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Interleukin-6 increases rat metalloproteinase-13 gene expression through Janus kinase-2-mediated inhibition of serine/threonine phosphatase-2A

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Abstract

Interleukin-6 (IL-6) increases metalloproteinase-13 (MMP-13) gene expression by increasing phosphorylated c-Jun and by inhibiting serine/threonine phosphatase-2A (PP2A) activity. We investigated the mechanisms by which IL-6 induces c-Jun phosphorylation and PP2A inactivation in Rat-1 fibroblasts. We show that IL-6 increased MMP-13 mRNA, phosphorylated c-Jun, and activator protein 1 (AP1) binding activity without increasing c-Jun-N-terminal kinase (JNK) activity. These effects did not seem to be mediated by ERK, p38 MAP kinase, phosphatidylinositol-3-kinase, calmoduline-dependent protein kinase, protein kinase C (PKC) or protein kinase A since inhibition with specific inhibitors did not abrogate these effects. IL-6 increases PP2A catalytic subunit tyrosine phosphorylation. Inhibition of the tyrosine kinase Jak2, with the specific inhibitor AG490, abrogated this effect. Likewise, this Jak2 inhibitor blocked the effects of IL-6 on c-Jun phosphorylation, AP1 binding activity and metalloproteinase-13 gene expression. We conclude that IL-6 increases MMP-13 gene expression by activation of Jak2, resulting in tyrosine phosphorylation of the catalytic subunit of PP2A, which in turn decreases PP2A activity and prolongs c-Jun phosphorylation.
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Keywords: Collagenase; Interleukin-6; Phosphatase 2A; JAK-2

Interleukin-6 (IL-6) is a multifunctional glycoprotein produced by activated monocytes, macrophages, endothelial cells, and hepatic stellate cells that mediates a wide variety of functions in different cells, including fibroblasts, hepatocytes, and hepatic stellate cells [1]. IL-6 promotes cell proliferation and differentiation and regulates specific gene expression [2], particularly the expression of the acute phase proteins in liver cells [1,3].

Acute and chronic liver diseases, particularly alcoholic liver diseases, are similar to the acute phase response in some respects. Thus, patients with alcoholic hepatitis show fever, muscle wasting, neutrophilia, and increased production of C-reactive protein, α_1-antitrypsin and amyloid A [4,5]. High levels of IL-6 have been detected in the sera of patients with alcoholic liver cirrhosis [6–8] and some authors have shown a correlation between circulating concentrations of IL-6 and serum concentrations of C-reactive protein [9,10], an IL-6-induced protein. Thus, IL-6 seems to be one of the most important factors regulating inflammatory response in the liver.

Matrix metalloproteinases (MMPs) constitute a family of structurally related zymogens capable of degrading a wide variety of extracellular matrix components [11], and, therefore, they may play a key role in the resolution of liver fibrosis upon withdrawal of the injurious agent. In rats and mice, there is only one interstitial MMP, the MMP-13, that shares 86% homology with human MMP-13. A variety of biologically active agents, such as tumor necrosis factor-α, interleukin-1, and tumor promoters (phorbol esters), mod-

Abbreviations: AP1, activator protein 1; IL-6, Interleukin-6; JAK, Janus kinase; JNK, c-Jun-N-terminal kinase; MAP, mitogen-activated protein; MMPs, Matrix metalloproteinases; PKC, protein kinase C; PP2A, serine/threonine phosphatase 2A; $PP2A_C$, catalytic subunit of PP2A; PP2A-A, structural subunit of PP2A.

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ulates the synthesis of these enzymes and their natural inhibitors, tissue inhibitors of MMPs [12–14].

In a previous study, we demonstrated that IL-6 stimulates MMP-13 gene expression by acting on an activator protein 1 (AP1) binding site in the MMP-13 promoter after inducing the synthesis and phosphorylation of AP1 proteins, including c-Jun [15]. Phosphorylation state of a protein is a dynamic process controlled by both protein kinases and protein phosphatases [16]. In our study, we provided evidence indicating that increase in phosphorylated c-Jun was not mediated by enhanced c-Jun-N-terminal kinase (JNK) activity but was associated with decreased serine/threonine phosphatase 2A activity (PP2A) [15].

Core structure of PP2A consists of a 36-kDa catalytic C subunit ($PP2A_C$) complexed with a structural A subunit of 65 kDa (PP2A-A). This dimer is associated with a third, variable regulatory B subunit, that likely influences substrate specificity or cellular localization [17]. Phosphatase activity is regulated by the phosphorylation on tyrosine-307 at the C-terminus of the catalytic subunit of PP2A. While this phosphorylation leads to a 90% loss in activity, dephosphorylation reactivates this enzyme [17]. Activation of cytokine receptors by tyrosine phosphorylation leads to transient inactivation of PP2A and thereby prolongs activation of protein kinases [17,18]. Considering this mechanism of regulation of the PP2A activity, we could hypothesize that binding of IL-6 to specific receptors on cell surface would inactivate PP2A by increasing tyrosine phosphorylation on its catalytic subunit which, in turn, would prolong activation of AP1. Similar sequence of events has been shown following addition of insulin to skeletal muscle cells [19,20]. Inhibition of PP2A with I_2^{PP2A}, a protein that binds to the catalytic subunit of this phosphatase, increased concentration and DNA binding of c-Jun and transcriptional activity of AP1 [21].

Although JNK is the major protein kinase involved in c-Jun phosphorylation at serines 63 and 73 [22], a variety of other protein kinases, including ERK-1, ERK-2, and p38 kinase, may also efficiently phosphorylate c-Jun and activate AP1 [23].

To investigate the mechanisms of c-Jun phosphorylation and PP2A inactivation by IL-6 in vitro, we examined the role played by non-JNK protein kinase in IL-6-induced c-Jun phosphorylation and the effect of IL-6 on tyrosine phosphorylation of the catalytic subunit of PP2A.

1. Materials and methods

1.1. Cell culture

Rat-1 fibroblasts obtained from American Type Culture Collection were grown at 37 °C in an atmosphere of 5% CO_2, 95% air in cell culture flasks using 10 ml of Dulbecco's minimum essential medium with Earle's salts containing 5% fetal bovine serum (Bio-Whittaker, Verviers, Belgium), 0.5 mg/ml L-glutamine, 100 units/ml penicillin G and 0.1 mg/ml streptomycin.

1.2. RNA preparation and Northern analysis

Total RNA was prepared from cultured Rat-1 fibroblasts as described by Chomczynski and Sacchi [24]. cDNA probes for rat MMP-13 and 18S RNA (*Eco*R1 fragment of the pBR322 plasmid) were labeled using random priming DNA labeling kit (Amersham Biosciences, Europe, Barcelona, Spain). Membranes were hybridized and washed with a final stringency of 0.1×SSC, 0.1% SDS, and then analyzed by autoradiography. The autoradiograms were quantitated by scanning laser densitometry (Desk Top Scanner Plus, Pharmacia Biotech, Barcelona. Spain).

1.3. Preparation of cytosolic and nuclear extracts and gel mobility shift assays

Cytosolic and nuclear proteins from Rat-1 fibroblasts untreated and treated with IL-6 were extracted by the method of Dignam et al. [25]. The pellet corresponding to the nuclei was resuspended in 50 μL Dignam C buffer and protein concentration was determined by the BioRad assay according to manufacturer's instructions. An oligonucleotide representing the consensus AP1 binding site from the human collagenase gene was synthesized and end labeled using the Klenow fragment and [^{32}P]dCTP (Amersham Biosciences) as described elsewhere [15]. For competition experiments, 200-fold unlabeled annealed oligonucleotide was added to binding reactions.

1.4. Western blot analysis

Whole cell protein extracts were prepared from Rat-1 fibroblasts cultured on plastic until confluence as previously described [15]. The filters were incubated with polyclonal antibodies to c-Jun, JNK, phosphorylated $PP2A_C$ or PP2A-A_α, monoclonal antibodies to serine-63 phosphorylated c-Jun, phosphorylated JNK (Santa Cruz Biotechnology, Santa Cruz, CA) or HRP-conjugated phosphotyrosine antibody (Upstate Biotechnology, Lake Placid, NY). The antibodies to c-Jun and phosphorylated c-Jun react with rat as well as with human and mouse protein. The antibody to phosphorylated JNK is raised against a peptide containing phosphorylated Thr-183 and Tyr-185 of JNK1 of human origin and is not cross-reactive with Erk1, Erk2 or p38 MAP kinases. Goat polyclonal antibody recognizing $PP2A_C$ is raised against an amino acid sequence containing phosphorylated Tyr-307 of $PP2A_C$. Anti-PP2A-A_α is a goat polyclonal IgG antibody non-cross-reactive against other PP2A subunits. HRP-conjugated antiphosphotyrosine is specific for phosphotyrosine-containing proteins of all species. Signals were detected using the ECL Western Blotting Detection Reagent (Amersham Biosciences).

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