Interleukin-1β upregulates matrix metalloproteinase-13 gene expression via c-Jun N-terminal kinase and p38 MAPK pathways in rat hepatic stellate cells

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Received June 9, 2013; Accepted October 7, 2013

DOI: 10.3892/mmr.2013.1719

Abstract. Matrix metalloproteinase-13 (MMP-13) is crucial in the cleavage and remodeling of the extracellular matrix (ECM), and its expression levels are decreased following the induction of liver fibrosis. The aim of the present study was to investigate the role of c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase (MAPK) in interleukin (IL)-1β-mediated MMP-13 gene expression in rat hepatic stellate cells (HSCs). In the present study, we demonstrated that IL-1β is capable of activating JNK and p38 in a time-dependent manner and the inhibition of the JNK pathway is able to increase MMP-13 mRNA expression; however, the inhibition of the p38 MAPK pathway is capable of inhibiting MMP-13 gene expression. These data demonstrate that IL-1β is able to promote MMP-13 mRNA expression in rat HSCs and the JNK and p38 MAPK pathways were involved in this process. In summary, IL-1β-induced MMP-13 mRNA expression is possibly mediated by cytoplasmic JNK and p38 MAPK pathways, and they play a distinct role in this process. Thus, the JNK and p38 MAPK pathway co-operatively mediate MMP-13 mRNA expression in rat HSCs.

Introduction

Following liver injury, the repair process comprises activation and proliferation of hepatic stellate cells (HSCs), which produce extracellular matrix (ECM) proteins (1-3). Once activated, HSCs upregulate gene expression of ECM components, matrix-degrading enzymes and their respective inhibitors, which in turn results in matrix remodeling. The balance between ECM deposition and remodeling determines whether fibrosis progresses or regresses (4,5). Antifibrogenic therapies aim to inhibit the activation of profibrogenic cells to prevent fibrillar collagen-I deposition, degrade the excessive ECM to recover the normal liver architecture and restore functional liver mass. Matrix metalloproteinases (MMPs) are important regulators of the ECM as they regulate cellular inflammation, ECM deposition and tissue reorganization. MMP-13, also called collagenase-3, is a member of the large family of MMPs and is important in the degradation of components of the ECM, particularly collagens. It degrades collagen type II efficiently and also collagen type I, III and X (6-7).

Mitogen-activated protein kinase (MAPK) cascades are signaling systems that transmit stimuli from outside the cell to the nucleus. Recent studies have demonstrated that MAPK signaling, converging on c-Jun NH2-terminal kinase (JNK) and p38 MAPK, plays a central role in liver injury and compensatory HSC proliferation, thus it has attracted considerable attention as a therapeutic target (8,9). JNKs and p38 MAPKs, also called stress-activated MAPKs, are preferentially activated by proinflammatory cytokines, including lipopolysaccharide endotoxin (LPS), tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1). Following activation, stress-activated MAPKs phosphorylate specific serine/threonine residues of target substrates and exert a variety of cellular functions, including cell death, survival, proliferation, migration and inflammation (10-13).

IL-1 is a pro-inflammatory cytokine and has long been implicated in promoting tissue fibrosis (14,15,16). Our previous studies have demonstrated that IL-1β is able to upregulate the gene expression of tissue inhibitors of matrix metalloproteinases (TIMPs) in rat HSCs via c-Jun N-terminal kinase and p38 MAPK pathways (17-20). In the present study, we demonstrate the association between the effects of IL-1β upregulating MMP-13 mRNA expression and JNK-p38 MAPK pathways in rat HSCs.

Materials and methods

Cell culture and treatments. The HSC cell line (characteristics of the fat-storing cell line, CFSC) was provided by

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Key words: matrix metalloproteinase-13, c-Jun N-terminal kinase, p38, MAPK, interleukin-1β, hepatic stellate cells

Professor Greenwell, Marion Bessin Liver Research Center, Albert Einstein College of Medicine (Bronx, NY, USA). The phenotype of the CFSC cell line is activated HSCs. Following spontaneous immortalization in culture, cells were seeded and grown in RPMI-1640 (Gibco-BRL, Grand Island, NY, USA) containing 10% fetal calf serum (FCS; Sijiqing Biological Products Company, Hangzhou, Zhejiang, China), 4 mmol/l glutamine, 1 mmol/l HEPES and 100 U/ml penicillin/streptomycin, at 37˚C and 5% CO_2. When the cells were 80-90% confluent, cell culture was continued in a non-serum medium for 12 h to synchronize the cells at G_0 phase. HSC proliferation was detected 24 h following the addition of IL-1β (10 μg/l; PeproTech Inc., Rocky Hill, NJ, USA). The JNK inhibitor SP600125 and p38 MAPK inhibitor SB203580 were purchased from Sigma Chemicals International (St. Louis, MO, USA).

Reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR was performed in order to measure the expression levels of MMP-13 mRNA in rat HSCs. The sequences of the primers for MMP-13 sense and antisense were 5'-GCG GGA ATC CTG AAG AAG TCT AC-3' (sense) and 5'-TTG GTC CAG GAG GAA AAG CG-3' (antisense), a 424 bp long fragment was amplified. The sequences of the primers for GAPDH sense and antisense were 5'-GGC CCC TCT GGA AAG CTG TG-3' (sense) and 5'-CCG CCT GCT TCA CCA CCT TCT-3' (antisense), a 239 bp long fragment was amplified. Total RNA was extracted from cultured HSCs using TRIzol reagent according to the manufacturer's instructions. Next, 2 μg RNA of each sample was reverse transcribed using a random primer and reverse transcriptase in 25 μl of volume. Subsequently, the PCR was performed in 25 μl of reaction mixture containing 5 μl cDNA templates, 2.5 μl 10X PCR-buffer, 1 μl 10 mmol/l dNTPs, 1.5 μl 15 pmol/l MMP-13 or GAPDH primers and 2.5 U Taq DNA polymerase. The cycle number was 35 cycles for MMP-13 and 30 cycles for GAPDH. PCR was conducted for 5 min at 94˚C for initial DNA denaturation, followed by individual cycles of denaturation (at 94˚C for 45 sec), annealing (at 56˚C for 35 sec), polymerization (at 72˚C for 45 sec) and then a final extension of 5 min at 72˚C. PCR products were electrophoresed on 2% agarose gel, stained with ethidium bromide (EB) and quantitated using Gel-Pro Analyzer Version 3.0. The intensity of the MMP-13 band was compared with the intensity of the GAPDH band, and the amount of MMP-13 mRNA was estimated.

Western blot analysis. The HSCs were lysed on ice by lysis buffer (RIPA, 50 mol/l Tris-HCl (pH 7.5), 150 mol/l NaCl, 10% glycerol, 1% Nonidet P-40, 1% SDS, 0.5% deoxycholate, 1.0 mol/l PMSF and 1 mol/l sodium orthovanadate). Protein samples (80 μg) were subjected to 10% SDS-PAGE gel electrophoresis and then transferred onto a nitrocellulose membrane by electroblotting. The membrane was incubated at 4˚C overnight in Tris-buffered saline/Tween-20 (20 mol/l Tris-HCl, pH 7.4, 150 mol/l NaCl, 0.05% Tween-20) with 5% nonfat milk. Following blocking, the membranes were incubated for 5 h at room temperature in Tris-buffered saline (TBS) buffer (50 mol/l Tris-HCl and 150 mol/l NaCl) containing a 1:100 dilution of mouse anti-phospho-JNK monoclonal antibody, anti-phospho-p38 monoclonal antibody (Santa Cruz Biotechnology, Inc., CA, USA) or rabbit anti-β-actin polyclonal antibody (Zhongshanjinqiao, Beijing, China). Next, membranes were incubated for 120 min at room temperature in TBS containing a 1:3,000 dilution of anti-mouse IgG (H+L)/HRP, 1:1,000 dilution of anti-mouse IgM (H+L)/HRP (Zhongshan Biotechnology, Beijing, China) or 1:3,000 dilution of goat anti-rabbit IgG antibody (Zhongshan Biotechnology). The level of phospho-JNK protein and phospho-p38 protein was normalized to the level of β-actin protein.

Statistical analysis. The data are presented as the mean ± SD of each group. The statistical software SPSS 11.0 (Bizinsight Information Technology Co., Ltd., Beijing, China) was used. Intergroup differences between two groups were analyzed by an independent t-test. The differences among multiple groups were analyzed by analysis of variance (ANOVA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

IL-1β upregulates mRNA expression of MMP-13. We examined the mRNA expression of MMP-13 in rat HSCs with RT-PCR. The ratio of MMP-13/GAPDH represents the expression of MMP-13 mRNA. The data demonstrate that MMP-13 mRNA expression (1.20±0.18) in the group treated with IL-1β (10 μg/l) for 24 h was significantly higher than that in the control group (0.68±0.17). There was statistical significance between the two groups ($P<0.01$; Fig. 1).

IL-1β activates JNK and p38 in a time-dependent manner. JNK activity was represented by the ratio of the mean value of JNK1 and JNK2/β-actin, and p38 activity was represented by the ratio of p38/β-actin. JNK activity following IL-1β treatment for 0, 5, 15, 30, 60, and 120 min was 0.982 (0.299), 1.501 (0.720), 2.133 (0.882), 3.360 (0.452), 2.181 (0.789) and 1.385 (0.368), respectively. We observed that JNK activity slightly increased following IL-1β treatment for 5 min, significantly increased following IL-1β treatment for 15 min and reached its peak following IL-1β treatment for 30 min. The activity returned to the initial level following IL-1β treatment for 120 min. The differences between JNK activity following treatment for 15, 30 and 60 min were significantly higher than the corresponding values in the control group ($P<0.01$, $P<0.01$, $P<0.01$). The p38 activity following IL-1β treatment for 0, 5, 15, 30, 60 and 120 min was 1.061 (0.310), 2.020 (0.863), 2.380 (0.573), 2.973 (0.953), 2.421 (0.793) and 1.755 (0.433), respectively. The p38 activity slightly increased following treatment for 5 min and reached its peak after 30 min. The activity returned to the initial level following treatment for 120 min. The differences between p38 activity following treatment for 5, 15, 30 or 60 min were significantly higher than the corresponding values in the control ($P<0.01$, $P<0.01$, $P<0.01$, $P<0.01$, respectively; Fig. 2).

Effect of SP600125 and SB203580 on IL-1β-induced expression of MMP-13 mRNA in rat HSCs. Following treatment with the inhibitor of the p38 MAPK pathway, SB203580, at 10, 20 and 40 μmol/l, the expression of MMP-13 mRNA was 0.81 (0.11), 0.64 (0.14) and 0.60 (0.10), respectively, compared

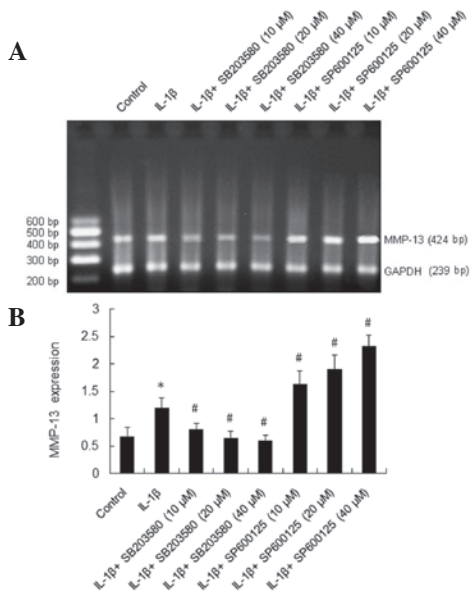

Figure 1. IL-1β upregulates MMP-13 mRNA expression and the effects of the inhibitors of p38 and JNK on IL-1β-induced MMP-13 mRNA expression in rat HSCs. (A) Representative RT-PCR results; (B) densitometry of RT-PCR analyzed by Gel-Pro software. Following incubation with SB203580 or SP600125 (10, 20, 40 μmol/l) for 30 min, the HSCs were continuously cultured with IL-1β (10 μg/l) for 24 h. IL-1β groups were treated with IL-1β (10 μg/l) for 24 h and the control group was treated with culture media only. Expression of MMP-13 mRNA was determined using RT-PCR. Data are expressed as the mean ± SD (n=6 per group). *P<0.01 vs. control; #P<0.01 vs. IL-1β. IL-1β, interleukin-1β; MMP-13, matrix metalloproteinase-13; JNK, c-Jun N-terminal kinase; HSCs, hepatic stellate cells; RT-PCR, reverse transcription-polymerase chain reaction.

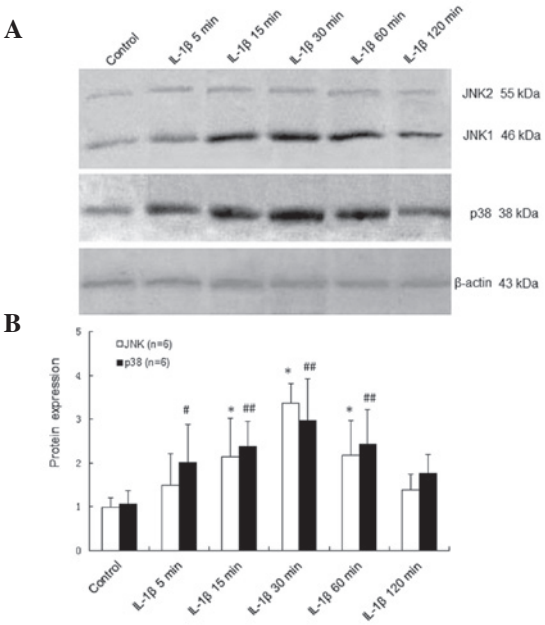

Figure 2. JNK and p38 activity following IL-1β stimulation in rat HSCs. (A) Respresentative Western blot analysis results of JNK and p38; (B) densitometry of Western blotting analyzed by Gel-Pro software. Control group was treated with culture media only, IL-1β group was treated with IL-1β (10 μg/l) for 0, 5, 15, 30, 60 and 120 min, then MAPK activity, including JNK and p38 in rat HSCs was evaluated in all groups by Western blotting. Data are expressed as the mean ± SD (n=6 per group). *P<0.01 vs. 0 of JNK; #P<0.05 vs. 0 of p38; ##P<0.01 vs. 0 of p38. IL-1β, interleukin-1β; JNK, c-Jun N-terminal kinase; HSCs, hepatic stellate cells; MAPK, mitogen-activated protein kinase.

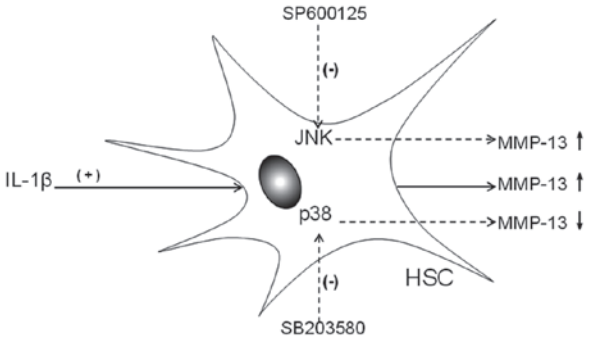

Figure 3. IL-1β upregulates MMP-13 gene expression via JNK and p38 pathways in rat HSCs. IL-1β, interleukin-1β; MMP-13, matrix metalloproteinase-13; JNK, c-Jun N-terminal kinase; HSC's, hepatic stellate cells.

with 1.20 (0.18) in the control group, thereby suggesting decreased MMP-13 mRNA expression. The inhibitory effect of SB203580 was concentration dependent [0.81 (0.11) vs. 1.20 (0.18), P<0.01; 0.64 (0.14) vs. 1.20 (0.18), P<0.01; and 0.60 (0.10) vs. 1.20 (0.18), P<0.01]. By contrast, following treatment with the inhibitor of the JNK pathway, SP600125, at 10, 20 and 40 μmol/l, the expression of MMP-13 mRNA was 1.63 (0.24), 1.91 (0.26), 2.33 (0.20), respectively, compared with 1.20 (0.18) in the control group, thereby suggesting increased MMP-13 mRNA expression (P<0.01, P<0.01, P<0.01, respectively; Fig. 1).

Discussion

At present, liver diseases constitute a major medical problem worldwide. Liver damage mainly occurs due to viral infections, excessive alcohol consumption, autoimmune reactions or as a consequence of adverse drug effects. Following liver tissue damage, HSCs undergo a transition from a quiescent to an activated phenotype and increase proliferation and synthesis of the ECM (21-23). Activated HSCs express MMPs, the key enzyme in the degradation of ECM; however, they also express TIMPs. Numerous cytokines may affect the activation of HSCs and regulate the secretion of MMPs and TIMPs.

There is increasing evidence supporting an important role for cytokines in various aspects of inflammatory liver diseases, including IL-1, IL-6 and TNF-α (24-26). These cytokines are produced in the liver by Kupffer cells and hepatocytes, playing roles in lipid metabolism and hepatic inflammation (27-29). We have recently demonstrated that IL-1β is able to promote the gene expression of TIMPs, proliferation and type I collagen synthesis in rat HSCs (17-20). In the present study, MMP-13 mRNA expression following treatment with IL-1β for 24 h was significantly higher than that in the control group. This suggests that IL-1β is also able to upregulate MMP-13 gene expression in rat HSCs. Therefore, our study revealed that IL-1β played a pivotal role in fibrosis regression, in part through the expression of MMP-13.

MAPKs, including extracellular signal-regulated kinases, JNK and p38 MAPK, belong to a family of Ser/Thr protein kinases that mediate numerous complex cellular programs, including gene expression, mitosis, differentiation, proliferation and cell survival in response to different

stimuli (12-14,30). IL-1 is able to activate JNK and p38 MAPK in various types of cells. It has been demonstrated previously that IL-1β is capable of promoting proliferation, type I collagen synthesis and TIMP-1 gene expression via c-Jun N-terminal kinase and p38 MAPK pathways in rat HSCs. Our present data demonstrated that following stimulation with IL-1β, the two MAPK pathways, JNK and p38 MAPK, were both activated. Following stimulation with IL-1β for 15 min, the level of phosphorylated JNK significantly increased compared with that in the control and reached the peak 30 min later. The level returned to the initial level 120 min later. By contrast, following stimulation with IL-1β for 5 min, the level of activated p38 significantly increased compared with that in the control and reached the peak 30 min later. The level returned to the initial level 120 min later. This finding suggested that IL-1β is able to activate JNK and p38 in a time-dependent manner and further confirmed that IL-1β is able to upregulate MMP-13 gene expression through JNK and p38 pathways in HSCs. To investigate the association between MAPK phosphorylation and MMP-13 gene expression induced by IL-1β, HSCs were pretreated with SB203580 and SP600125. Our study demonstrated that inhibition of the JNK pathway is able to increase MMP-13 gene expression; however, inhibition of the p38 pathway is able to inhibit MMP-13 gene expression. Taken together with the results of the present study, these data suggest that upregulation of JNK appears to be deleterious, whereas p38 MAPK may protect IL-1β-mediated MMP-13 gene expression in HSCs. This finding further suggested that JNK and p38 played important roles in IL-1β-induced MMP-13 gene expression in HSCs. Although these two pathways have opposite functions, their co-regulation may increase MMP-13 gene expression (Fig. 3). MMP-13 gene expression is usually controlled by the co-effects of different signaling pathways rather than any single pathway. JNK and p38 constitute a small part of the complex intracellular signaling pathway through which IL-1β promotes HSC MMP-13 gene expression. Further studies are needed in order to elucidate other pathways, which may also be involved.

In conclusion, we provided evidence that IL-1β is capable of promoting MMP-13 mRNA expression in rat HSCs and the JNK and p38 MAPK pathways were involved in this process. In summary, IL-1β-induced MMP-13 mRNA expression is possibly mediated by cytoplasmic JNK and p38 MAPK pathways and they play a distinct role in this process. Thus, JNK and p38 MAPK pathways co-operatively mediate MMP-13 mRNA expression in rat HSCs. A better understanding of these pathways may be useful in order to identify targets for hepatic fibrosis therapy.

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