Gene 573 (2015) 321–327

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Induction of human ADAMTS-2 gene expression by IL-1α is mediated by a multiple crosstalk of MEK/JNK and PI3K pathways in osteoblast like cells

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ARTICLE INFO

Article history:
Received 29 April 2015
Received in revised form 2 June 2015
Accepted 19 July 2015
Available online 29 July 2015

Keywords:
ADAMTS-2
ADAMTS-3
IL-1α
Saos-2
MG-63
MEK/JNK/PI3K

ABSTRACT

Up-regulation of ADAMTS genes with proinflammatory cytokines is important for some pathological conditions such as osteoarthritis (OA) that is a disease based on ECM degradation in cartilage. IL-1α is a proinflammatory cytokine and important both to normal and pathophysiologic conditions in cartilage and bone. Effects of some proinflammatory cytokines such as TNF-α and IL-1β on the some members of ADAMTS family have been investigated in some chondrocyte tissues or cell lines. However the effect of the IL-1α on the expression of ADAMTS-2 and ADAMTS-3 gene expression in osteoblast like cell lines, remains unclear. Therefore, the aim of this study is to investigate the effect of IL-1α on ADAMTS-2 and ADAMTS-3 gene expression in osteoblast like cells, Saos-2 and MG-63.

The present study, for the first time, demonstrated that IL-1α increases ADAMTS-2 and ADAMTS-3 gene expressions in both Saos-2 and MG-63 cells. Having correlation to mRNA induction, the upregulation of ADAMTS-2,-3 protein levels by IL-1α stimulation is also observed. The inhibition studies showed that this upregulation occurred at the level of transcription, and there was no effect of IL-1α on ADAMTS-2 mRNA half-life in Saos-2 cells. Transactivation potential of IL-1α on ADAMTS-2 promoter was investigated by transient transfection assay. Specifically, IL-1α strongly increased −658/+112 and −530/+112 ADAMTS-2 promoter constructs. Further, we analyzed signaling pathways involved in ADAMTS-2 induction. Pathway inhibition studies revealed that this upregulation depends on the activation of MEK, JNK and PI3K pathways. These findings suggested that IL-1α is a strong positive regulator of ADAMTS-2 and ADAMTS-3 expression. These findings would provide novel insight into the pathophysiology of OA.

1. Introduction

Osteoarthritis (OA) development and progression are now thought to involve inflammation even in the early stages of the disease (Lawrence et al., 1998; Cicuttini and Spector, 1995). Proinflammatory cytokines, such as interleukin-1 alpha (IL-1α), tumor necrosis factor alpha (TNF-α) are critical mediators of the disturbed metabolism and enhanced catabolism of the joints affected by OA. Interleukin 1 (IL-1) is considered to be one of the most important catabolic factors in joint diseas issues (Van den Berg, 2001; Goldring, 1999; Goldring, 2000). IL-1 promotes collagen degradation by stimulating metalloproteinases (Gowen et al., 1984; Martel-Pelletier et al., 1991). Matrix metalloproteinases (MMPs), such as MMP-1, 3 and 9, were considered as key enzymes in the degradation of the extracellular matrix (ECM) in bone. However, some studies showed that ADAMTS-5 (a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs)) was more efficient than MMPs in cleaving aggrecan core protein. It was reported that, in comparison to ADAMTS-5, MMP-3 was about 10–100 times less efficient on cleaving the aggrecan. Therefore, researchers suggested that ADAMTS-5 may be a suitable target for the development of new drugs to inhibit the development of cartilage destruction in arthritis (Wang et al., 2010; Durigova et al., 2011; Stanton et al., 2005; Glasson et al., 2005). Also, RUNX-2 (Runt-related transcription factor 2) contributes to the pathogenesis of OA through chondrocyte hypertrophy and matrix breakdown after the induction of joint instability (Tetsunaga et al., 2011; Pereira et al., 2009). To date, there are 19 ADAMTS family members that have been identified (Cal et al., 2002). Along with ADAMTS-5, few members of ADAMTS family have been implicated in osteoblast and cartilage. It was shown that another member of ADAMTS family, ADAMTS-1 protein, accumulates in osteoblast ECM during differentiation. Furthermore, the

Abbreviations: ADAMTS, A Disintegrin And Metalloproteinase with Thrombospondin Motifs; OA, Osteoarthritis; IL-1α, Interleukin-1 alpha; TNF-α, Tumor Necrosis Factor alpha; ECM, Extracellular Matrix; MMP, Matri Metalloproteinase; NT, Not treated; ActD, Actinomycin D; Chx, Cycloheximide.

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http://dx.doi.org/10.1016/j.gene.2015.07.064
0378-1119/

ADAMTS-1 expression in regions of osteogenesis implicates this multifunctional protein to be involved in mineralized nodule and bone formation (Lind et al., 2005; Miles et al., 2000). Another major group of ADAMTS proteins involved in cartilage remodeling are procollagen N proteinases ADAMTS-2, ADAMTS-3 and ADAMTS-14. ADAMTS-3 has already been reported to play a role in the processing of type II fibrillar collagen, the specific component of the articular cartilage (Porter et al., 2005). Increased ADAMTS-3 gene expression was also observed in some pathological conditions such as osteoarthritis, myocardial infarction, and breast cancer. In our previous study, the IL-6 cytokine that is the main regulator of bone resorption, upregulates ADAMTS-2 and ADAMTS-3 genes which mediated the JNK pathway in osteoblast (Alper and Kockar, 2014).

Despite its importance in biosynthetic processing of fibrillar procollagens and extracellular matrix, little is known about the role of the *ADAMTS-2* and *ADAMTS-3* genes in ECM degradation in osteoblasts. The objective of this study is to better understand the role of IL-1α cytokine that is the main regulator of cartilage destruction in OA on *ADAMTS-2* and *ADAMTS-3* gene expressions in osteoblastic cells, MG-63, and Saos-2. We determined that IL-1α stimulation results in a significant increase *ADAMTS-2* and *-3* gene expressions at mRNA and protein levels in two osteoblastic cell models. Moreover, this cytokine transactivates human *ADAMTS-2* gene promoter in osteoblastic cells. Pathway inhibition studies show that this upregulation depends on the activation of MEK, JNK and PI3K pathways.

2. Results

2.1. IL-1α elevates steady state levels of ADAMTS-2 and ADAMTS-3 mRNA in a time-dependent and cycloheximide-sensitive way but does not effect mRNA stability in osteosarcoma cells

Ephithelial-like Saos-2 cells have widely been used as models for human osteoblasts (Dinarello, 1997). These cells possess high affinity receptors for human recombinant IL-1α (Marmiroli et al., 1994; Rodan et al., 1990). IL-1α is a pro-inflammatory cytokine produced by macrophages as well as many other types of cells, which is able to modulate biosynthetic activities in bone cells (Bodo et al., 1992). To date, it has been reported that some pro inflammatory cytokines such as IL-6 can promote both differentiation and the activation of osteoblasts (Goff et al., 2010). It has also been reported by in vitro and in vivo studies that TNFα and IL-1α/β maintain osteosarcoma cells in an undifferentiated state and promote tumor growth (Mori et al., 2014). Firstly, we investigated the proliferative effect of the proinflammatory cytokine IL-1α on Saos-2 cells by MTT experiments. At a concentration of 500 U/ml of IL-1α was added serum starved Saos-2 cells and incubated 1 h, 24 h, 48 h and 72 h. We determined that the administration of the IL-1α increased cellular proliferation in Saos-2 cells up to ~1.8 fold with a statistically important manner at 48 h (Supplementary data).

In order to examine the effect of the proinflammatory mediator IL-1α on the steady-state levels of ADAMTS-2 and ADAMTS-3 mRNA, serum starved Saos-2 cells were treated with 500 U/ml of IL-1α for different time intervals, namely 1 h, 3 h, 6 h, 24 h, 48 h and 72 h. mRNA levels were quantitated by qRT-PCR and normalized to hβ-2. In each situation, ADAMTS-2 and ADAMTS-3 expression were compared to control cells not treated with IL-1α. Stimulation of Saos-2 with IL-1α resulted in up-regulation of ADAMTS-2 mRNA starting approx. 3 h after treatment reaching 3 fold of the initial levels (Fig. 1A). ADAMTS-3 mRNA level was also up-regulated up to 3 fold after 3 h of treatment (Fig. 1B). We further extended our study to another human osteosarcoma cell model, MG-63 that have the fibroblastic origin. We confirmed the upregulation of both ADAMTS-2 and ADAMTS-3 mRNA levels stimulation with IL-1α in MG-63 cells. ADAMTS-2 and ADAMTS-3 mRNA levels increased about 2.7 fold and 2.5 fold respectively (Fig. 1C and D) after 3 h of treatment (mean of three experiments). We, then, treated Saos-2 cells with actinomycin D and cycloheximide prior to 500 U/ml IL-1α treatment and examined the effect of IL-1α on ADAMTS-2 transcription. To directly assess whether this upregulation in mRNA level was due to altered transcription or mRNA stability we examined the half-life of the ADAMTS-2 mRNA in osteoblastic cells. Interestingly after the transcriptional blockade with Actinomycin D, in the first set of experiments; Actinomycin-D, with or without IL-1α, were added to the cells at different time points (15 min., 45 min., 60 min., 90 min. and 120 min). Total RNA was isolated at indicated time points. The level of ADAMTS-2 mRNA was analyzed by qRT-PCR relative of the level of transcripts for the housekeeping gene, hβ-2. Following the addition of Actinomycin D ADAMTS-2 mRNA decayed over 2 hour time course in the absence of IL-1α. The half-life of human ADAMTS-2 mRNA is relatively short (15 min) in osteoblast-like cells treated with Actinomycin D. Importantly, the addition of IL-1α did not increase the stability of ADAMTS-2 mRNA over 2 hour time points (Fig. 2A). Induction of ADAMTS-2 mRNA by IL-1α was observed in the presence of cycloheximide, indicating that de novo protein synthesis is not required for this response (Fig. 2B).

2.2. IL-1α increases ADAMTS-2 and ADAMTS-3 protein levels in Saos-2 and MG-63 cells

We also looked for changes in protein levels of the corresponding genes in response to 500 U/ml IL-1α stimulation in Saos-2 and MG-63 cells by western blotting. ADAMTS-2 protein level was induced in time-dependent manner starting from 3 h up to 48 h by reaching 5 fold in Saos-2 cells (Fig. 3A). In MG-63 cells, ADAMTS-2 induction started from 3 h about 2.3 fold reaching maximum level at 24 h (Fig. 3C). ADAMTS-3 protein level was induced up to about 7 fold after 1 h of stimulation and this effect maintained for 72 h in Saos-2 cells. In MG-63 cells, ADAMTS-3 protein level was induced up to 2.6 fold after 1 h of stimulation (Fig. 3D). The response of IL-1α in MG-63 cells was started at early hours and more obvious compared to Saos-2 cells. We validated upregulation of ADAMTS-2 and ADAMTS-3 protein levels by immunofluorescence assay. Saos-2 cells were treated 500 U/ml IL-1α for 24 h. Untreated groups were used as control. Consistent with the immunoblot analysis, IL-1α stimulation increased ADAMTS-2 and ADAMTS-3 protein expressions (Fig. 4.)

2.3. IL-1α activates human ADAMTS-2 promoter through MEK, JNK and PI3K pathways in Saos-2 cells

We then tested whether the upregulation of ADAMTS-2 with IL-1α occurs at the level of transcription. We have previously cloned and characterized the human *ADAMTS-2* gene promoter (Miles et al., 2000). We, therefore, decided to use these DNA constructs to rapidly delineate the minimal regulatory sequence(s) required for the action of IL-1α. Thus, four DNA constructs, containing 5′ truncations of the *ADAMTS-2* gene promoter in the pMetLuc vector, pMET_TS2_180 (−180/+112), pMET_TS2_324 (−324/+112), pMET_TS2_530 (−530/+112), pMET_TS2_760 (−658/+112), were transiently transfected into the Saos-2 cells and the reporter gene activity in cells that were either left untreated or exposed to IL-1α was determined. As shown in Fig. 5A, an IL-1α dependent induction in reporter gene activity was obtained with pMET_TS2_530 (−530/+112) and pMET_TS2_760 (−658/+112) promoter constructs. To evaluate roles of MEK, JNK, and PI3K signaling pathways in IL-1α mediated transactivation of ADAMTS-2 gene, selective inhibitors were introduced into pMET_TS2_760 transiently transfected Saos-2 cells prior to IL-1α stimulation. Luciferase activities were measured after 48 h of incubation from the cell culture media as described above and normalized to SEAP values. Fold change was estimated by comparison with indicated groups in Fig. 5B. It was determined that MEK, JNK, and PI3K inhibitor pre-treatments blocked IL-1α induced ADAMTS-2 promoter activity with a statistical significance (Fig. 5B).