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Mechanical stress loading induces CD44 cleavage in human chondrocytic HCS-2/8 cells

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ABSTRACT

Although excessive mechanical stress loading is known to induce articular cartilage degradation, the mechanism underlying this process is unclear. The interaction between hyaluronan (HA) and its primary receptor CD44 maintains the homeostasis of articular chondrocytes. CD44 cleavage and the generation of CD44-intracellular domain (ICD) can lead to the loss of extracellular matrices in chondrocytes. Here we studied the effects of cyclic tensile strain (CTS) loading, a representative mechanical stress, on CD44 cleavage. CTS loading (1 Hz and 20% elongation for 48 h) increased ADAM10 expression and CD44 cleavage in HCS-2/8 cells, a human chondrocytic cell line. Co-treatment with a chemical ADAM10 inhibitor significantly suppressed CTS loading-induced CD44 cleavage. Chemical inhibition of transient receptor potential vanilloid 4 (TRPV4) significantly suppressed CTS loading-induced ADAM10 expression and CD44 cleavage. Conversely, chemical activation of TRPV4 increased ADAM10 expression and enhanced CD44 cleavage. Our findings suggest that CTS loading significantly increases the expression of ADAM10, which in turn enhances CD44 cleavage in HCS-2/8 cells. The primary mechanoreceptor mediating this process is TRPV4. This signature event could provide an avenue for intervention in the prevention of cartilage degradation leading to OA.

1. Introduction

Hyaluronic acid (HA) is essential for maintaining the homeostasis of articular chondrocytes. The HA/proteoglycan-rich cell-associated matrix is anchored to chondrocytes via the binding of HA to CD44, the principal cell surface receptor for HA [1,2]. Intracellular signaling via CD44 facilitates the expression of genes induced by the loss of HA-CD44 binding and promotes matrix metabolism and the expression of genes involved in matrix repair in chondrocytes [3,4].

CD44 is a transmembrane protein and an HA receptor [5]. Upon cleavage, CD44 loses its function as a receptor. This signature event includes cleavage of the extracellular domain of CD44 by metalloproteinases (ADAM10, ADAM17, and MT1-MMP) and release of the CD44 ecto-domain. An 18–20 kD C-terminal fragment remains within the membrane (CD44-EXT) [6,7]. This CD44-EXT fragment is then cleaved by γ-secretase within the intramembrane domain, leading to the release of a 15 kD intracellular domain (CD44-ICD) into the cytoplasm [8].

We previously found that CD44 cleavage is significantly enhanced in articular cartilage obtained from osteoarthritis (OA) patients [9]. Cleavage of CD44 not only reduces the amount of functional CD44, but also increases generation of the soluble CD44 ecto-domain, which can act as a decoy receptor for HA. The release of CD44-ICD into the cytoplasm of chondrocytes exerts a competitive effect on interactions between full-length CD44 and a cytoskeletal adaptor protein. These interactions are required to stabilize HA and the pericellular matrix. Moreover, forced expression of CD44-ICD reduces the amount of extracellular matrices in articular chondrocytes [10]. These data suggest that CD44 cleavage and CD44-ICD production could be involved in the pathogenesis or development of OA.

Osteoarthritis (OA) is caused by multiple factors such as genetics, aging, obesity, and mechanical stress. In particular, dynamic mechanical stress loading is an important factor that induces articular cartilage degradation [11,12]. A previous study demonstrated that mechanical stress loading significantly reduces the

Abbreviations: HA, Hyaluronan; OA, osteoarthritis; CTS, cyclic tensile strain; TRPV4, transient receptor potential vanilloid 4.

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expression of matrix constituents in chondrocytes [13]. However, the mechanism underlying how mechanical stress loading causes articular cartilage degradation is unknown.

Recently, transient receptor potential vanilloid 4 (TRPV4), a member of the vanilloid subfamily of the transient receptor potential (TRP) superfamily of ion channels, was shown to function as a mechanoreceptor in articular chondrocytes [14]. One of the earliest events in response to mechanical stress loading in these cells is Ca influx [15]. TRPV4 is a Ca^{2+}-permeable ion channel, is highly expressed in articular chondrocytes, and can be gated by osmotic stimuli.

We hypothesized that excessive mechanical stress loading would induce CD44 cleavage via the activation of TRPV4 and increase the expression of MMPs as the 'first-step' in cartilage degradation. To this end, we investigated the induction of and mechanism underlying CD44 cleavage under mechanical stress loading using HCS-2/8 cells, a human chondrocytic cell line.

2. Materials and methods

2.1. Cells and cell culture

HCS-2/8, a human chondrocytic cell line, is a continuous long-term culture cell line derived from a human chondrosarcoma [16]. HCS-2/8 cells were cultured on 6-well plates in Dulbecco's Modified Eagle's Medium (DMEM, SIGMA) supplemented with 5% fetal bovine serum (FBS) and 1% antibiotics at 37 °C in a 5% CO_2 environment. HCS-2/8 cells were passaged at sub-confluence using 0.5% trypsin and 0.2% ethylenediaminetetraacetic acid (EDTA) every 10–14 days. For mechanical stress loading experiments, 2×10^5 cells were cultured on 10 cm^2 silicon culture chambers precoated with type 1 collagen (Cellmatrix®, Nitta Gelatin, Japan). After 48 h of static incubation in 5% FBS followed by overnight serum deprivation, silicon chambers were subjected to cyclic tensile strain (CTS) loading using the automated cell stretching system STB-140 (STREX, Japan) under serum-free conditions. In some experiments, chemical pre-treatment (24 h) was applied between overnight serum starvation and CTS loading.

HCS-2/8 cells were collected after mechanical stress loading or static culture (control). Collected cells were then processed for real-time PCR and Western blot analysis.

2.2. Western blot

Total protein was extracted using Cell Lysis Buffer (Cell Signaling Technology, USA) containing a protease inhibitor cocktail. Protein samples were loaded and separated on NuPAGE Bis-Tris Mini Gels (Invitrogen, USA) under reducing conditions. Samples were then transferred onto a nitrocellulose membrane and blocked in 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T). CD44, ADAM10, ADAM17, membrane type 1 (MT1-MMP), and β-actin were detected with primary antibodies, followed by the appropriate secondary antibody. Detection was performed using chemiluminescence (Thermo, USA). The following primary antibodies were used: anti-ADAM10 antibody ab1997 (Abcam, Tokyo, Japan), anti-ADAM17 antibody ab2051 (Abcam, Tokyo, Japan), and anti-MT1-MMP1 antibody ab3644 (Abcam, Tokyo, Japan). A polyclonal antibody specific for CD44-ICD (cytotail) was used to detect CD44 cleavage [9].

2.3. Real-time PCR

Total RNA was extracted using the RNeasy Mini Kit (Qiagen, Germany) according to the manufacturer's instructions. Reverse transcription was performed with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, USA) at 37 °C for 120 min. Real time PCR was carried out using the Light cycler System with FastStart Master SYBR Green I ^{PLUS} (Roche, USA). Primers for ADAM10, ADAM17, MT1-MMP, and GAPDH were all designed and synthesized by Abcam plc, Cambridge, UK. The following primers were used: ADAM10, forward primer 5′-TGCAGTGCCCACAGCAA-3′, reverse primer 5′-AGAGCGACCAGTTCCCTACAA-3'; ADAM17, forward primer 5′- AACCGCAGGACTCTTTGTTC-3′, reverse primer 5′-GTGGTCTTGTTGACAATCCG-3'; MT1-MMP, forward primer 5′-TCAGGGCAGTGGATAGCGA-3′, reverse primer 5′-CCCGGTTCTACCTTCAGCTTC-3'; GAPDH, forward primer 5′-TGCACCACCAACTGCTTAGC-3′, reverse primer 5′-GGCATGGACTGTGGTCATGAG-3'.

PCR conditions were as follows: 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C, with data collected in the last 30 s. Real-time PCR efficiencies and fold increase in mRNA copy number were calculated as previously described [17].

2.4. Statistical analysis

All experiments were repeated at least three times and similar results were obtained. The Kruskal-Wallis H test was used for two-group comparisons. $P < 0.05$ was considered statistically significant. All statistical analyses were carried out using SPSS for Windows version 21 (SPSS, Chicago, IL).

3. Results

3.1. Induction of CD44 cleavage by CTS loading

We studied CD44 cleavage induced by CTS loading using the stretch device STB-14. Fig. 1A is a representative Western blot using an antibody that detects CD44-ICD. Full length CD44 was detected at around 85 kD, CD44-EXT as doublet bands at 18–20 kD, and CD44-ICD as a single band at around 15 kD. Since the antibody against CD44-ICD recognizes the intracellular domain of CD44, every form of CD44 can be detected with it. Enhanced cleavage was reflected by an increase in band intensity of CD44-EXT and CD44-ICD after 48 h of CTS loading (1 Hz, 20% elongation) relative to the control.

We next determined the appropriate conditions of CTS that most efficiently led to CD44 cleavage. A longer period of CTS loading led to more pronounced CD44 cleavage (see 48 h data point in Fig. 1B). HCS-2/8 cells became unhealthy at 72 h, and most cells detached from the silicone chamber surface. We also examined CD44 cleavage at various CTS intensities: 0.2 Hz and 5% elongation, 0.5 Hz and 10% elongation, and 1 Hz and 20% elongation. The most pronounced cleavage was observed at 48 h with CTS at 1 Hz and 20% elongation (Fig. 1C). This condition was used for subsequent CD44 cleavage experiments.

3.2. Changes in expression of ADAM10, ADAM17, and MT1-MMP by CTS loading

Expression of ADAM10 mRNA was significantly increased at 48 h with CTS at 1 Hz and 20% elongation relative to the untreated control ($P < 0.001$). No significant change was observed in the expression of ADAM17 and MT1-MMP mRNA (Fig. 2A). Similar results were obtained with protein levels. CTS loading (1 Hz at 20% elongation for 48 h) increased the expression of ADAM10 protein, but not ADAM17 or MT1-MMP (Fig. 2B). Importantly, pre-treatment for 24 h with GI254023X (Tocris Bioscience, USA), a selective ADAM10 inhibitor, significantly suppressed CD44 cleavage induced by CTS loading in a dose-dependent manner (Fig. 2C).

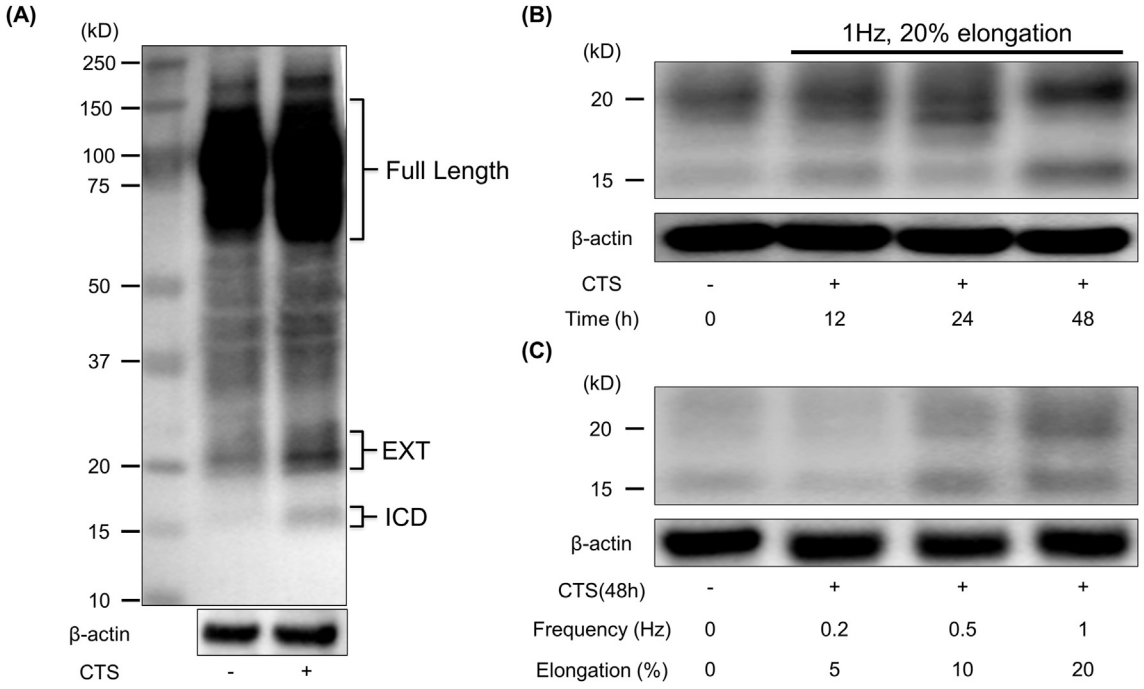

Fig. 1. Induction of CD44 cleavage by mechanical stress loading in HCS-2/8 cells. (A) A representative Western blot showing CD44 cleavage. CD44 cleavage was enhanced upon cyclic tensile strain (CTS) loading (1 Hz and 20% elongation for 48 h). The enhanced cleavage included an increase in intensity of the 18–20 kD doublet CD44-EXT and 15 kD CD44-ICD bands. (B) CD44 cleavage was time-dependently enhanced up to 48 h under CTS loading at 1 Hz and 20% elongation. (C) Different intensities of CTS loading for 48 h were applied. CD44 cleavage was most strongly enhanced at the highest intensity of CTS loading (1 Hz and 20% elongation).

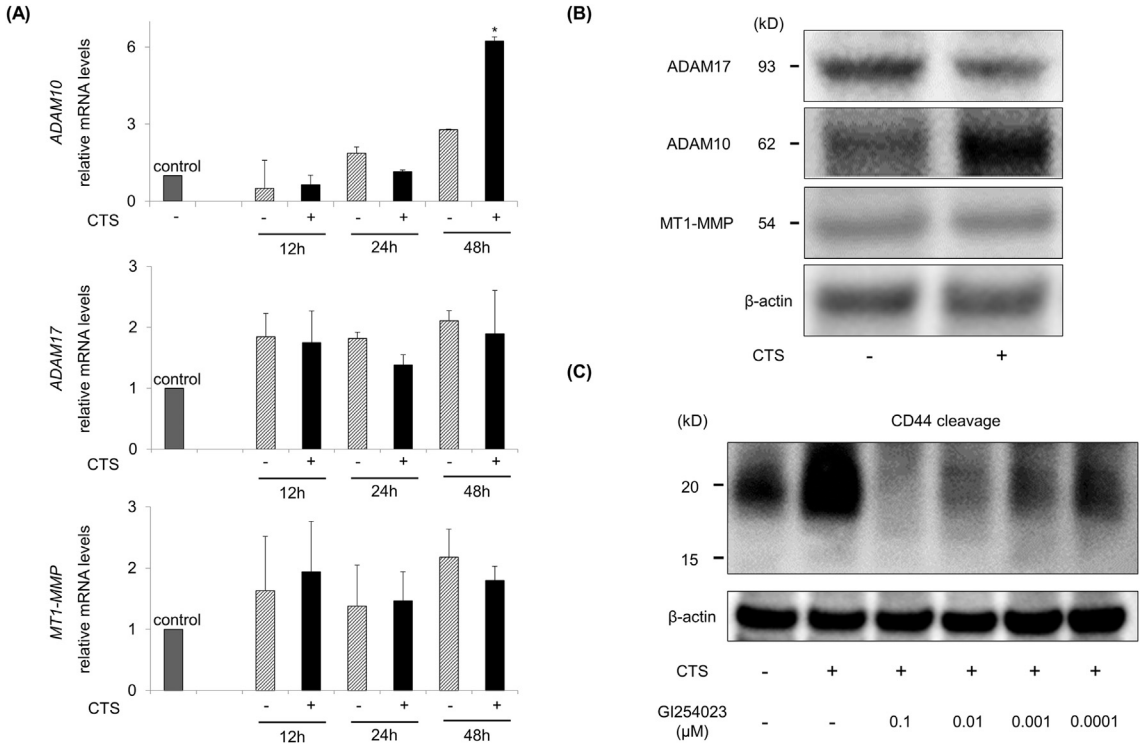

Fig. 2. Effects of mechanical stress loading on MMP expression and CD44 cleavage. (A) Changes in mRNA expression of ADAM10, ADAM17, and MT1-MMP. The far left bar shows the control (cells immediately after overnight serum starvation and before application of cyclic tensile strain (CTS) (i.e., 0 h)). mRNA expression of ADAM10 was significantly increased at 48 h of CTS loading compared with the control ($P = 0.001$). No significant changes were observed in mRNA expression of ADAM17 or MT1-MMP throughout the 48 h of treatment. *$P < 0.05$ vs control sample at each time-point (Kruskal-Wallis H test). (B) Western blot analysis of protein expression of ADAM10, ADAM17, and MT1-MMP. CTS loading for 48 h at 1 Hz and 20% elongation increased the protein expression of ADAM10, but not ADAM17 or MT1-MMP. (C) The effect of a chemical ADAM10 inhibitor (GI254023X) on CD44 cleavage. Pre-incubation for 24 h and co-incubation with GI254023X markedly suppressed CD44 cleavage induced by CTS loading for 48 h at 1 Hz and 20% elongation.

3.3. Effects of chemical activation of TRPV4 on CD44 cleavage and MMP expression

We next studied the effects of chemical activation of TRPV4, which mimics mechanical stress loading, on CD44 cleavage and mRNA expression of ADAM10, ADAM17, and MT1-MMP. TRPV4 was activated with a small molecule agonist, GSK1016790A (GSK101). Fully confluent cells were subjected to overnight serum starvation and followed by 24 h of GSK101 treatment at various concentrations (0–2000 nM). Similar to the results of CTS loading, GSK101 significantly increased ADAM10 mRNA expression in a dose-dependent manner, while no significant change was found in ADAM17 or MT1-MMP mRNA expression (Fig. 3A). The induction reached significance at ≥500 nM GSK101. Both 500 and 1000 nM GSK101 also significantly increased the expression of ADAM10 protein (Fig. 3B). CD44 cleavage was also increased by stimulation at both 500 and 1000 nM GSK101 (Fig. 3C).

3.4. Effects of TRPV4 antagonist on CTS loading-induced CD44 cleavage and MMP expression

Fully confluent HCS-2/8 cells in silicon culture chambers were pre-treated for 24 h with 10 μM GSK205 (AOBIOUS INC), a selective TRPV4 antagonist. Following this, CTS loading at 1 Hz and 20% elongation was applied for 48 h in the presence of GSK205. As expected, blocking TRPV4 with GSK205 effectively suppressed the increase in ADAM10 mRNA and protein expression (Fig. 4A, B). No significant changes were observed in ADAM17 and MT1-MMP mRNA expression. CTS loading-induced CD44 cleavage was also suppressed by pre-treatment and co-treatment with GSK205 (Fig. 4C).

4. Discussion

In this study, we found that excessive mechanical stress loading induces CD44 cleavage in human chondrocytic cells. Previous studies have reported that excessive mechanical stress loading on the joint surface resulted in degeneration of articular cartilage, which led to symptomatic OA [18,19]. However, the detailed mechanisms underlying cartilage degeneration are not fully understood. CD44 cleavage and CD44-ICD production have been reported to induce the loss of extracellular matrices in articular chondrocytes [10]. Our current results showing that CD44 cleavage is induced by CTS loading could, at least partially, explain the initial changes that occur in cartilage degeneration in response to excessive mechanical stress loading.

The significance of CD44 cleavage in chondrocytes is not fully understood. However, it is assumed to be important for OA pathogenesis. In a previous study, CD44 cleavage was significantly enhanced in chondrocytes obtained from OA cartilage and de-differentiated chondrocytes, a cellular model of OA chondrocytes [9]. CD44-ICD, an end product of CD44 cleavage, is known to competitively inhibit the interaction between CD44 and the cytoskeleton. The loss of this interaction leads to a loss of CD44-HA binding, and subsequently, a loss of extracellular matrices. Thus, CD44 cleavage and CD44-ICD accumulation could potentially be involved in the initial degenerative changes in articular cartilage in OA patients [10].

TRPV4 activation and increased ADAM10 expression were key factors that led to CD44 cleavage in this study. Christopher et al. reported that the activation of TRPV4 plays a critical role in the mechanoregulation of chondrocyte physiology and matrix metabolism in response to dynamic compressive loading [14]. We

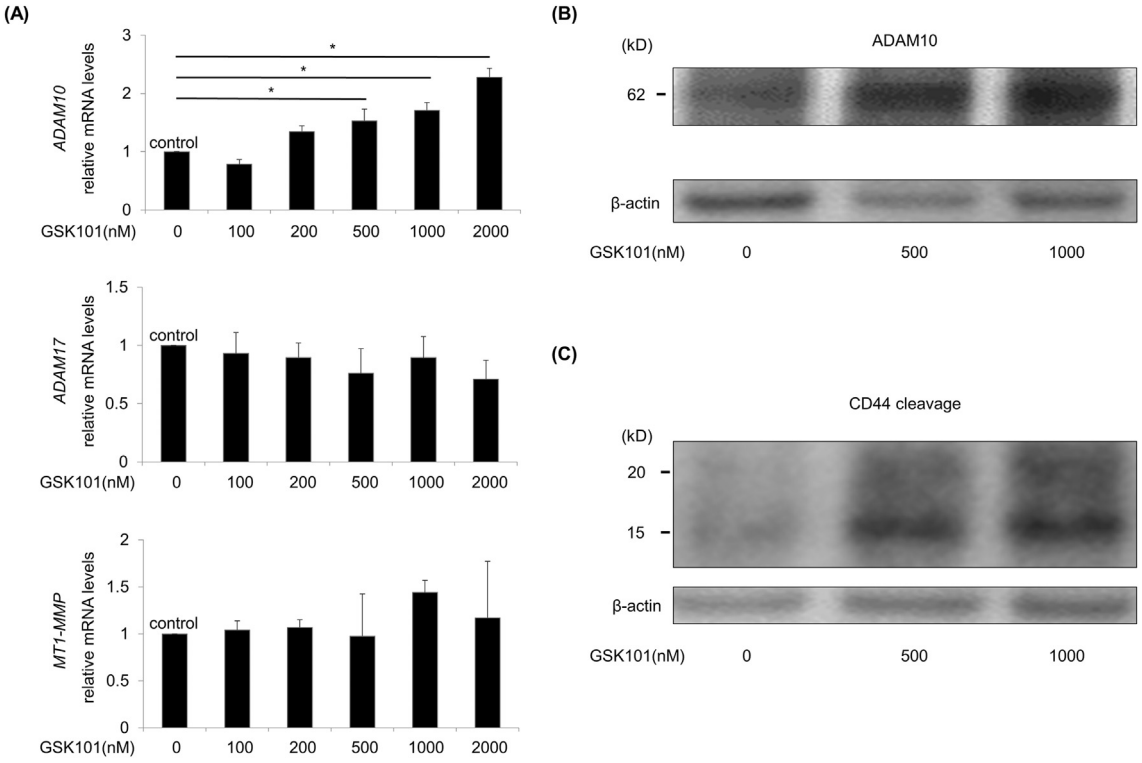

Fig. 3. Effects of chemical activation of TRPV4 on MMP expression and CD44 cleavage. (A) Effects of stimulation with GSK101, a selective agonist of TRPV4, for 24 h on ADAM10, ADAM17, and MT1-MMP mRNA expression. GSK101 increased the mRNA expression of ADAM10 in a dose-dependent manner. Treatments at concentrations ≥500 nM GSK101 were statistically significant. No significant changes were observed in ADAM17 or MT1-MMP. *P < 0.05, Kruskal-Wallis H test. (B) Both 500 and 1000 nM GSK101 treatment for 24 h increased the protein expression of ADAM10. (C) CD44 cleavage was significantly enhanced by both 500 and 1000 nM GSK101 treatment for 24 h.

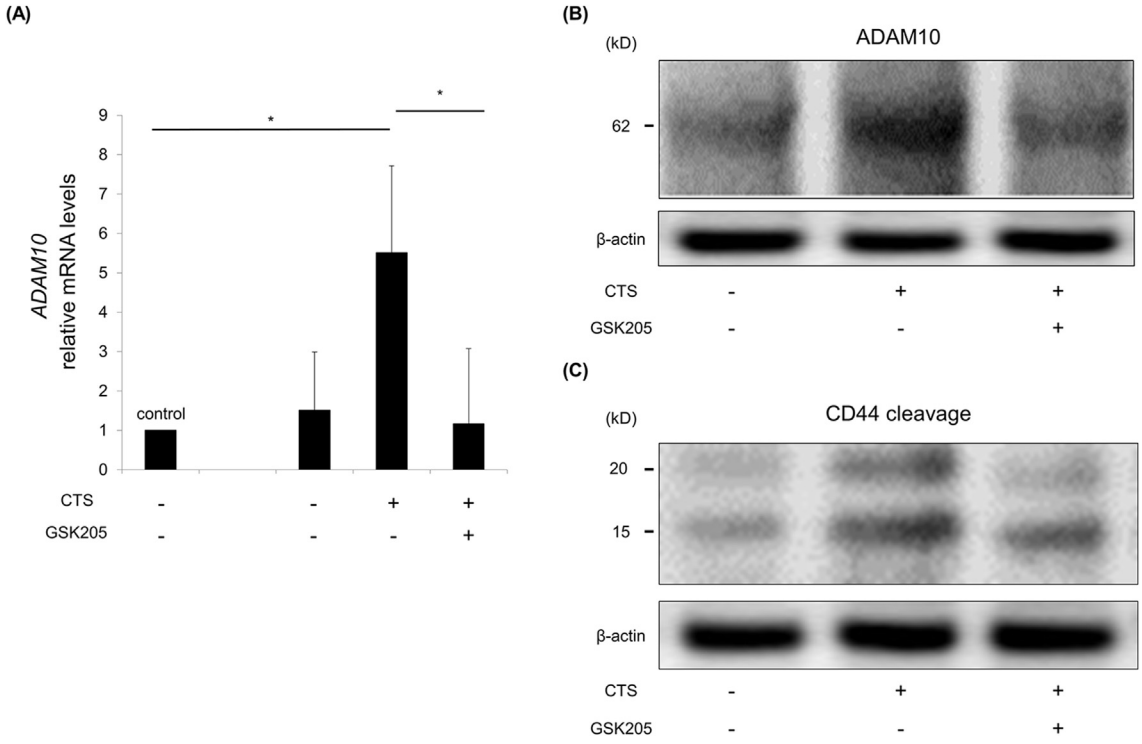

Fig. 4. Effects of chemical inhibition of TRPV4 on MMP expression and CD44 cleavage induced by mechanical stress loading. (A) Effects of 24 h of pre-treatment and co-incubation with GSK205, a selective TRPV4 antagonist, during cyclic tensile strain (CTS) loading on mRNA expression of ADAM10. The far left bar shows the control (immediately after overnight serum starvation and before pre-treatment with GSK205). GSK205 treatment effectively suppressed the induction of ADAM10 mRNA by CTS loading. *P < 0.05, Kruskal-Wallis H test. (B) Treatment with GSK205 also suppressed the protein expression of ADAM10 induced by CTS loading. (C) CD44 cleavage induced by CTS loading was effectively suppressed with GSK205 treatment.

confirmed that TRPV4 plays a pivotal role as a mechanoreceptor in monolayer HCS-2/8 cells under CTS loading. Both the chemical activation of TRPV4 with GSK101 and chemical inhibition with GSK205 demonstrated that the primary mechanoreceptor involved was TRPV4.

ADAM10, ADAM17, and MT1-MMP are potential mediators of CD44 ectodomain shedding. In a previous study using cancer cells, Nagano et al. reported that Ca^{2+} influx activated ADAM10, which led to CD44 ectodomain cleavage in U251MG cells derived from a malignant glioblastoma tumor [7]. We previously reported that ADAM10 was the primary mediator of CD44 ectodomain shedding in HCS-2/8 cells and bovine articular chondrocytes treated with IL-1β + Oncostatin M [20]. Similar to previous studies that used different cell types and/or stimuli, the present study found that Ca^{2+} influx through TRPV4 and increase in ADAM10 expression were the primary processes leading to CD44 cleavage in HCS-2/8 cells under CTS loading.

Some previous studies have reported on the catabolic effects of CTS loading. For example, Beckman et al. reported that CTS (0.5 Hz and 16% elongation for 12 h) increased the expression of vascular endothelial growth factor (VGEF), which is involved in the progression of OA in human chondrocytes [21]. Das et al. reported that CTS loading (0.5 Hz and 3% elongation for 1 h, twice a day for three days) down-regulated the expression of aggrecan and COL2, major components of extracellular matrices in articular cartilage, in human articular chondrocytes [13]. Doi et al. reported that CTS loading (0.5 Hz and 7% elongation for 24 h) significantly increased the expression of MMP-13 and cathepsin B in rat chondrocytes [22]. In addition to these data, we found that CTS loading induces CD44 cleavage, which could lead to the loss of extracellular matrices in human chondrocytic cells. This finding provides some mechanistic insight into how cartilage degradation can be induced by excessive mechanical stress loading.

This study has some limitations. First, our analysis was limited to investigating the effects of CTS loading. Cellular responses differ depending on the type of mechanical stress. Future studies should assess the effects of different types of mechanical stress, such as compressive stress, shear stress, fluid pressure stress, and shaking stress. Second, this study used simple monolayer HCS-2/8 cell cultures. Further studies using primary chondrocytes cultured in monolayer or three dimensions would be informative. Moreover, the use of *in vivo* animal models will be needed to confirm the association between mechanical stress loading and CD44 cleavage in articular cartilage.

In summary, CTS loading increased the expression of ADAM10, which in turn led to enhanced CD44 cleavage in HCS-2/8 cells. The primary mechanoreceptor that mediated this effect was TRPV4. This signature event could provide an avenue for intervention in the prevention of cartilage degradation leading to OA.

Conflict of interest

All authors have no conflict of interest.

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References

[1] W. Knudson, D.J. Aguiar, Q. Hua, et al., CD44-anchored hyaluronan-rich pericellular matrices: an ultrastructural and biochemical analysis, Exp. Cell Res.

228 (1996) 216–228.
[2] C.B. Knudson, Hyaluronan receptor-directed assembly of chondrocyte pericellular matrix, J. Cell Biol. 120 (1993) 825–834.
[3] R.F. Thorne, J.W. Legg, C.M. Isacke, The role of the CD44 transmembrane and cytoplasmic domains in co-ordinating adhesive and signalling events, J. Cell. Sci. 117 (2004) 373–380.
[4] T. Mori, K. Kitano, S. Terawaki, R. Maesaki, Y. Fukami, T. Hakoshima, Structural basis for CD44 recognition by ERM proteins, J. Biol. Chem. 283 (2008) 29602–29612.
[5] A. Aruffo, I. Stamenkovic, M. Melnick, C.B. Underhill, B. Seed, CD44 is the principal cell surface receptor for hyaluronate, Cell 61 (1990) 1303–1313.
[6] H. Nakamura, N. Suenaga, K. Taniwaki, H. Matsuki, K. Yonezawa, M. Fujii, Y. Okada, M. Seiki, Constitutive and induced CD44 shedding by ADAM-like proteases and membrane-type 1 matrix metalloproteinase, Cancer Res. 64 (2004) 876–882.
[7] O. Nagano, D. Murakami, D. Hartmann, B. De Strooper, P. Saftig, T. Iwatsubo, M. Nakajima, M. Shinohara, H. Saya, Cell-matrix interaction via CD44 is independently regulated by different metalloproteinases activated in response to extracellular Ca(2+) influx and PKC activation, J. Cell Biol. 165 (2004) 893–902.
[8] S. Lammich, M. Okochi, M. Takeda, C. Kaether, A. Capell, A.K. Zimmer, D. Edbauer, J. Walter, H. Steiner, C. Haass, Presenilin-dependent intramembrane proteolysis of CD44 leads to the liberation of its intracellular domain and the secretion of an Abeta-like peptide, J. Biol. Chem. 277 (2002) 44754–44759.
[9] N. Takahashi, C.B. Knudson, S. Thankamony, W. Ariyoshi, L. Mellor, H.J. Im, W. Knudson, Induction of CD44 cleavage in articular chondrocytes, Arthritis Rheum. 62 (2010) 1338–1348.
[10] L. Mellor, C.B. Knudson, D. Hida, E.B. Askew, W. Knudson, Intracellular domain fragment of CD44 alters CD44 function in chondrocytes, J. Biol. Chem. 288 (2013) 25838–25850.
[11] R.L. Sah, Y.J. Kim, J.Y. Doong, A.J. Grodzinsky, A.H. Plaas, J.D. Sandy, Biosynthetic response of cartilage explants to dynamic compression, J. Orthop. Res. 7 (1989) 619–636.
[12] F. Guilak, B.C. Meyer, A. Ratcliffe, V.C. Mow, The effects of matrix compression on proteoglycan metabolism in articular cartilage explants, Osteoarthr. Cartil. 2 (1994) 91–101.
[13] R.H. Das, H. Jahr, J.A. Verhaar, J.C. van der Linden, G.J. van Osch, H. Weinans, In vitro expansion affects the response of chondrocytes to mechanical stimulation, Osteoarthr. Cartil. 16 (2008) 385–391.
[14] C.J. O'Conor, H.A. Leddy, H.C. Benefield, W.B. Liedtke, F. Guilak, TRPV4-mediated mechanotransduction regulates the metabolic response of chondrocytes to dynamic loading, Proc. Natl. Acad. Sci. U.S.A. 111 (2014) 1316–1321.
[15] N. Tanaka, S. Ohno, K. Honda, K. Tanimoto, T. Doi, M. Ohno-Nakahara, E. Tafolla, S. Kapila, K. Tanne, Cyclic mechanical strain regulates the PTHrP expression in cultured chondrocytes via activation of the Ca2+ channel, J. Dent. Res. 84 (2005) 64–68.
[16] M. Takigawa, K. Tajima, H.O. Pan, M. Enomoto, A. Kinoshita, F. Suzuki, Y. Takano, Y. Mori, Establishment of a clonal human chondrosarcoma cell line with cartilage phenotypes, Cancer Res. 49 (1989) 3996–4002.
[17] S. Ohno, H.J. Im, C.B. Knudson, W. Knudson, Hyaluronan oligosaccharides induce matrix metalloproteinase 13 via transcriptional activation of NFkappaB and p38 MAP kinase in articular chondrocytes, J. Biol. Chem. 281 (2006) 17952–17960.
[18] H.B. Sun, Mechanical loading, cartilage degradation, and arthritis, Ann. N. Y. Acad. Sci. 1211 (2010) 37–50.
[19] S. Tanaka, C. Hamanishi, H. Kikuchi, K. Fukuda, Factors related to degradation of articular cartilage in osteoarthritis: a review, Semin. Arthritis Rheum. 27 (1998) 392–399.
[20] K. Terabe, N. Takahashi, T. Takemoto, W. Knudson, N. Ishiguro, T. Kojima, Simvastatin inhibits CD44 fragmentation in chondrocytes, Arch. Biochem. Biophys. 604 (2016) 1–10.
[21] R. Beckmann, A. Houben, M. Tohidnezhad, N. Kweider, A. Fragoulis, C.J. Wruck, L.O. Brandenburg, B. Hermanns-Sachweh, M.B. Goldring, T. Pufe, H. Jahr, Mechanical forces induce changes in VEGF and VEGFR-1/sFlt-1 expression in human chondrocytes, Int. J. Mol. Sci. 15 (2014) 15456–15474.
[22] H. Doi, K. Nishida, M. Yorimitsu, T. Komiyama, Y. Kadota, T. Tetsunaga, A. Yoshida, S. Kubota, M. Takigawa, T. Ozaki, Interleukin-4 downregulates the cyclic tensile stress-induced matrix metalloproteinases-13 and cathepsin B expression by rat normal chondrocytes, Acta Med. Okayama 62 (2008) 119–126.