EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 37, No. 5, 488-491, October 2005

Transcription repression of a CCAAT-binding transcription factor CBF/HSP70 by p53

Hee-Don Chae[1], Jeanho Yun[1] and Deug Y. Shin[1,2]

[1]National Research Laboratory for Cell Cycle Regulation
Department of Microbiology
Dankook University College of Medicine
Cheonan 330-714, Korea
[2]Corresponding author: Tel, 82-41-550-3878;
Fax, 82-41-550-3864; E-mail, dreamer@dku.edu

Accepted 11 July 2005

Abbreviations: CBF, CCAAT-binding factor; HSP70, heat shock protein 70; NF-Y, Nuclear factor-Y; cdk2, cyclin-dependent kinase 2; RIPA butter, radioimmunoprecipitation assay buffer

Abstract

NF-Y transcription factor binds to CCAAT boxes on promoters of cell cycle regulatory genes such as *cdc2*, *cyclin B*, *cdc25C*, and *cyclin A*. We previously reported that the DNA binding activity of NF-Y is regulated by p53-p21-cdk2 pathway. CBF/HSP70 was originally identified as a transcription factor binding to the CCAAT box on the hsp70 promoter and mediates transcription repression of hsp70 promoter by p53. Recently it was demonstrated that CBF/HSP70 interacts and cooperates with NF-Y. In this study, we found that p53 represses the transcription of CBF/HSP70. Since transactivation ability of NF-Y is regulated in a cell cycle-dependent manner, we examined the transcription of CBF/HSP70 during the cell cycle. After synchronization of a human bladder carcinoma cell lacking functional p53 at early S phase, we infect the cells with adenovirus encoding p53. Cells infected with control virus progressed to S and G2 after release from the arrest. In contrast, cells expressing p53 enter S and G2 phases, but arrest at G2/M. The expression of CBF/HSP70 was induced at S/G2 phase in cells infected with a control virus, but kept to be repressed in cells expressing p53. Thus, these results suggest that p53 suppresses the expression of cell cycle regulatory genes though inhibiting both CCAAT binding factors, CBF/HSP70 and NF-Y.

Keywords: cell cycle; cell cycle proteins; protein p53; transcription factors

Introduction

Inactivation of p53 tumor suppressor gene occurs in over half of all human tumors, implying that loss of this gene represents a fundamentally important step in genomic instability and susceptibility to malignant transformation (Hollstein *et al.*, 1991; Kastan *et al.*, 1995; Seo and Jung, 2004). The underlying mechanism of tumor suppressor activity of p53 resides in part in its ability to bind DNA in a sequence-specific manner to activate gene transcription (Kastan *et al.*, 1995). It has been reported that a substantial number of genes involving in cell cycle, apoptosis, and senescence are activated by p53 (Kastan *et al.*, 1995).

In addition to playing a role as a DNA-binding dependent transcription activator, p53 has also been reported to negatively regulate the transcription of a number of genes. We previously reported that p53 inhibits cell cycle dependent transcription of cell cycle regulatory genes such as cdc2 and cyclin B genes, which, in turn, results in inactivation of cdc2 kinase and cell cycle arrest at G2 checkpoint (Park *et al.*, 2000). Also, NF-Y is involved in transcription repression of G2-specific genes after p53 induction and DNA damage (Yun *et al.*, 1999; Manni *et al.*, 2001). Furthermore, the DNA binding activity of NF-Y is decreased by p53 (Jung *et al.*, 2001). Moreover, we recently reported that cdk2 phosphorylates two serine residues near DNA binding domain of YA subunit of NF-Y (Yun *et al.*, 2003). Although YA phosphorylation had no effect on heterotrimer formation with the YB-YC dimer, it is necessary for efficient DNA binding of NF-Y (Yun *et al.*, 2003).

Another CCAAT-binding transcription factor, CBF/HSP70 was originally identified as a transcription factor binding to the CCAAT box on the hsp70 promoter and mediates transcription repression of hsp70 promoter by p53 (Agoff *et al.*, 1993). Recent studies have reported that CBF/HSP70 also participates in transcription regulation on CCAAT boxes from several promoters and directly binds with NF-Y (Imbriano *et al.*, 2001). Since CBF/HSP70 mediates p53-dependent transcription repression of the hap70 promoter, in this study, we examined whether p53 regulates CBF/HSP70 as well as NF-Y.

Materials and Methods

Cell culture and virus infection

The human bladder cancer cells, EJ, were cultured in

Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Life Technologies, Inc., Gaithersburg, MD) and were synchronized at G1-S with a modified double-thymidine block protocol (Park *et al.*, 2000). After synchronization, cells were infected with an adenovirus that encodes p53 or the control virus (Ad-ΔE1), each at a multiplicity of infection of 50 plaque-forming units per cell for 3 h. After 8 h, cells were washed twice in Dulbecco's PBS, which released them from the G1-S block.

Cell cycle analysis

For cell cycle analyses, 1×10^6 cells were harvested by trypsinization and fixed by rapid submersion in 1 ml cold 70% ethanol. After fixation at -20°C for at least 1 h, cells were pelleted and subsequently resuspended in 1 ml staining solution (50 μg/ml propidium iodide, 50 μg/ml RNase, 0.1% Triton X-100 in citrate buffer, pH 7.8), and analyzed with Facscan (BD Biosciences, San Diego, CA).

Western blot analysis

Cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 7.5; 50 mM NaCl, 0.5% Triton X-100, 1 mM EDTA, 10 mM Na pyrophosphate, 50 mM Na fluoride, 5 mM Na orthovanadate, 10 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatinA) (Chung *et al.*, 2003), and 20 μg of proteins from each lysate was separated on 10% or 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels, and transferred to a polyvinylidene difluoride (PVDF) membrane (NEN, Boston, MA). Westernblot analysis was performed using the ECL Western blotting detection system (Amersham). Mono- clonal antibody against p53 and goat polyclonal antibody against actin were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Northern blot analysis

Total cellular RNA was isolated with the use of Trizol solution (Life Technologies). RNA (15 μg) was analyzed by electrophoresis in 1% agarose-formaldehyde gels and transferred to nitrocellulose filters. Hybridizations were performed with the following ^{32}P-labeled DNA probes. Hybridization conditions and washing procedures were optimized according to the manufacturer's instructions (ExpressHyb; Clontech Inc., Palo Alto, CA).

Results and Discussion

To examine whether p53 regulate the expression of CBF.HSP70, we infected EJ cells with recombinant adenovirus encoding p53 (Ad-p53) or its control virus (Ad-ΔE1) and examined mRNA levels of CBF/HSP70. Figure 1 showed that mRNA levels of CBF/HSP70 were decreased at 24 h and undetectable at 48 h after infection of Ad-p53 (Figure 1A), which is paralleled with protein levels of p53 (Figure 1B). As a control experiment, we infected the cells with the control virus and found that mRNA levels of CBF/HSP70 was not changed (Figure 1A). We also analyzed mRNA levels of cyclin B1, which is one of NF-Y target gene and repressed by p53 (Yun *et al.*, 1999; Chae *et al.*, 2004). Cyclin B1 expression was also repressed by infection with the p53-virus, but not in cells infected with the control virus (Figure 1A).

We next examined whether CBF/HSP70 transcription is regulated in a cell cycle-dependent manner. Cells were synchronized at G1/S phase of the cell cycle by double thymidine block method (Park *et al.*, 2000). After release from cell cycle arrest by removal of thymidine, the cell cycle was re-initiated and progressed into S, G2, and M phases in orderly (Figure 2A). As a control, we examined cdc2, which is known to be regulated in a cell cycle-dependent manner (Figure 2B). While the expression of CBF/ HSP70 was increased 10 h after release cell from cell cycle arrest, which is corresponding to S and G2 phase (Figure 2B), cdc2 transcription was induced 8h after the release (Figure 2B), implying that CBF/HSP70 expression followed by cdc2. However,

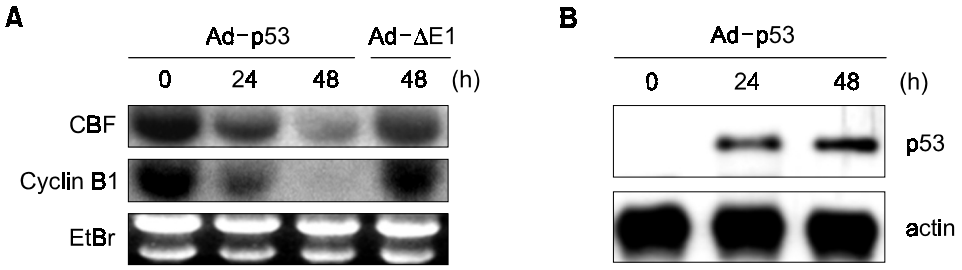

Figure 1. Repression of CBF/HSP70 by p53. (A) EJ cells were infected with recombinant adenoviruses (Ad-p53, Ad-ΔE1) and harvested at the indicated time points. Total RNA (15 μg) was separated on a 1% agarose-formaldehyde gel and transferred to nylon membranes. The filter was hybridized with CBF/HSP70 or cyclin B1. Ethidium bromide (*EtBr*) staining is presented as loading control. (B) Cell lysates prepared as in (A) were subjected to Western blotting with antibodies against p53 and actin.

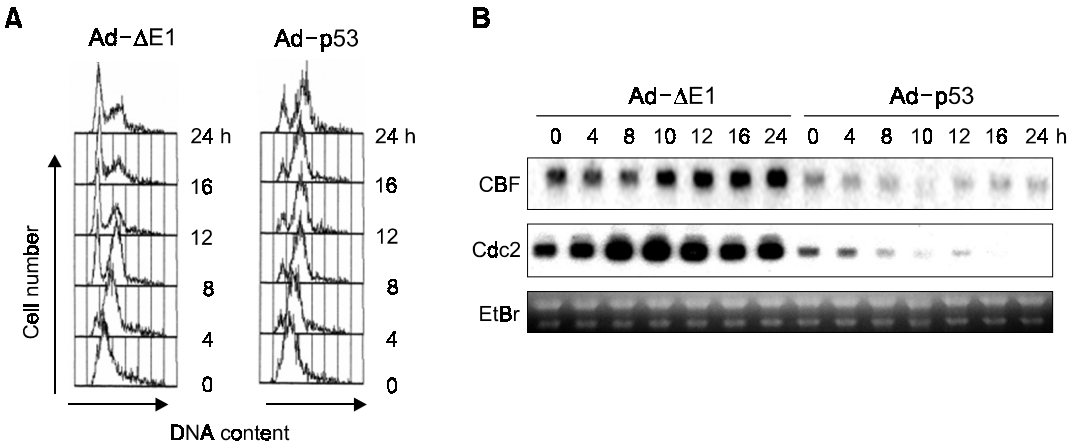

Figure 2. p53 represses cell cycle-dependent transcription of CBF/HSP70. (A) EJ cells were synchronized at G_1.S boundary and infected with a recombinant adenovirus that encodes p53 (Ad-p53) or a control virus (Ad-ΔE1). After release from the growth arrest, cells were harvested at the indicated time points, fixed in 70% ethanol and analyzed by flow cytometry for DNA content. (B) EJ cells were infected with adenoviruses and harvested at the indicated time points as in Figure 2. Total RNA (15 μg) was separated on a 1% agarose-formaldehyde gel and transferred to nylon membranes. The membranes were hybridized with CBF/HSP70 or cdc2. Ethidium bromide (*EtBr*) staining is presented as loading control.

CBF/HSP70 expression was inhibited in cells expressing p53, in which cdc2 transcription was also repressed (Figure 2B). Thus, these results imply that p53 represses cell cycle-dependent expression of CBF/HSP70.

It was reported that CBF/HSP70 interacts in solution and on DNA with the NF-Y (Imbriano *et al.*, 2001). In yeast two-hybrid assays CBF/HSP70 interacts with YB subunit of NF-Y (Imbriano *et al.*, 2001). Since we previously reported that p53 leads to the dephosphorylation of YA subunit in the NF-Y through p21 induction and cdk2 inactivation. In this study, we suggest that p53 inhibits transactivation ability of another CCAAT-binding transcription factor, CBF/HSP70 by repression of its transcription (Figure 1). We also showed that CBF/HSP70 expression is regulated in a cell cycle dependent manner and reaches at maximum levels at G2 phase (Figure 2). Since previous study suggests that CBF/HSP70 interacts and cooperates with NF-Y, both CCAAT-binding transcription factors are responsible to express cell cycle regulatory genes, such as cdc2 and cyclin B, in a cell cycle dependent manner. Thus, NF-Y works for their expression at G1/S, and then together with CBF/HSP70 at G2 phase of the cell cycle.

Acknowledgement

This research was supported by fund of Alumni of Dankook University.

References

Agoff SN, Hou J, Linzer DI, Wu B. Regulation of the human hsp70 promoter by p53. Science 1993;259:84-7

Chae HD, Yun J, Bang YJ, Shin DY. Cdk2-dependent phosphorylation of the NF-Y transcription factor is essential for the expression of the cell cycle-regulatory genes and cell cycle G1/S and G2/M transitions. Oncogene 2004;23:4084-8

Chung HS, Park SR, Choi EK, Park HJ, Griffin RJ, Song CW, Park H. Role of sphingomyelin-MAPKs pathway in heat-induced apoptosis. Exp Mol Med 2003;35:181-8

Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991;253:49-53

Imbriano C, Bolognese F, Gurtner A, Piaggio G, Mantovani R. HSP-CBF Is an NF-Y-dependent Coactivator of the Heat Shock Promoters CCAAT Boxes. J Biol Chem 2001;276:26332-9

Jung MS, Yun J, Chae HD, Kim JM, Kim SC, Choi TS, Shin DY. p53 and its homologues, p63 and p73, induce a replicative senescence through inactivation of NF-Y transcription factor. Oncogene 2001;20:5818-25

Kastan MB, Canman CE, Leonard CJ. P53, cell cycle control and apoptosis: implications for cancer. Cancer Metastasis Rev 1995;14:3-15

Manni J, Mazzaro G, Gurtner A, Mantovani R, Haugwitz U, Krause K, Engeland K, Sacchi A, Soddu S, Piaggio G. NF-Y mediates the transcriptional inhibition of the cyclin B1, cyclin B2, and cdc25C promoters upon induced G2 arrest. J Biological Chemistry 2001;276:5570-6

Park M, Chae HD, Yun J, Jung M, Kim YS, Kim SH, Han MH, Shin DY. Constitutive activation of cyclin B1-associated cdc2 kinase overrides p53-mediated G2-M arrest. Cancer Res 2000;60:542-5

Seo YR, Jung HJ. The potential roles of p53 tumor suppressor in nucleotide excision repair (NER) and base excision repair (BER). Exp Mol Med 2004;36:505-9

Yun J, Chae HD, Choy HE, Chung J, Yoo HS, Han MH, Shin DY. p53 negatively regulates cdc2 transcription via the CCAAT-binding NF-Y transcription factor. J Biol Chem 1999;274: 29677-82

Yun J, Chae H-D, Choi T-S, Kim E-H, Bang Y-J, Chung J-K, Choi K-S, Mantovani R, Shin DY. Cdk2-dependent phosphorylation of the NF-Y transcription factor and its involvement in the p53-p21 signaling pathway. J Biol Chem 2003; M305178200