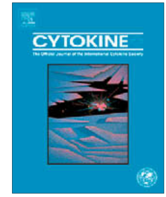
Cytokine 46 (2009) 332–338

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/issn/10434666



Role of a LIF antagonist in LIF and OSM induced MMP-1, MMP-3, and TIMP-1 expression by primary articular chondrocytes

Aradhana Upadhyay [a], Gaurav Sharma [a], Satu Kivivuori [b], Warren S. Raye [a], Ebrahim Zabihi [d], Graeme J. Carroll [c], Jalal A. Jazayeri [a,*]

[a] Medicinal Chemistry and Drug Action, Monash Institute of Pharmaceutical Sciences, Monash University, 381 Royal Parade, Parkville, Vic. 3052, Australia
[b] Smerud Medical Research Finland AB/Oy, Pihatörmä 1 C, FIN-02240 Espoo, Finland
[c] Department of Rheumatology, Fremantle Hospital, P.O. Box 480, Fremantle WA 6959, Australia
[d] Cellular and Molecular Biology Research Centre, Babol University of Medical Sciences, Iran

ARTICLE INFO

Article history:
Received 11 September 2008
Received in revised form 16 January 2009
Accepted 2 March 2009

Keywords:
Oncostatin M (OSM)
Leukemia inhibitory factor (LIF)
Cartilage
Matrix metalloproteinases (MMPs)

ABSTRACT

Cartilage degradation is mediated by matrix metalloproteinases (MMPs) and their inhibitors, tissue metalloproteinases (TIMPs), which are transcriptionally regulated by a variety of growth factors and cytokines. The levels of various MMPs as well as TIMPs have been shown to increase in response to certain cytokines. These include leukaemia inhibitory factor (LIF) and Oncostatin M (OSM), both of which have been detected in the synovial fluids of patients with rheumatoid arthritis (RA). However, the role of LIF and OSM in the regulation of various MMPs and TIMPs is still incompletely understood. The aims of this study were to examine the effects of LIF and OSM on MMP-1, MMP-3, and TIMP-1 production. In addition, the capacity of the LIF antagonist, MH35-BD, to block LIF and OSM induced MMP expression was examined. Primary chondrocytes, isolated from porcine metacarpophalangeal cartilage, were cultured in the presence and absence of LIF and OSM, with and without a predetermined concentration of the LIF antagonist. We analysed the levels of MMP-1, MMP-3 and TIMP-1 expression using qRT-PCR, Northern blot, and ELISA assays. The results indicate that LIF and OSM increase the expression of MMP-1, MMP-3, and TIMP-1 several fold. Furthermore their expression is reduced to basal levels in the presence of the LIF antagonist MH35-BD.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Cartilage degradation is a major pathological feature of inflammatory diseases of the joints such as RA. Resorption of cartilage is also a hallmark of osteoarthritis (OA), although here the etiologic and pathogenic processes may be quite different. A number of mechanisms are believed to be involved in the destruction of cartilage. These can be predominantly categorised as intrinsic or extrinsic mechanisms. Intrinsic resorption takes place when chondrocytes, the only cells present in the articular cartilage, exert their capacity to affect extracellular matrix resorption. Under the influence of cytokines, in particular TNF-α, IL-1, IL-6, LIF,[1] and OSM, these cells switch into a catabolic mode and degrade the surrounding extracellular matrix [1]. In contrast, extrinsic resorption is mediated by tissues or cells that lie outside the articular cartilage and which may be contiguous with it or more distant. For example in RA, synovial pannus may overlay and/or invade the cartilage from above or below the chondral surfaces [2]. In this context, resorption may be mediated by factors operating at the tissue interfaces or perhaps in response to humoral factors such as cytokines, enzymes, and oxygen radicals, which diffuse into the adjacent cartilage.

The MMPs are a family of enzymes which facilitate cartilage turnover and breakdown and whose levels are elevated in joint tissues of patients with RA and OA [3,4]. These proteolytic enzymes attack and degrade components of the extracellular matrix. Importantly, they contribute to collagen and other matrix protein breakdown. In addition to chondrocytes, other cell types synovial fibroblasts, monocytes, and macrophages also release MMPs, as well as their natural inhibitors, TIMPs [5–8]. TIMPs are produced in response to stimulation by many pro-inflammatory cytokines, TNF-α [9], IL-1 [10], OSM [11–13] amongst many others.

LIF shares the same receptor complex with other IL-6 subfamily cytokines. The receptor is comprised of an LIF receptor (LIF-R) subunit and a signal transduction element, notably glycoprotein 130 (gp130) [14]. LIF binds to the LIF-R α chain, the LIF/LIF-R complex is then able to bind to gp130 to form an LIF:LIF-R:gp130 signalling

* Corresponding author. Fax: +61 3 9903 9638.
E-mail address: jalal.jazayeri@pharm.monash.edu.au (J.A. Jazayeri).

[1] *Abbreviations used:* LIF, leukaemia inhibitory factor; OSM, oncostatin M; MMP matrix metalloproteinases; TIMP, tissue inhibitor matrix metalloproteinases; gp130, glycoprotein 130; IL-6, interleukin-6; qRT-PCR, quantitative reverse transcriptase-polymerase chain reaction; TNF-α, tumour necrosis factor-alpha; RA, rheumatoid arthritis; OA, osteoarthritis; ANOVA, analysis of variance; CI, confidence interval.

1043-4666/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.cyto.2009.03.001

complex [15]. OSM, on the other hand, binds to gp130 and signals through either a gp130:LIF-R or gp130 and OSM receptor (OSM-R) hetero-dimer [16]. The LIF mutant, MH35-BD will complex with LIF-R but has a reduced gp130 binding affinity and thus has a capacity to inhibit LIF-R/gp130 signalling [24]. LIF, OSM and other IL-6 subfamily cytokines, such as IL-6 are expressed in the cartilage and synovial membrane and also regulate MMP expression in the joint [17]. Using both human and bovine chondrocytes, OSM stimulation has been shown to increase most of the major MMPs implicated in cartilage degradation, in particular MMP-1, 3 and 13 [18].

LIF and OSM are suspected to contribute to the pathogenesis of RA. For instance, elevated concentrations of LIF and OSM have been observed in the synovial fluid aspirated from the joints of subjects with RA [19–21]. In addition, LIF and OSM have also been shown to be produced by cultured synovial cells and articular chondrocytes; and in combination with other cytokines, stimulate cartilage and bone resorption [22,23].

In this study, we used porcine primary articular chondrocytes to evaluate the effect of LIF and OSM on the expression of two of the proteolytic enzymes, MMP-1 and MMP-3, and one tissue metalloproteinase inhibitor, TIMP-1. The impact of a LIF antagonist, designated MH35-BD, on LIF and OSM induction, was also examined. MH35-BD is a mutant hybrid form of human LIF (hLIF) and murine LIF (mLIF) [24]. The overall and more general aim is to determine whether this antagonist can be safely and expeditiously used as a therapeutic drug for treatment of RA and OA and perhaps other inflammatory diseases in which LIF and OSM stimulate proteolytic enzyme production leading in turn to tissue degradation.

2. Materials and methods

2.1. Reagents

Recombinant human OSM and hLIF were purchased from Sapphire Bioscience (Sydney, Australia) and Chemicon (Melbourne, Australia), respectively. Nystatin, foetal calf serum (FCS), streptomycin, penicillin, L-glutamine and DMEM were all obtained from GIBCO (Gaithersburg, USA). MMP-1, MMP-3, and TIMP-1 ELISA assay kits were from Ray Biotech (Georgia, USA) while MMP-1/TIMP-1 complex kit was from R&D Systems (Sydney Australia). Hyaluronidase, bacterial collagenases, and trypsin were from Sigma–Aldrich (Sydney, Australia). All other reagents were obtained commercially and were of molecular biology grade. The LIF mutant, MH35BD/Q29AG124R (hereafter called MH35-BD) and the Ba/F3-hLIF-R/hgp130 cells were kindly provided by Dr. N. Nicola from the Walter and Elisa Hall Institute of Medical Research (WEHI), Melbourne Australia.

2.2. Protein expression and purification

MH35-BD was cloned and expressed as a glutathione-*S*-transferase (GST) fusion protein in *Escherichia coli*, strain BL21. After purification of the recombinant fusion protein by glutathione-agarose (Sigma–Aldrich, USA), the protein was cleaved from GST using thrombin (Sigma–Aldrich, USA), essentially following the method described by Smith and Johnson [25]. The purity of all proteins was confirmed by SDS–PAGE and silver staining.

2.3. Cell proliferation assays

To determine the biological activity of MH35-BD, a cell proliferation assay employing Ba/F3-hLIF-R/hgp130 cells was used. This cell line originates from an IL-3-dependent cell line derived from murine pro B lymphocytes, which do not normally express LIF-R or gpl30 [16]. To generate the stable Ba/F3-hLIF-R/hgp130 cell line, Ba/F3 cells were stably transfected with the human LIF-R (hLIF-R) and human gp130 (hgp130), after which they proliferated rapidly in the presence of hLIF [26]. Their proliferation is markedly decreased in the presence of hLIF antagonists. The assay procedure was conducted as described previously [27].

2.4. Isolation of primary chondrocytes and cell culture

Porcine metacarpophalangeal joint cartilage were cut into approximately 2–3 mm pieces and washed three times in phosphate buffered saline (PBS) containing penicillin (100 U/mL), streptomycin (100 mg/mL) and nystatin (40 U/mL). The cartilage pieces were digested by hyaluronidase (1 mg/mL, 15 min, 37 °C), trypsin (2.5 mg/mL, 30 min, 37 °C), and bacterial collagenases (3 mg/mL, 2 h, 37 °C). Cells were centrifuged at 200*g* for 5 min, washed in PBS, and resuspended in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% (v/v) FCS. Cells were cultured in monolayer in standard T-25 tissue culture flasks at $\sim 1.0 \times 10^6$ cells per flask. After 48 h, cells were subcultured into 6-well plates, in triplicate, and then incubated for 21 days to reach a confluency of 70%. Media was changed every 4 days.

Chondrocyte monolayers were grown to ~80% confluency and then treated with LIF or OSM at 10, 20, 50, and 100 ng/mL, in the presence and absence of a constant concentration 2.2 μg/mL (100 nM) of MH35-BD, and incubated for 12, 24, and 48 h, respectively. At the end of the incubation period lysis buffer (RLT; Qiagen, USA) was added to the cells and they were incubated for 10 min at 37 °C. The cell lysate was transferred to a 1.5 mL microcentrifuge tube and stored at −80 °C or used immediately for RNA extraction.

2.5. RNA isolation and cDNA synthesis

RNA was isolated from cell lysates using an RNeasy Mini Kit (Qiagen, Australia) according to the manufacturer's instructions. The concentration of total RNA was determined by measuring the A_{260} (1 A_{260} = 40 μg RNA/mL). The A_{260}/A_{280} ratios for the samples used were greater than 1.8. Synthesis of cDNA was conducted using SuperScript™ III Reverse Transcriptase (Invitrogen, Australia) in a 20 μL reaction according to the manufacturer's instructions. The starting amount of total RNA was 100 ng for all samples and the supplied reaction buffer contained oligo $(dT)_{20}$ (1.25 μM) and random hexamers (1.25 ng/μL). Tubes were incubated at 25 °C for 10 min followed by incubation at 42 °C for 50 min. The reaction was then terminated by incubating at 85 °C for 5 min before chilling all tubes on ice. To each reaction, 1 μL (2U) of *E. coli* RNase H (Invitrogen, Australia) was added and incubated for a further 20 min at 37 °C. Samples were either used directly in qRT-PCR or stored at −20 °C until used.

2.6. Cloning of the MMP genes

Genes were amplified using their respective primers and annealing temperatures (Table 1) in separate PCRs. Briefly, each PCR was carried out in 25 μL and contained reaction buffer (20 mM Tris–HCl pH 8.4, 50 mM KCl), 200 μM dATP, 200 μM dGTP, 200 μM dCTP, 200 μM dTTP, 3 mM $MgCl_2$, 0.5 U Platinum® *Taq* DNA Polymerase (Invitrogen, USA), 12.5 pmol each primer, PCR-grade water and 2 μL cDNA. PCR products were ligated into the pGEM®-T Easy Vector (Promega, USA). The ligation reaction consisted of Rapid Ligation Buffer (30 mM Tris–HCl pH 7.8, 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 1% polyethylene glycol; Promega, USA), 50 ng pGEM®-T Easy Vector, 3 Weiss units T4 DNA ligase and 3 μL PCR product. Ligation reactions were incubated at room temperature for 1 h and then transformed into Library Efficiency® DH5α™ Competent Cells (Gibco, USA). Successful cloning of an insert into the pGEM®-T Easy vector interrupts the coding sequence of β-galactosidase. Thus recombinant clones can be identified by

Download English Version:

https://daneshyari.com/en/article/2795238

Download Persian Version:

https://daneshyari.com/article/2795238

Daneshyari.com