Osteoarthritis and Cartilage 19 (2011) 222–232

Osteoarthritis and Cartilage

Regulation of mechanical stress-induced MMP-13 and ADAMTS-5 expression by RUNX-2 transcriptional factor in SW1353 chondrocyte-like cells

T. Tetsunaga †, K. Nishida ‡*, T. Furumatsu †, K. Naruse §, S. Hirohata ||, A. Yoshida †, T. Saito †, T. Ozaki †

† Department of Orthopaedic Surgery, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama City, Okayama 700-8558, Japan
‡ Department of Human Morphology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama City, Okayama 700-8558, Japan
§ Department of Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama City, Okayama 700-8558, Japan
|| Department of Molecular Biology and Biochemistry, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama City, Okayama 700-8558, Japan

ARTICLE INFO

Article history:
Received 7 July 2010
Accepted 10 November 2010

Keywords:
Chondrocyte
Mechanical stress
RUNX-2
ADAMTS
Aggrecanase

SUMMARY

Objective: To investigate the mechanism of mechanical stress-induced expression and regulation of aggrecanases and examine the role of runt-related transcription factor 2 (RUNX-2) in chondrocyte-like cells.
Methods: SW1353 cells were seeded onto stretch chambers at a concentration of 5×10^4 cells/chamber, and a uni-axial cyclic tensile strain (CTS) (0.5 Hz, 10% stretch) was applied for 30 min. Total RNA was extracted, reverse transcribed, and analyzed by polymerase chain reaction (PCR) and real-time PCR. RUNX-2 overexpression and small interfering RNA (siRNA) targeting RUNX-2 were used to investigate the role of RUNX-2 in CTS-induced gene expression. The involvement of diverse mitogen-activated protein kinase (MAPK) pathways in the activation of RUNX-2, MMP-13 and ADAMTS-5 during CTS was examined by Western blotting.
Results: CTS induced expression of *RUNX-2*, *MMP-13*, *ADAMTS-4*, *-5*, and *-9*. Overexpression of RUNX-2 up-regulated expression of *MMP-13* and *ADAMTS-5*, whereas RUNX-2 siRNA resulted in significant down-regulation of mechanically-induced *MMP-13* and *ADAMTS-5* expression. CTS induced activation of p38 MAPK, and CTS induction of *RUNX-2*, *MMP-13* and *ADAMTS-5* mRNA was down-regulated by the selective p38 MAPK inhibitor SB203580 but not by the p44/42 MAPK inhibitor U0126, or the JNK MAPK inhibitor JNK inhibitor II.
Conclusions: RUNX-2 might have a role as a key downstream mediator of p38's ability to regulate mechanical stress-induced *MMP-13* and *ADAMTS-5* expression.

Introduction

Osteoarthritis (OA) is a common joint disease characterized by cartilage destruction, subchondral bone sclerosis, and osteophyte formation. The development of OA is related to genetic factors, environmental factors, metabolic disorders, and biochemical and/or biomechanical abnormality of the joint. Although mechanical stimulation which represents the physiological environment of cartilage increases matrix synthetic activity and up-regulates proteinase enzymes, repeated excessive mechanical strain may alter chondrocyte metabolism and induce structural failure of the extracellular matrix (ECM)[1,2]. However, the precise mechanisms of mechanical stress-induced cartilage matrix degradation are not fully understood.

The main components of the articular cartilage ECM are type II collagen and aggrecan. Aggrecan loss resulting from decreased synthesis by chondrocytes and activation of enzymes that degrade the cartilage matrix is recognized as one of the earliest events in the course of OA, followed by mechanical injury of collagen fibrils[3]. OA disease progression is a highly complicated process involving multiple events such as aggrecan loss, resulting from decreased synthesis by chondrocytes and activation of enzymes that degrade the cartilage matrix. Two classes of enzymes – matrix metalloproteinases (MMPs) and aggrecanases – contribute to aggrecan depletion by cleaving proteins in arthritic cartilage. Various MMPs play important roles in collagen degradation. MMP-1, -8, -13

* Address correspondence and reprint requests to: Keiichiro Nishida, Department of Human Morphology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 2-5-1 Shikata-cho, Okayama 700-8558, Japan. Tel: 81-86-235-7273; Fax: 81-86-223-9727.
E-mail address: knishida@md.okayama-u.ac.jp (K. Nishida).

1063-4584/$ – see front matter © 2010 Osteoarthritis Research Society International. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.joca.2010.11.004

comprise the collagenase subfamily in humans, involved in cartilage destruction. MMP-1 is expressed in a broad range of normal tissue types, such as fibroblast and macrophages[4]. MMP-13 has higher affinity for type II collagen than MMP-1 or -8 but can also cleave aggrecan at specific sites[5]. Although having less catalytic efficiency than the classic collagenase, MMP-14 has been shown to make the specific collagen cleavage[6]. In the current study, we focused on MMP-13, of which close relationship with runt-related transcription factor (RUNX)-2 has been reported previously[7].

Aggrecanase-1 and -2 belong to the ADAMTS (a disintegrin and metalloproteinase with thrombospondin type 1 motifs) family of zinc metalloproteinases[8,9]. ADAMTS-1, -8, and -9 have weak aggrecan degrading activity[10–12], whereas ADAMTS-4 (aggrecanase-1) and -5 (aggrecanase-2) are known to be efficient cleavers of aggrecan *in vitro* and have been implicated in the structural damage seen in human OA. Which of the ADAMTSs is the primary aggrecanase responsible for aggrecan degradation in OA has been intensively investigated[13–18], but has not yet reached the general consensus in human OA. Elucidation of the mechanisms by which ADAMTSs are activated in OA has important implications for drug design. Previous reports and our recent report suggest that stimulation by pro-inflammatory cytokines such as interleukin (IL)-1 induces expression of *ADAMTS-4* and *-9* in chondrocytes[19,20]. Although mechanical stress-induced aggrecanase expressions have been clarified partially[21,22], overall has not been fully elucidated.

RUNX family members regulate the expression of the genes involved in cellular differentiation and cell cycle progression. RUNX-2 plays an essential role in bone mineralization (by stimulating osteoblast differentiation)[23]. Recent studies have demonstrated that RUNX-2 contributes to the pathogenesis of OA through chondrocyte hypertrophy and matrix breakdown after the induction of joint instability[24]. In Runx-2-deficient (Runx-$2^{+/-}$) mice, the pathology of instability-induced experimental OA was ameliorated and both type X collagen and *MMP-13* expressions were decreased compared with wild-type mice[24]. *In vitro*, cyclic tensile strain (CTS) is known to up-regulate *MMP-13* expression *via* the Runx-2/Cbfa1 pathway in primary chondrocytes[25]. These findings led us to investigate the effect of mechanical stress on RUNX-2 expression using SW1353 chondrocytes-like cells and the signal transduction pathways underlying mechanical stress-induced RUNX-2 expression. Our findings may contribute to the development of novel disease-modifying anti-OA drugs targeting the mechanical stress/RUNX-2 pathway.

Methods

Cells and cell culture

SW1353 cells were cultured in 10 mL Dulbecco's Modified Eagle Medium (DMEM, Wako, Osaka, Japan) containing 10% fetal bovine serum (FBS, HyClone, South Logan, UT), 100 U/mL penicillin, and 100 mg/mL streptomycin (Sigma, Tokyo, Japan) at 37°C in a humidified atmosphere of 5% CO_2 in air. The cells were subcultured at split ratios of 1:3–1:4 using trypsin plus ethylenediaminetetraacetic acid (EDTA) every 7–10 days. The medium was changed every 3 days. For most experiments, SW1353 cells were transferred to serum-free DMEM for 24 h before being exposed to the different stimuli.

Stretching experiments

SW1353 cells were seeded onto stretch chambers, each having a culture surface of 2×2 cm, at a concentration of 5×10^4 cells/chamber. The silicon chamber has a 200 μm-thick transparent bottom and the side wall is 400 μm thick to prevent narrowing of the bottom center. One end of the chamber is firmly attached to a fixed frame, while the other end is held on a movable frame. The movable frame is connected to a motor driven shaft. The amplitude and the frequency of stretch were controlled by a programmable microcomputer, ST-140 (STREX, Osaka, Japan). Silicon membrane was uniformly stretched over the whole membrane area and using this system, we could apply a known, uniform stretch to most of the cells[26,27]. After 48 h culture on stretch chamber, SW1353 cells increased to 50% confluence. CTS (0.5 Hz, 10% stretch) was applied for 30 min using ST-140. Unstretched cells cultured on stretch chambers were used as controls. To test the effect of 5% and 10% CTS (0.5 Hz) on anabolic gene expression by SW1353 cells, as the half life of α1 chain of type II collagen (*COL2A1*) mRNA is reported to be approximately 15 h, we examined the *COL2A1* expression at 15 and 30 h after CTS by real-time polymerase chain reaction (PCR). Next, we examined the effect of 10% CTS (0.5 Hz) on *RUNX-2*, *MMP-13* and *ADAMTS-4*, *-5*, *-9* expressions by SW1353 cells up to 24 h after CTS by reverse transcription (RT)-PCR and real-time PCR.

RNA preparation and RT-PCR

Following stimulation, the cells were washed once with phosphate buffered saline (PBS), and total RNA was extracted using ISOGEN (Nippon Gene, Toyama, Japan) reagent according to the manufacturer's instructions. Concentration and purity were assayed with spectrophotometry. One microgram of total RNA was reverse transcribed to complementary DNA (cDNA) using ReverTra Ace with Oligo-dT primers according to the manufacturer's protocol (TOYOBO, Tokyo, Japan). The cDNAs underwent PCR amplification in the presence of 10 pmol of each specific primer using ExTaq DNA polymerase (TAKARA BIO, Shiga, Japan). Each specific primer was optimized for concentrations before RT-PCR. The specific primer sets described in Table I were used. The cycle number was selected from the linear part of the amplification curve. For all the RT-PCR fragments, the reactions were allowed to proceed for 32 cycles for *COL2A1*, *RUNX-2*, *MMP-13*, *ADAMTS-5*, 31 cycles for *ADAMTS-4* and *-9*, 25 cycles for glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) in an iCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA). RT-PCR was performed with incubation at 94°C for 30 s, 60°C (55°C for *RUNX-2*) for 30 s, and 72°C for 30 s, with the final incubation at 72°C for 7 min.

Real-time PCR analyses

Real-time PCR was performed using a LightCycler Rapid Thermal Cycling system (Roche Diagnostics, Indianapolis, IN), using the LightCycler-FastStart DNA Master SYBR Green I kit (Roche Diagnostics) according to a previously reported protocol[28]. The PCR mixture consisted of 1 × SYBR Green PCR Master Mix, which included DNA polymerase, SYBR Green I dye, dNTPs (including dUTP), PCR buffer, 10 pmol of forward and reverse primers, and cDNA of samples, in a total volume of 15 μL. Amplification of a housekeeping gene,

Table I
Primer sequences of genes used for RT-PCR and real-time PCR analyses

Gene name	Primer sequence (5′–3′)	Primer sequence (3′–5′)
COL2A1	AAT TCC TGG AGC CAA AGG AT	AGG ACC AGT TGC ACC TTG AG
RUNX-2	CTC TAC CAC CCC GCT GTC TT	CAC CTG CCT GGC TCT TCT TAC
MMP-13	CTT GAT GCC ATT ACC AGT C	GGT TGG GAA GTT CTG GCC A
ADAMTS-4	AGG CAC TGG GCT ACT ACT AT	GGG ATA GTG ACC ACA TTG TT
ADAMTS-5	TAT GAC AAG TGC GGA GTA TG	TTC AGG GCT AAA TAG GCA GT
ADAMTS-9	GGA CAA GCG AAG GAC ATC C	ATC CAT CCA TAA TGG CTT CC
G3PDH	CAT CAA GAA GGT GGTGAA GCA G	CGT CAA AGG TGG AGG AGT GG