs undergo fibrous dedifferentiation accompanying by collagen components remolding by collagen I accumulation and collagen II reduction. Therefore, we treated CHs with 10% CTS for 24 h to induce fibrosis in the following experiments.

Silencing TGF-β1 Expression Weakens CTS Induced CHs Fibrosis

After 24 h of 10%-CTS, we found the mRNA expression of TGF-β1, CCN2, and integrin-α5β1 was significantly increased (Figure 2A). Therefore, we silenced the TGF-β1 gene expression

Table I. Primer sequences for RT-PCR.

Gene name	Forward (5′>3′)	Reverse (5′>3′)
Collagen I	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC
Collagen II	TGGACGATCAGGCGAAACC	GCTGCGGATGCTCTCAATCT
Collagen III	ATGTTGTGCAGTTTGCCCAC	TCGTCCGGGTCTACCTGATT
Runx-2	TGGTTACTGTCATGGCGGGTA	TCTCAGATCGTTGAACCTTGCTA
MMP-13	ACTGAGAGGCTCCGAGAAATG	GAACCCCGCATCTTGGCTT
ADAMTS-5	GAACATCGACCAACTCTACTCCG	CAATGCCCACCGAACCATCT
TGF-β1	GGCCAGATCCTGTCCAAGC	GTGGGTTTCCACCATTAGCAC
CCN2	CAGCATGGACGTTCGTCTG	AACCACGGTTTGGTCCTTGG
Integrin-α5β1	GGCTTCAACTTAGACGCGGAG	TGGCTGGTATTAGCCTTGGGT
GAPDH	ACAACTTTGGTATCGTGGAAGG	GCCATCACGCCACAGTTTC

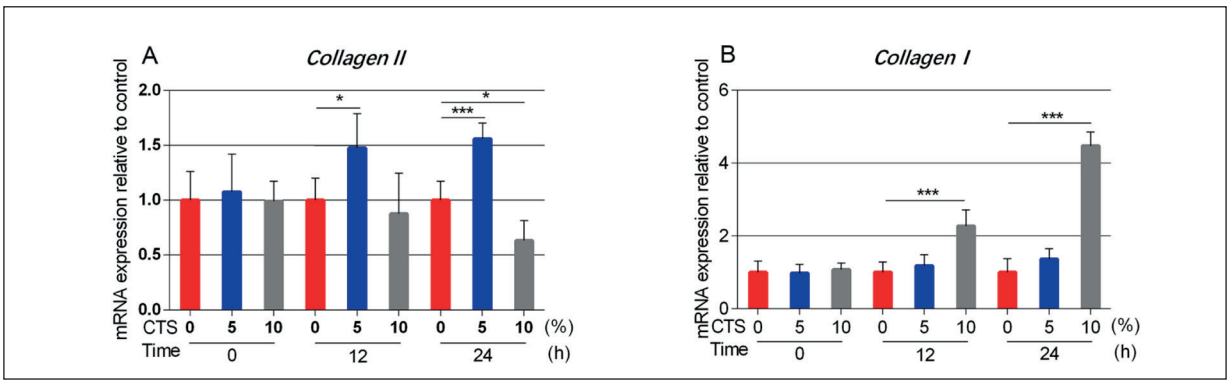

Figure 1. High level of CTS disorders CHs collagen II and I components. CHs were grouped into non-CTS, 5%, and 10% tension groups, and each group was sub-classified into 0 h, 12 h-CTS, and 24 h-CTS subgroups. RT-PCR analysis for the mRNA expression level of (**A**) collagen II and (**B**) collagen I from CHs normalized to GAPDH expression. Results are expressed as mean ± SD. (*$p < 0.05$, ***$p < 0.001$).

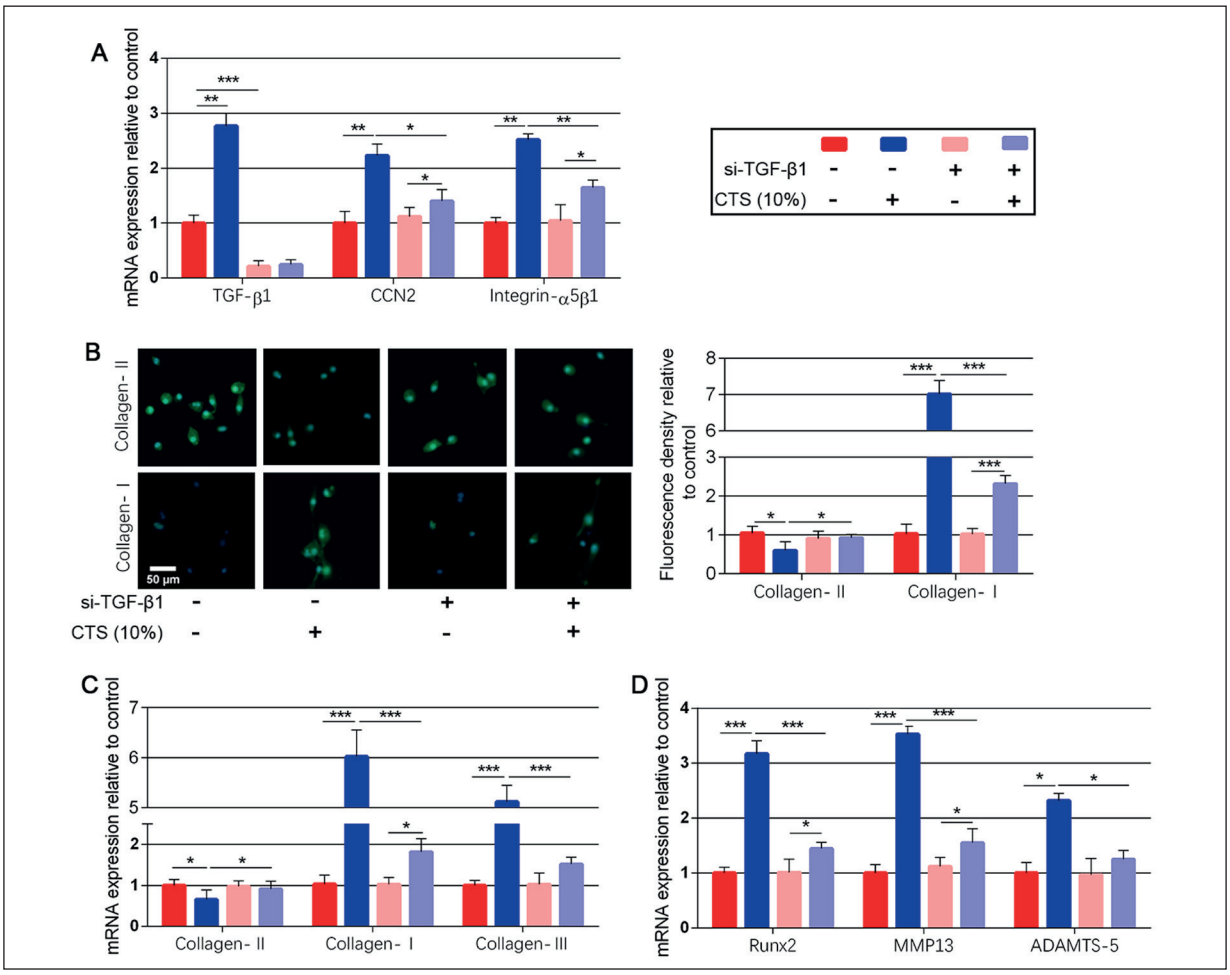

Figure 2. Silencing of the TGF-β1 gene alleviates CTS induced CHs fibrosis. Normal CHs or TGF-β1-silenced CHs were subjected to 10%-CTS for 24 h. The cells without CTS and siRNA transfection were set as control. **A,** RT-PCR analysis for the mRNA expression level of TGF-β1, CCN2, and integrin-α5β1. **B,** IF analysis for the protein expression of collagen II and collagen I, and its quantification measured by Image J software, (magnification: 200×). **C,** RT-PCR analysis for the mRNA expression level of collagen II, collagen I, collagen III. **D,** Runx2, MMP13, ADAMTS-5 normalized to GAPDH expression. Results are expressed as mean ± SD. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

of CHs and then treated with 10%-CTS for 24 h to clear whether it changed CCN2 and integrin-α5β1expression and helped to weaken the fibrosis progress. The result indicated that TGF-β1 deficiency alleviated the CCN2 and integrin-α5β1 increase caused by CTS. Besides, the TGF-β1 silenced CHs reserved the collagen II content after CTS, and the collagen I level was also inhibited under the 10% CTS, which was consistent in both protein and mRNA level (Figure 2B and 2C). Meanwhile, the collagen III mRNA was also increased after CTS, which was rejected by TGF-β1 silencing compared to the control (Figure 2C). In addition, we also analyzed the fibrosis-related gene of CHs, such as Runt-related transcription factor 2 (Runx2), Matrix Metallopeptidase 13 (MMP13), and ADAMTS-5 (a disintegrin and metalloproteinase with thrombospondin motifs-5). CTS induced Runx2, MMP13, and ADAMTS-5 overexpression compared to the control, and TGF-β1 silencing was efficient to suppress their expression (Figure 2D).

Blocking CCN2 or Integrin-α5β1 Weakens CTS Induced CHs Fibrosis

Since CCN2 and integrin-α5β1 were upregulated by CTS, we further used the FG-3190 (100 μg/mL) and ATN-161 (1 μM) in the culture medium while CTS to blocking CCN2 and integrin-α5β1 expression, respectively. Interestingly, the inhibiting of CCN2 led to the downregulation of CCN2 and integrin-α5β1, and TGF-β1 also slightly reduced compared to the non-FG-319 treated CTS CHs. Besides, the ATN-161 significantly decreased integrin-α5β1 expression compared to the non-ATN-161 treated CTS CHs, and it did not affect the TGF-β1 and CCN2 expression (Figure 3A). The protein and mRNA levels of collagen II were both increased after the supplement of FG-3190 or ATN-161. On the contrary, the collagen I level was suppressed due to the inhibition of CCN2 or TGF-β1 (Figure 3B and 3C). In addition, the collagen III, Runx2, MMP13, and ADAMTS-5 expression were decreased after the blocking of CCN2 or integrin-α5β1 compared to the non-drug treated CTS CHs (Figure 3D). Although the efficacy of stimulation of FG-3190 and ATN-161 was different in CHs, it seemed that it was more useful to inhibit integrin-α5β1 than to inhibit CCN2 from delaying CHs fibrosis.

TGF-β1 and CCN2 Overexpression Trigger Integrin-α5β1 Expression of CHs

In these experiments, the high level of CTS triggered the TGF-β1, CCN2, and integrin-α5β1 gene expression. Besides, the suppression of TGF-β1 resulted in the reduction of CCN2 and integrin-α5β1. The data suggested crosstalk between TGF-β1, CCN2, and integrin-α5β1 in the mechanical stress condition. However, whether this crosstalk exists independent of CTS remains unclear. Instead of the CTS, we cultured the CHs with rh-TGF-β1 or rh-CCN2 to upregulate the cellular TGF-β1 or CCN2 expression. Additionally, we used the FG-3019 and ATN-161 to reveal the relation among TGF-β1, CCN2, and integrin-α5β1 without mechanical stress. As shown in Figure 4A, compared to the control, rh-TGF-β1 stimulation contributed to the upregulation of TGF-β1, CCN2, and integrin-α5β1 mRNA expression; rh-CCN2 increased the CCN2 and integrin-α5β1 mRNA expression; FG-3019 and ATN-161 only affected the CCN2 and integrin-α5β1 expression, respectively. The data suggested that TGF-β1 overexpression could increase CCN2 and integrin-α5β1 mRNA content, and CCN2 overexpression also caused the upregulation of integrin-α5β1. However, the blocking of CCN2 did not change the integrin-α5β1 expression. Therefore, we concluded that TGF-β1 and CCN2 overexpression trigger integrin-α5β1 expression, but the suppression of CCN2 did not result in a decrease of integrin-α5β1. In addition, we found TGF-β1 overexpression promoted both collagen II and I protein production, and rh-CCN2 was only able to increase collagen I expression (Figure 4B). However, the blocking of CCN2 and integrin-α5β1 made no difference in collagen II and I protein expression (Figure 4C and 4D).

Discussion

Articular cartilage is under endogenous and exogenous mechanical stimulation, and appropriate mechanical stimulation is significant for maintaining the steady-state of articular cartilage[22]. However, excessive mechanical stimulation can cause articular cartilage damage and even OA. Articular cartilage is composed of CHs and ECM, and CHs are responsible for the production and maintenance of ECM stability. Typically, the mechanical stimuli to which CHs subjects are mainly pressured, but tension and shear forces are also fundamental in some areas[23]. Kelly et al[24] found that tension occurs mostly around the compressed area of articular cartilage and is also at the boundary between articular cartilage and subchondral bone. CTS is closer to the physiolo-

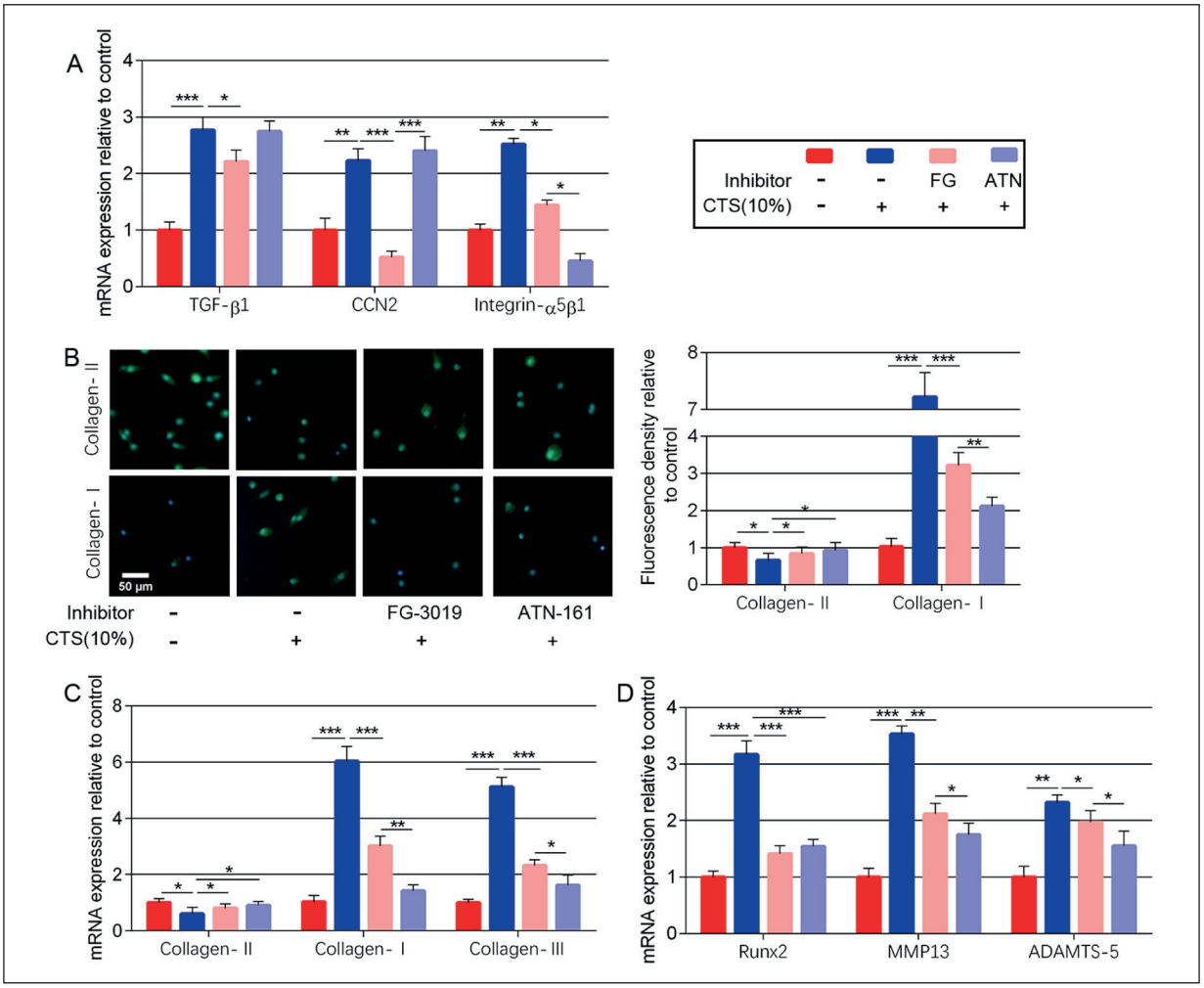

Figure 3. Blocking CCN2 or integrin-α5β1 weakens CTS induced CHs fibrosis. Normal CHs were subjected to 10%-CTS for 24 h. Two groups of them were cultured in the FG-3019, or ATN-161 contained medium while CTS. The cells without CTS in the normal medium were set as control. **A,** RT-PCR analysis for the mRNA expression level of TGF-β1, CCN2, and integrin-α5β1. **B,** IF analysis for the protein expression of collagen II and collagen I, and its quantification measured by Image J software, (magnification: 200×). **C,** RT-PCR analysis for the mRNA expression level of collagen II, collagen I, collagen III. **D,** Runx2, MMP13, ADAMTS-5 normalized to GAPDH expression. Results are expressed as mean ± SD. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

gical state of articular cartilage stress than static tension, and its effect on CHs is more evident[25]. Many studies have proved that different tensile strengths have different effects on the synthesis of ECM. Fukuda et al[26] indicated the 5% CTS loaded on CHs promotes proteoglycan synthesis, and 17% magnitude CTS suppresses its synthesis. Besides, Connelly et al[27] declared that 5% CTS of 1 Hz had no effect on the proteoglycan synthesis of CHs, but 10% and 20% significantly interrupted its integration. However, whether CTS affects CHs fibrosis is not fully understood.

CH fibrosis is characterized by dysregulation of ECM metabolism, manifested by changes in collagen composition, increased expressions of matrix catabolic genes, such as MMP13 and ADAMTS-5, and overexpression of the hypertrophic transcription factor Runx2[28,29]. As the previous studies, our research has demonstrated that 5% of CTS promoted collagen II expression. Differently, 10% CTS decreased the collagen II and was capable of triggering the collagen I and III formation, which was associated with the upregulation of TGF-β1, CCN2, and integrin-α5β1. TGF-β1 overexpression is the inducer of the onset of tissue fibrosis[30], which usually causes the upregulation of type I and type III collagen[31]. Additionally, the exogenic stimulation of TGF-β1 or

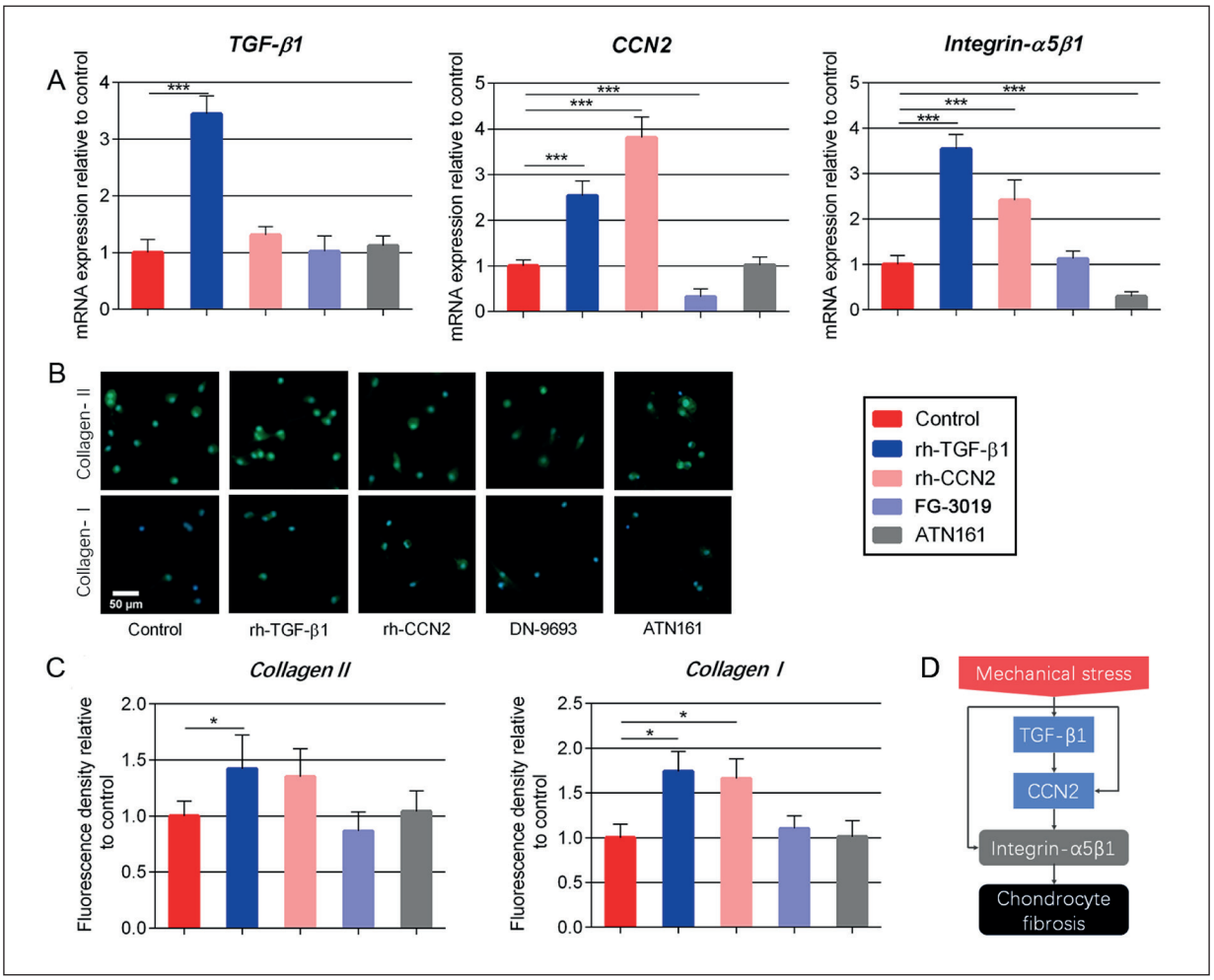

Figure 4. TGF-β1 and CCN2 overexpression trigger integrin-α5β1 expression of CHs. Normal CHs were stimulated in the medium containing rh-TGF-β1, rh-CCN2, FG-3019, or ATN-161 for 24 h without CTS. **A,** RT-PCR analysis for the mRNA expression level of TGF-β1, CCN2, and integrin-α5β1 normalized to GAPDH expression. **B,** IF analysis for the protein expression of collagen II and collagen I, and (**C**) its quantification measured by Image J software, (magnification: 200×). **D,** Graphical abstract of this study. Results are expressed as mean ± SD. (*$p < 0.05$, ***$p < 0.001$).

CCN2 has been used in the establishment of CHs fibrosis *in vitro*[32,33]. Because mechanical stress can induce the generation of TGF-β1[34] and also CCN gene expression in fibroblasts, endothelial cells, bone cells, and other cell types[35,36], therefore, the mechanism by which mechanical stretch induces the orientation of CHs fibrosis should be associated with the upregulation of TGF-β1 and CCN2. In our study, the upregulation of TGF-β1 did promote the CCN2 expression. However, CTS increased the CCN2 expression in the TGF-β1 silenced CHs, which suggested CCN2 could be regulated directly by mechanical stress that independent of TGF-β1. Even though we showed the upregulation of TGF-β1 triggering the CCN2 expression in both CTS and relaxed condition. To alleviate the CTS induced fibrous process of CHs, suppressing TGF-β1 or CCN2 expression might be a feasible way.

Integrins are transmembrane receptors that mediate various biomechanical signal transductions inside and outside cells. Among the multiple subunits, integrin-α5β1 is very sensitive to the extracellular mechanical signals. Kurakawa et al[37] concluded that mechanical-stress upregulates integrins, particularly the α5β1 subtype, in nucleus pulposus and annulus fibrosus cells that lead to the intervertebral disc degeneration. Lucchinetti et al[38] elucidated integrin-α5β1 acts as a mechanical signal transducer to modulate the cellular physiology between the CH and the ECM. Whereas, the effect of integrin-α5β1 on the CHs

fibrosis and whether it interacts with TGF-β1 and CCN2 has not been fully explained. From our findings, blocking the expression of integrin-α5β1 alleviated the CTS induced collagen disorders and the Runx2, MMP13, and ADAMTS-5 excess production. Meanwhile, the TGF-β1 and CCN2 expression were not interrupted. TGF-β1 and CCN2 played the pro-fibrous role in the CHs metabolism via the mediation of integrin-α5β1. Because integrin-α5β1 is the cellular receptor of TGF-β1[39] and CCN2[19], our observation verified that TGF-β1 and CCN2 regulate CHs fibrosis through the intervention of integrin-α5β1. Notably, our results only proved the upregulation of TGF-β1 and CCN2 could activate integrin-α5β1, but no evidence was noticed that inactivation of TGF-β1 or CCN2 inhibited the integer-α5β1 expression. Moreover, the expression of integrin could also be directly upregulated by the influence of high mechanical-stimulation, and this pathway is independent of TGF-β1 and CCN2.

Conclusions

Our results show that a high level of mechanical-stress can induce CHs fibrosis by the mechanisms of TGF-β1, CCN2, and integrin-α5β1 activation, which is shown as a graphical abstract in Figure 4D. The novelty of this study underlies disrupting the crosstalk among TGF-β1, CCN2, and integrin-α5β1 plays a meaningful effect on delaying the formation of CHs fibrosis caused by mechanical-stress. Further, we propose to explore the axial compressive force on the process of CHs fibrosis and the potential mechanism related to TGF-β1/CCN2/integrin-α5β1 signaling, which would help us to understand the pathology and find the therapies of OA.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Tang LL, Wang YL, Sun CX. The stress reaction and its molecular events: splicing variants. Biochem Biophys Res Commun 2004; 320: 287-291.
2) Bleuel J, Zaucke F, Bruggemann GP, Niehoff A. Effects of cyclic tensile strain on chondrocyte metabolism: a systematic review. PLoS One 2015; 10: e119816.
3) Johnson VL, Hunter DJ. The epidemiology of osteoarthritis. Best Pract Res Clin Rheumatol 2014; 28: 5-15.
4) Sanchez-Adams J, Leddy HA, McNulty AL, O'Conor CJ, Guilak F. The mechanobiology of articular cartilage: bearing the burden of osteoarthritis. Curr Rheumatol Rep 2014; 16: 451.
5) Schwartz MA, Ginsberg MH. Networks and crosstalk: integrin signalling spreads. Nat Cell Biol 2002; 4: E65-E68.
6) Roca-Cusachs P, Iskratsch T, Sheetz MP. Finding the weakest link: exploring integrin-mediated mechanical molecular pathways. J Cell Sci 2012; 125: 3025-3038.
7) Tian J, Zhang FJ, Lei GH. Role of integrins and their ligands in osteoarthritic cartilage. Rheumatol Int 2015; 35: 787-798.
8) Perera PM, Wypasek E, Madhavan S, Rath-Deschner B, Liu J, Nam J, Rath B, Huang Y, Deschner J, Piesco N, Wu C, Agarwal S. Mechanical signals control SOX-9, VEGF, and c-Myc expression and cell proliferation during inflammation via integrin-linked kinase, B-Raf, and ERK1/2-dependent signaling in articular chondrocytes. Arthritis Res Ther 2010; 12: R106.
9) Cheng K, Xia P, Lin Q, Shen S, Gao M, Ren S, Li X. Effects of low-intensity pulsed ultrasound on integrin-FAK-PI3K/Akt mechanochemical transduction in rabbit osteoarthritis chondrocytes. Ultrasound Med Biol 2014; 40: 1609-1618.
10) Parra-Torres NM, Cazares-Raga FE, Kouri JB. Proteomic analysis of rat cartilage: the identification of differentially expressed proteins in the early stages of osteoarthritis. Proteome Sci 2014; 12: 55.
11) Brenner DA, Rippe RA, Rhodes K, Trotter JF, Breindl M. Fibrogenesis and type I collagen gene regulation. J Lab Clin Med 1994; 124: 755-760.
12) George J, Tsutsumi M. siRNA-mediated knockdown of connective tissue growth factor prevents N-nitrosodimethylamine-induced hepatic fibrosis in rats. Gene Ther 2007; 14: 790-803.
13) Fonseca C, Lindahl GE, Ponticos M, Sestini P, Renzoni EA, Holmes AM, Spagnolo P, Pantelidis P, Leoni P, McHugh N, Stock CJ, Shi-Wen X, Denton CP, Black CM, Welsh KI, du Bois RM, Abraham DJ. A polymorphism in the CTGF promoter region associated with systemic sclerosis. N Engl J Med 2007; 357: 1210-1220.
14) Leask A, Chen S, Pala D, Brigstock DR. Regulation of CCN2 mRNA expression and promoter activity in activated hepatic stellate cells. J Cell Commun Signal 2008; 2: 49-56.
15) Xu H, Li P, Liu M, Liu C, Sun Z, Guo X, Zhang Y. CCN2 and CCN5 exerts opposing effect on fibroblast proliferation and transdifferentiation induced by TGF-beta. Clin Exp Pharmacol Physiol 2015; 42: 1207-1219.
16) Furumatsu T, Matsumoto E, Kanazawa T, Fujii M, Lu Z, Kajiki R, Ozaki T. Tensile strain increases expression of CCN2 and COL2A1 by activat-

ing TGF-beta-Smad2/3 pathway in chondrocytic cells. J Biomech 2013; 46: 1508-1515.

17) Almonte-Becerril M, Gimeno-LLuch I, Villarroya O, Benito-Jardon M, Kouri JB, Costell M. Genetic abrogation of the fibronectin-alpha5beta1 integrin interaction in articular cartilage aggravates osteoarthritis in mice. PLoS One 2018; 13: e198559.
18) Loeser RF. Integrins and chondrocyte-matrix interactions in articular cartilage. Matrix Biol 2014; 39: 11-16.
19) Lau LF. Cell surface receptors for CCN proteins. J Cell Commun Signal 2016; 10: 121-127.
20) Ohara Y, Chew SH, Misawa N, Wang S, Somiya D, Nakamura K, Kajiyama H, Kikkawa F, Tsuyuki Y, Jiang L, Yamashita K, Sekido Y, Lipson KE, Toyokuni S. Connective tissue growth factor-specific monoclonal antibody inhibits growth of malignant mesothelioma in an orthotopic mouse model. Oncotarget 2018; 9: 18494-18509.
21) Hata M, Naruse K, Ozawa S, Kobayashi Y, Nakamura N, Kojima N, Omi M, Katanosaka Y, Nishikawa T, Naruse K, Tanaka Y, Matsubara T. Mechanical stretch increases the proliferation while inhibiting the osteogenic differentiation in dental pulp stem cells. Tissue Eng Part A 2013; 19: 625-633.
22) Ma CH, Chiua YC, Wu CH, Jou IM, Tu YK, Hung CH, Hsieh PL, Tsai KL. Homocysteine causes dysfunction of chondrocytes and oxidative stress through repression of SIRT1/AMPK pathway: A possible link between hyperhomocysteinemia and osteoarthritis. Redox Biol 2018; 15: 504-512.
23) Martire LM, Hemphill RC, Zhaoyang R, Stephens M, Franks MM, Stanford AM. Daily marital tension and symptom severity in older adults with diabetes or osteoarthritis. Ann Behav Med 2018; 52: 842-853.
24) Kelly PA, O'Connor JJ. Transmission of rapidly applied loads through articular cartilage. Part 1: Uncracked cartilage. Proc Inst Mech Eng H 1996; 210: 27-37.
25) Liu Q, Hu X, Zhang X, Duan X, Yang P, Zhao F, Ao Y. Effects of mechanical stress on chondrocyte phenotype and chondrocyte extracellular matrix expression. Sci Rep 2016; 6: 37268.
26) Fukuda K, Asada S, Kumano F, Saitoh M, Otani K, Tanaka S. Cyclic tensile stretch on bovine articular chondrocytes inhibits protein kinase C activity. J Lab Clin Med 1997; 130: 209-215.
27) Connelly JT, Vanderploeg EJ, Levenston ME. The influence of cyclic tension amplitude on chondrocyte matrix synthesis: experimental and finite element analyses. Biorheology 2004; 41: 377-387.
28) Meng XM, Nikolic-Paterson DJ, Lan HY. TGF-beta: the master regulator of fibrosis. Nat Rev Nephrol 2016; 12: 325-338.
29) Hunter DJ, Bierma-Zeinstra S. Osteoarthritis. Lancet 2019; 393: 1745-1759.
30) van der Kraan PM, Blaney DE, Blom A, van den Berg WB. TGF-beta signaling in chondrocyte terminal differentiation and osteoarthritis: modulation and integration of signaling pathways through receptor-Smads. Osteoarthritis Cartilage 2009; 17: 1539-1545.
31) Kim SG, Akaike T, Sasagaw T, Atomi Y, Kurosawa H. Gene expression of type I and type III collagen by mechanical stretch in anterior cruciate ligament cells. Cell Struct Funct 2002; 27: 139-144.
32) Xue M, Gong S, Dai J, Chen G, Hu J. The treatment of fibrosis of joint synovium and frozen shoulder by smad4 gene silencing in rats. PLoS One 2016; 11: e158093.
33) Blaney DE, Vitters EL, Mooren FM, Oliver N, Berg WB, van der Kraan PM. Connective tissue growth factor/CCN2 overexpression in mouse synovial lining results in transient fibrosis and cartilage damage. Arthritis Rheum 2006; 54: 1653-1661.
34) Zhang RK, Li GW, Zeng C, Lin CX, Huang LS, Huang GX, Zhao C, Feng SY, Fang H. Mechanical stress contributes to osteoarthritis development through the activation of transforming growth factor beta 1 (TGF-beta1). Bone Joint Res 2018; 7: 587-594.
35) Yamashiro T, Fukunaga T, Kobashi N, Kamioka H, Nakanishi T, Takigawa M, Takano-Yamamoto T. Mechanical stimulation induces CTGF expression in rat osteocytes. J Dent Res 2001; 80: 461-465.
36) Takano-Yamamoto T, Fukunaga T, Takeshita N. Gene expression analysis of CCN protein in bone under mechanical stress. Methods Mol Biol 2017; 1489: 283-308.
37) Kurakawa T, Kakutani K, Morita Y, Kato Y, Yurube T, Hirata H, Miyazaki S, Terashima Y, Maeno K, Takada T, Doita M, Kurosaka M, Inoue N, Masuda K, Nishida K. Functional impact of integrin alpha5beta1 on the homeostasis of intervertebral discs: a study of mechanotransduction pathways using a novel dynamic loading organ culture system. Spine J 2015; 15: 417-426.
38) Lucchinetti E, Bhargava MM, Torzilli PA. The effect of mechanical load on integrin subunits alpha5 and beta1 in chondrocytes from mature and immature cartilage explants. Cell Tissue Res 2004; 315: 385-391.
39) Tian H, Mythreye K, Golzio C, Katsanis N, Blobe GC. Endoglin mediates fibronectin/alpha5beta1 integrin and TGF-beta pathway crosstalk in endothelial cells. EMBO J 2012; 31: 3885-3900.