Biochimie 94 (2012) 1390–1397

Contents lists available at SciVerse ScienceDirect

Biochimie

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

Research paper

Transcription factors Sp1 and Sp3 regulate basal transcription of the human IRF-3 gene

Hua-Guo Xu [a,b,1], Rui Jin [a,1], Wei Ren [a], Li Zou [a], Yi Wang [a], Guo-Ping Zhou [a,*]

[a] Department of Pediatrics, The First Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu Province 210029, China
[b] Department of Clinical Laboratory, The First Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu Province 210029, China

ARTICLE INFO

Article history:
Received 14 November 2011
Accepted 12 March 2012
Available online 17 March 2012

Keywords:
Interferon regulatory factor 3 (IRF-3)
Transcriptional activity
Promoter
Sp1
Sp3

ABSTRACT

Interferon regulatory factor 3 (IRF-3) plays a crucial role in initiation and development of the IFN antiviral response. The expression level of human IRF-3 is thought to be closely related to antiviral state of cells. However, the mechanisms of the transcription regulation of IRF-3 have remained largely unknown. We previously reported that transcription factor E2F1 negatively regulates the basal transcriptional activity of IRF-3. Here we demonstrate that transcription factors Sp1 and Sp3 up-regulate the basal transcriptional activity of IRF-3 and increase IRF-3 expression at mRNA level. By transient transfection analysis we revealed that mutation of Sp1/NRF-1 binding site resulted in a profound reduction of IRF-3 promoter activity. Overexpression of Sp1 and Sp3, but not NRF-1, transactivated the IRF-3 promoter activity in reporter gene assays while knocking-down of endogenous Sp1 and Sp3 by a shRNA strategy markedly inhibited IRF-3 promoter activity. Chromatin immunoprecipitation (ChIP) assays showed that Sp1 and Sp3 interact with the IRF-3 promoter in vivo. These results suggest that basal expression level of IRF-3 is regulated by transcription factors Sp1 and Sp3.

1. Introduction

Interferon regulatory factors (IRFs), a family of transcription mediators, have been shown to play crucial roles in the transcriptional regulation of type I IFN genes, IFN-stimulated genes (ISG), and other cytokines and chemokines [1–4]. So far, nine IRF members, IRF-1 to -9, have been found and identified on different human chromosomes [5]. IRF-3, an important member of the IRF family, plays an essential role in virus and double-stranded RNA mediated induction of type I IFN genes [6]. IRF-3 is a ubiquitously expressed phosphoprotein of 427 amino acids. Under normal conditions, IRF-3 is constitutively present as a monomer in the cell cytoplasm. Viral infection can trigger the phosphorylation of IRF-3, mediated by cellular TBK-1 and IKKε, which leads to its conformational change and activation. The activated IRF-3 translocate to the nucleus, and form homodimerization and heterodimerization, and further associate with the coactivator CBP/p300, which leads to the transcriptional activation of IFN-β and other IFN-stimulated genes [6–8].

Recent studies have shown that the expression level of human IRF-3 is closely related to antiviral state of cells. Decrease of endogenous IRF-3 RNA and protein levels suppresses induction of type I IFN, specifically IFN-β, and increases susceptibility to infection with virus [9]. Overexpression of IRF-3 significantly increases virus-mediated expression of type I IFN genes and results in the induction of an antiviral state [10]. However, the mechanisms of the transcription regulation of IRF-3 have remained largely unknown. Previously, we reported that the transcription of the human IRF-3 gene is controlled by a minimal promoter which is located within a region of 56 bp at a position of -149/-93 bp relative to the transcription start site (TSS) in HEK 293 cells and transcription factor E2F1 negatively regulates human IRF-3 gene promoter through binding to this region [11,12]. However, we did not find any transcription factor that positively regulates IRF-3 transcription activity.

In this study, we show exogenous Sp1 and Sp3 expression, but not NRF-1, leads to a significant increase in promoter activity and IRF-3 mRNA expression. We demonstrate here that Sp1 and Sp3 positively regulate IRF-3 transcription through binding to the Sp1/NRF-1 binding site in the minimal promoter region of IRF-3 gene.

* Corresponding author. Nanjing Medical University, Department of Pediatrics, The First Affiliated Hospital, 300 Guang Zhou Road, Nanjing, Jiangsu Province 210029, China. Tel.: +86 25 51716487; fax: +86 25 83724440.
E-mail address: gpzhou2003@126.com (G.-P. Zhou).
[1] Hua-Guo Xu and Rui Jin have contributed equally to this work.

0300-9084/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2012.03.011

2. Materials and methods

2.1. Cell culture

Human embryonic kidney (HEK) 293T cells and Human cervical cancer HeLa cells were obtained firstly from the American Type Culture Collection (ATCC). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS), 100 unit/ml penicillin and 100 μg/ml streptomycin at 37 °C with 5% CO_2 humidified air.

2.2. Plasmids

The cloning of the human IRF-3 gene promoter region was performed as described previously [11]. The primers and final PCR products were listed in Table 1 and Fig. 1. The expression plasmids pN3-Sp1, pN3-Sp3, pN3 empty vector were kindly provided by Dr. Guntram Suske. Sp1 shRNA, Sp3 shRNA and the negative control were gifted by Dr. Grace Gill [13]. Sequences targeted in the Sp1 and Sp3 mRNAs as well as the negative control sequence are listed below:

Sp1i: 5′GGGAACATCACCTTGCTACCT3′
Sp3i: 5′GGGACCAACAACATCAAGAAG3′
control: 5′GGGAATTAATATGCACAGGCC3′

pcDNA3 was purchased from Invitrogen (Carlsbad, CA, USA). pcDNA3-Flag-NRF-1 which contains the full length human NRF-1 was a gift from Dr. Raymond J. Deshaies (Howard Hughes Medical Institute, Pasadena, CA, USA). A dominant negative pcDNA3-Flag-dnNRF-1 construct which expresses amino acids 1–342 of NRF-1 but lacks the transactivation domain was a generous gift from Dr. Andres J. Klein-Szanto (Fox Chase Cancer Center, Philadelphia, PA, USA). pcDNA3-HA-hPGC-1α was a gift from Dr. A. Kralli (The Scripps Research Institute, La Jolla, CA, USA).

2.3. Site-directed mutagenesis

Mutations of the C/EBP-α, Sp1/NRF-1 or Sp1 binding sites found in the IRF-3 promoter were performed by the generation of point mutations from the wild-type promoter region. The point mutations were generated by using the QuikChange Site-directed Mutagenesis kit. The mutations were confirmed by sequencing.

Table 1
Oligonucleotide sequences used for generation of reporter constructs.

Constuct name	Primer sequence (5′–3′): sense + antisense
pGL-149	F1 (sense): CGGGGTACCGTCTCCTCCACTGAACTCGTAC + R1 (antisense): GGAAGATCTGCCCTTTTTTGGGTTTCC
pGL-131	F2 (sense): CGGGG7/ACCGTACAAACTTTATTAGTTCAA + R1
pGL-115	F3 (sense): CGGGGTACCGTTCAACTTTCCCGCGCCTG + R1
pGL-109	F4 (sense): CGGGG7ACCCTTTCCCGCGCCTGCGC + R1
pGL-105	F5 (sense): CGGGGVACCCCCGCGCCTGCGCACTGG + R1
pGL-102	F6 (sense): CGGGGVACCGCGCCTGCGCACTGGGCC + R1
pGL-97	F7 (sense): CGGGGTACCTGCGCACTGGGCCGGGTC + R1
pGL-93	F8 (sense): CGGGG7ACCACTGGGCCGGGTCGATAAC + R1
pGL-67	F9 (sense): CGGGG7ACCGCGTAGAAAGGGCGGAAC + R1
pGL-149C/EBPamut	F10 (sense): ACTTTCCCGCGCCTGCGCACT + R10 (antisense): AAGTTTGTACGAGTTCAGTGGAGG
pGL-149Sp1/NRF-1mut	F11 (sense): TTCAACTTTCCCGCTGATGCGC + R11 (antisense): GTTGAACTAATAAAGTTTGTA
pGL-149Sp1 mut	F12 (sense): TGCGCACTGGAATGGGTCGAT + R12 (antisense): GGCGCGGGAAAGTTGAACTA

Restriction sites used for subcloning are in italics.

2.4. Transient transfections and dual-luciferase reporter assays

Transfections were carried out in HeLa cells by using Lipofectamine™2000 according to the manufacturer's suggestion. HeLa cells were seeded into 96-well plates 24 h before transfection. Cells were cotransfected with 200 ng of each of the luciferase-containing plasmids together with 2 ng of a control pRL-TK plasmid as an internal control. Luciferase assay was performed 24 h after transfection by using the Dual Reporter assay system (Promega) and TD-20/20 Turner Designs luminometer according to the manufacturer's protocol. Results were representative of at least three independent experiments performed in triplicate. For overexpression or RNAi, the expression plasmid or shRNA (100 ng) was individually cotransfected into HeLa cells, together with appropriate IRF-3 promoter reporter plasmids (100 ng) by using Lipofectamine™2000 (Invitrogen). Luciferase assay was performed 24 h after transfection.

2.5. Chromatin immunoprecipitation assay

The chromatin immunoprecipitation assay was performed by using the Chip-IT kit (Active Motif) following the manufacturer's instructions. Briefly, three 100 cm^2 dishes of 80–90%-confluent HeLa cells were treated with 1% formaldehyde in PBS for 10 min at room temperature. The formaldehyde was inactivated by the addition of 0.125 M glycine in PBS to the cells for 5 min at room temperature. The cells were then washed in ice-cold PBS and then lysed with lysis buffer containing 1% SDS. Sonication of cross-linked chromatin was performed at 200 W with five rounds of 20s pulses so that chromatin fragments thus were obtained ranged from 200 to 1000 bp in size. Soluble chromatin was subjected to overnight immunoprecipitation with antibodies against Sp1, Sp3 or IgG antibody (Santa Cruz Biotechnology). A portion of the chromatin solution was kept to check the amount of input DNA in different samples before immunoprecipitation. For each immunoprecipitation, 2 μg of the appropriate antibody was incubated with a pre-cleared chromatin aliquot overnight at 4 °C. Following immunoprecipitation and elution, the eluent was heated to 65 °C for 6 h to reverse the cross-link and then DNA was purified by using mini-columns provided with the kit. The purified DNA was amplified by the promoter-specific primers (ChIP-F, 5′-CAC CCC TCG TCA ACA CCC-3′ and ChIP-R, 5′-CGC GGG AAA GTT GAA CTA ATA-3′), and PCR was performed under the following conditions: 1 cycle at 94 °C for 5 min; 36 cycles of 30 s at 94 °C, 30 s at 59 °C, 30 s at 72 °C; and a final extension step for 10 min at 72 °C. The PCR products were analyzed by electrophoresis on a 2% agarose gel.

2.6. RNA purification and quantitative real-time RT-PCR

Total RNA extraction was performed by using Trizol reagent followed by chloroform-isopropanol extraction and ethanol precipitation. Subsequently, duplicate samples of 1 μl of each cDNA were used as a template. The quantification of gene transcripts was performed by real-time PCR using SYBR green I dye (Invitrogen) and the ABI PRISM 7700 sequence detection system (PE Applied Biosystems, Wellesley, MA, USA). The specificity of amplification was assessed for each sample by melting curve analysis. Expression values were normalized with control GAPDH. The primers used are as follows: sense primer 5′-GG ACC CTC ACG ACC CAC ATA-3′ and antisense primer 5′-C CAT GTT ACC CAG TAA CTC ATC CAG -3′ for IRF-3 and sense primer 5′-AGG TCG GAG TCA ACG GAT -3′ and antisense primer 5′-TCC TGG AAG ATG GTG ATG-3′ for GAPDH [12].