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Signaling pathways implicated in oncostatin M-induced aggrecanase-1 and matrix metalloproteinase-13 expression in human articular chondrocytes

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Abstract

Molecular mechanisms of oncostatin M (OSM)-stimulated cartilage extracellular matrix catabolism and signaling pathways were investigated in human arthritic chondrocytes. OSM, alone or with Interleukin-1 (IL-1β), increased glycosaminoglycan release and induced ADAMTS-4 and MMP-13 protein expression in human cartilage explants. OSM dose- and time-dependently increased ADAMTS-4 mRNA and MMP-13 protein expression in human femoral head chondrocytes. Extracellular signal-regulated kinases (ERK1/2)-MAPK pathway inhibitor, U0126, down-regulated ADAMTS-4 and MMP-13 induction by OSM. Janus kinase 2 (JAK2) inhibitor, AG490, suppressed OSM-induced ADAMTS-4 mRNA expression but did not affect MMP-13 levels while JAK3 pharmacological inhibitor and siRNA transfection suppressed both. Parthenolide, a signal transducer and activator of transcription (STAT1 and STAT3) phosphorylation inhibitor, reduced OSM-induced ADAMTS-4 and MMP-13 gene expression and prevented STAT1/3 DNA binding activity. Additionally, OSM-enhanced ADAMTS-4 mRNA and MMP-13 expression was down-regulated by phosphatidylinositol 3-kinase (PI3K) and Akt/PKB inhibitors, LY294002 and NL-71-101. Furthermore, JAK3 inhibition time-dependently down-regulated Akt but not ERK1/2 phosphorylation suggesting that Akt is a downstream target of JAK3. These results suggest that OSM-stimulated ADAMTS-4 and MMP-13 expression is mediated by ERK1/2, JAK3/STAT1/3 and PI3K/Akt and by cross talk between these pathways. The inhibitors of these cascades could block OSM-evoked degeneration of cartilage by ADAMTS-4 and MMP-13.

Keywords: Arthritis; Human chondrocytes; Matrix metalloproteinase; ADAMTS; Oncostatin M; Cell signaling

1. Introduction

The integrity of cartilage depends upon balance between matrix-degrading matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) that is controlled by the levels of proinflammatory and anti-inflammatory cytokines. These homeostatic balances are altered in arthritic joints due to excessive proinflammatory cytokines derived from endogenous and exogenous cells in synovium and cartilage [1,2]. Oncostatin M (OSM), a major cytokine produced by macrophages, neutrophils [3] and T cells, has both pro- and anti-inflammatory activities *in vivo*. Its levels are elevated in the patients with rheumatoid arthritis (RA) [4]. OSM by itself contributes to cartilage and bone loss in RA [5]. Blocking antibodies to OSM in mouse models of RA ameliorate arthritis [6]. Although not elevated in osteoarthritic (OA) synovial fluid [4], specific interaction of OSM with interstitial collagens [7] may contribute to its availability and local activities.

Loss of principal cartilage proteoglycan, aggrecan (which gives compressive stiffness) in arthritis occurs by multiple proteases. By measuring the activity with an aggrecan neoepitope antibody, aggrecanases or ADAMTS (*A D*isintegrin *A*nd *M*etalloproteinase with *t*hrombo*s*pondin motifs) were shown to cleave efficiently at multiple sites of the aggrecan core protein including between Glu^{373}–Ala^{374} [8]. ADAMTS-4 also cleaves at MMP site, Asn^{341}–Phe^{342} [9]. The c-terminal thrombospondin motif (TSP-1) of aggrecanase-1 binds with glycosaminoglycans of aggrecan and is critical for substrate recognition and cleavage [10]. ADAMTS-4-generated products are found in the cartilage and synovial fluid

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of OA patients indicating its prominent role in aggrecan catabolism [11]. OSM and IL-1 combination induces ADAMTS-4 RNA in human chondrocytes [12]. Both ADAMTS-4 and-5 are expressed in human arthritic synovium, though only former is inducible [13] and ADAMTS-4 cleaves aggrecan 2 times faster than ADAMTS-5 [14]. However, ADAMTS-5 is important for cartilage degradation in the mouse models of OA and RA [15,16]. The role of this enzyme in human arthritis remains to be elucidated. Thus, proteases from both synovial linings and chondrocytes can cause cartilage degradation. Aggrecanases are key targets for developing cartilage protective therapies, however, nothing is known about the proinflammatory/catabolic cytokine signaling and regulatory mechanisms controlling their expression in human femoral chondrocytes.

MMP-13 (collagenase-3) is another major enzyme that cleaves and denatures type II collagen 5–10 times more effectively than collagenase-1 in OA cartilage [17,18]. It also cleaves aggrecan at a different site [19]. Its expression is increased in OA cartilage [20]. Direct injection into knee or cartilage-specific overexpression of active human MMP-13 in joints led to tissue damage in transgenic mice, suggesting major implication of this enzyme in arthritis [21]. OSM alone and with other proinflammatory cytokines induces MMP-13 in articular chondrocytes [22]. Cytokine-stimulated proteolytic actions of aggrecanases followed by MMP-13 result in degradation of matrix components in human arthritic tissues and their inhibition is a valid therapeutic approach.

OSM stimulates ERK1/2, JNK1/2, p38 kinase and JAK/STAT activation in chondrocytes [22]. The PI3 kinase and its downstream kinases such as Akt/PKB kinase play a fundamental role in many biological functions such as cell growth, differentiation, survival, cytoskeletal organization, cell motility and inflammation [23]. The current study was aimed to identify the signal transduction pathways implicated in ADAMTS-4 induction by OSM compared to those of MMP-13. We demonstrate that OSM-induced ADAMTS-4 and MMP-13 expression is mediated by ERK, JAK/STAT and PI3K/Akt pathways in primary human chondrocytes from arthritic patients.

2. Experimental procedures

2.1. Materials

Recombinant human OSM and IL-1β were from R&D Systems (Minneapolis, MN). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum were purchased from Invitrogen (Burlington, ON). Human chondrosarcoma cell line, SW1353 was obtained from American Type Culture Collection (Manassas, VA). The phospho-specific p44/42 ERK (T202/Y204) E10 monoclonal and Akt/PKB antibodies were purchased from Cell Signaling (Pickering, ON). ADAMTS-4 (ab-16297) antibody was from Abcam (Cambridge, MA). Double-stranded STAT3 consensus oligonucleotides were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). U126, LY-294002, NL-71-101, JAK3 inhibitor I, and AG490 were from Calbiochem (La Jolla, CA). Nitrocellulose membranes were from Bio-Rad (Mississauga, ON). Enhanced chemiluminescence (ECL) reagent, leupeptin, pepstatin, aprotinin and fungal (*Streptomyces griseus*) pronase were from Roche Diagnostics (Laval, QC). MMP-13 (M4052) antibody (that detects both pro- and active forms of enzyme), bacterial (*Clostridium histolyticum*) collagenase type 1A and all other reagents were from Sigma (St. Louis, MO). JAK3 SMART pool siRNAs were purchased from Upstate (Lake Placid, NY). TransAM™ AP-1 assay kit was from ActiveMotif (Carlsbad, CA).

2.2. Methods

2.2.1. Human cartilage explants, culture of primary chondrocytes and SW1353 chondrocytic cells

The use of human tissues was approved by the institutional ethics committee. Human articular cartilage was obtained from the femoral heads of patients undergoing hip replacement surgery due to late-stage OA (female $n=15$ and age between 46 and 97 years, male $n=12$ and age between 47 and 89 years). Cartilage fragments (40–45 mg) were excised from the superficial and medial layers and cultured in 2 ml of DMEM supplemented with 10% fetal calf serum (FCS), penicillin (100 U/ml) and streptomycin (100 μg/ml) for 24 h prior to stimulating with different cytokines. For cell culture, immediately after harvesting the cartilage, the pieces were digested first with pronase for 60 min and then with collagenase for 9 h in DMEM. The chondrocytes were washed with phosphate buffered saline (PBS) and grown in DMEM supplemented with 10% FCS as high-density primary monolayer cultures until confluent growth. Cells were distributed in 6-well plates, grown to confluence, washed with PBS and kept in serum-free DMEM for 48 h. Human chondrosarcoma cell line, SW1353, was treated as described below for primary chondrocytes.

2.2.2. Glycosaminoglycan measurement by DMMB assay

Explants of human cartilage (40–45 mg) were stimulated with OSM (50 ng/ml) and/or IL-1β for 3 days in DMEM. Conditioned media were used to evaluate MMP-13 expression by Western blot and glycosaminoglycan (GAG) content by the dimethylmethylene blue (DMMB) assay based on the procedure of Farndal et al. [24].

2.2.3. RNA extraction and RT-PCR

Total RNA was extracted as previously described and aliquots of 3–5 μg analyzed by electrophoretic fractionation in 1.2% formaldehyde-agarose gels [22]. The integrity and quantity of applied RNA were verified by ethidium bromide staining of the gels and photography of the 28S and 18S ribosomal RNA bands. For RT-PCR, 2 mg of RNA was heated for 5 min at 65 °C and reverse transcribed in the mixture consisting of oligo d (T) 12–18mer, dNTPs, RNase inhibitor (Pharmacia), acetylated BSA (Promega) with Moloney murine leukemia-virus reverse transcriptase (MMLV-RT) (Invitrogen) according to the protocols of Clontech Laboratories Inc. (Paolo Alto, CA). Conditions of RT-PCR were same as described before for aggrecanase-1-specific primers [25]. The amplification profile was one cycle at 94 °C for 1 min, 35 cycles of 94 °C for one min, hybridization at 60 °C for 2 min and extension at 72 °C for 3 min., followed by one extension cycle of 7 min at 72 °C. The PCR was performed in a DNA cycler (Techne, Princeton, NJ) in a 50-ml reaction with 1.25 mM dNTPs, Taq DNA polymerase (Pharmacia) and respective primers. Aliquots of 10 ml from the 50 ml PCR reaction were analyzed on 1.4% agarose gels to detect ADAMTS-4 and glyceraldehyde phosphate dehydrogenase (GAPDH) cDNA amplification products of 692 and 226 bp respectively. Negative controls included either all the RT-PCR reagents except cDNA or additionally, RT was omitted before PCR. None of these controls gave any bands. Semi-quantitative estimates were obtained with NIH ImageJ 1.32j (National Institute of Health, USA) software by dividing ADAMTS-4 band intensities with those of GAPDH.

2.2.4. Western blot analysis

For measuring MMP-13 expression levels, chondrocytes were preincubated with different inhibitors (U0126 or parthenolide 5, 10 μM), JAK3 inhibitor (10, 30 μg/ml), LY-294002 and NL-71-101 (10, 20 μM) for 30 min. Cells were then stimulated with OSM (50 ng/ml) for 24 h. Total secreted proteins from 2 ml of conditioned medium of chondrocytes were concentrated by precipitation with trichloroacetic acid (TCA) and quantified by the Bio-Rad protein assay system. Proteins (5 μg) were separated by 10% SDS-PAGE. Blots were blocked in TBS-T containing 5% dry milk for 20 min at 37 °C. Thereafter, blots were probed with a polyclonal antibody against MMP-13 (1/1000 dilution) in blocking buffer at 4 °C overnight. Subsequently, membranes were washed in TBS-T buffer five times for 5 min. Detection was carried out using anti-rabbit