

Direct Transcriptional Activation of Human Caspase-1 by Tumor Suppressor p53*

Received for publication, January 18, 2001, and in revised form, February 9, 2001
Published, JBC Papers in Press, February 13, 2001,
DOI 10.1074/jbc.C100025200

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The tumor suppressor protein p53 is a sequence-specific DNA-binding protein, and its biological responses are very often mediated by transcriptional activation of various target genes. Here we show that caspase-1 (interleukin-1 β converting enzyme), which plays a role in the production of proinflammatory cytokines and in apoptosis, is a transcriptional target of p53. Caspase-1 mRNA levels increased upon overexpression of p53 by transfection in MCF-7 cells. Human caspase-1 promoter showed a sequence homologous to the consensus p53-binding site. This sequence bound to p53 in gel shift assays. A caspase-1 promoter-reporter construct was activated 6–8-fold by cotransfection with normal p53 but not by mutant p53 (His²⁷³) in HeLa, as well as MCF-7, cells. Mutation of the p53-binding site in caspase-1 promoter abolished transactivation by p53. Treatment of p53-positive MCF-7 cells with the DNA-damaging drug, doxorubicin, which increases p53 levels, enhanced caspase-1 promoter activity 4–5-fold, but similar treatment of MCF-7-mp53 (a clone of MCF-7 cells expressing mutant p53) and p53-negative HeLa cells with doxorubicin did not increase caspase-1 promoter activity. Doxorubicin treatment increased caspase-1 mRNA levels in MCF-7 cells but not in MCF-7-mp53 or HeLa cells. These results show that endogenous p53 can regulate caspase-1 gene expression.

The tumor suppressor protein p53 plays an important role in mediating response to stress such as that induced by DNA damage and hypoxia resulting in either growth arrest or apoptosis (1–3). It is a sequence-specific DNA-binding protein, and its biological effects are generally mediated by transcriptional activation of various target genes (1–3). The p53 gene is mutated in over 50% of human tumors and in some inflammatory disorders like rheumatoid arthritis (2–4). These p53 mutations are clustered in the sequence-specific DNA-binding domain of the molecule leading to inactivation of its sequence-specific transactivation function (2).

* This work was supported by a research grant from the Department of Biotechnology, Government of India (to G. S.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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Caspase-1, also known as interleukin-1 β converting enzyme, is a member of the cysteine protease family, which cleaves cellular substrates after aspartic acid (5–7). The primary function of caspase-1 is the proteolytic processing of the precursors of proinflammatory cytokines such as interleukin-1 β into active cytokines (5–7). In addition caspase-1 is also involved in some forms of apoptosis (5–7). Caspase-1 knockout mice are developmentally normal but are defective in the production of mature cytokines interleukin-1 β and interleukin-18. These mice are resistant to septic shock and show a partial defect in apoptosis (8, 9).

Several p53-responsive genes have been identified by using different approaches and various cell types (10, 11). These p53-responsive genes include various functional categories such as those involved in apoptosis, cell cycle, signal transduction, angiogenesis, etc. (11). Induction of various genes by p53 is dependent on the type of inducer used, and even with the same inducer it may be cell type-dependent (11). However none of the members of the caspase family have been identified as a transcriptional target of p53. Here we report that human caspase-1 is a transcriptional target of exogenous, as well as endogenous, p53. In addition we have identified a site in the caspase-1 promoter that is required for transcriptional activation by p53.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—The cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 °C in a CO₂ incubator. The transfections were done using LipofectAMINE PLUSTM reagent (Life Technologies, Inc.) according to manufacturer's instructions. All the plasmids for transfections were prepared by using Qiagen columns.

Reverse Transcriptase Polymerase Chain Reaction Analysis—Total RNA was isolated using TRIZOLTM reagent (Life Technologies, Inc.). Semiquantitative RT-PCR¹ was carried out essentially as described previously (12). RNA was reverse transcribed using reagents from an RNA-PCR kit (PerkinElmer Life Sciences). The GAPDH and caspase-1 mRNAs were amplified for 23 and 40 cycles, respectively, in the same reactions. The PCR products were analyzed on a 1.2% agarose gel containing ethidium bromide followed by Southern blot analysis for caspase-1. Primers for amplification of GAPDH mRNA have been described (12). Primers C1P2, 5'-CGAATTCAATGCTCTGGGAAGAGG-TAGAA-3', and C1P3, 5'-CGAATTCAAGGACAAACCGAAGGTGATC-3', were used for amplification of human caspase-1 mRNA. Primers C1P4, 5'-AAGGAGAAGAGAAAGCTGTTTATC-3', and C1P5, 5'-AT-TATTGGATAAATCTCTGCCGAC-3', were used to distinguish among α -, β -, and γ - or δ -isoforms of caspase-1.

CAT Assay—Cells grown in 35-mm dishes were transfected with 250 ng of pCAT-ICE, 150 ng of pCMV-SPORT- β GAL (Promega), and 500 ng of wild-type p53, mutant p53 (His²⁷³), or control plasmids. Lysates were prepared 30 h post-transfection from HeLa cells and 48 h post-transfection from MCF-7 cells using reporter lysis buffer from Promega according to the manufacturer's instructions. For CAT assay 40 μ l of lysate was mixed with 2 μ l of ¹⁴C-labeled chloramphenicol (25 μ Ci ml⁻¹; 54 mCi mmol⁻¹) and 10 μ l of acetyl coenzyme A (3.5 mg ml⁻¹) in a total volume of 60 μ l and incubated at 37 °C for 3 h. Relative CAT activities were calculated after normalizing with β -galactosidase enzyme activities.

Electrophoretic Mobility Shift Assay—Double-stranded oligonucleotide corresponding to the putative p53-binding site in caspase-1 pro-

¹ The abbreviations used are: RT-PCR, reverse transcriptase polymerase chain reaction; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CAT, chloramphenicol acetyltransferase; CMV, cytomegalovirus; β GAL, β -galactosidase; Casp-1, caspase-1; wt, wild-type; mt, mutated; bp, base pairs; cmk, chloromethylketone.

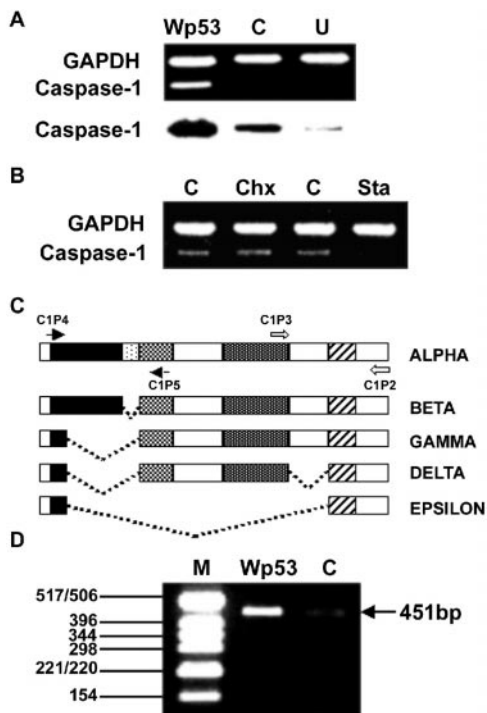


FIG. 1. p53-dependent expression of caspase-1. *Panel A*, RT-PCR analysis of total RNA from MCF-7 cells transfected with wild-type human p53 (*Wp53*), control plasmid (*C*), and untransfected (*U*) cells using primers for human caspase-1 and GAPDH. *Upper panel* shows an ethidium bromide-stained agarose gel of PCR products as indicated, and *lower panel* shows a Southern blot for caspase-1. *Panel B*, effect of cycloheximide (*Chx*) and staurosporine (*Sta*) on caspase-1 mRNA levels in MCF-7 cells. The cells were treated with 100 $\mu\text{g ml}^{-1}$ of cycloheximide for 24 h or 0.1 μM staurosporine for 8 h or was untreated (*C*). *Panel C*, schematic representation of splice variants of human caspase-1. The positions of primers used for amplifying caspase-1 are indicated by arrows. *Panel D*, RT-PCR analysis for caspase-1 expression using primers C1P4 and C1P5. A PCR product of 451 bp is produced from α -isoform. β -Isoform would give a PCR product of 388 bp whereas γ - and δ -isoforms would give a PCR product of 172 bp. *M*, molecular weight markers; *C*, control; *Wp53*, wild-type p53.

motor (Casp-1; see Fig. 3A) was end-labeled with polynucleotide kinase using [γ - ^{32}P]ATP. Nuclear extracts were prepared by high salt extraction of nuclei (13). Binding reactions with labeled oligonucleotide and nuclear extracts were performed essentially as described (14) in 10 mM Tris-HCl, pH 7.5, 0.1% Triton X-100, 4.5% glycerol, 1 mM EDTA, 0.05 mM dithiothreitol, 1 μg of poly(dI-dC), 100 mM sodium chloride. Nuclear extract (4 μg of protein) was then added followed by addition of 2 ng of labeled probe (50,000 cpm). The reaction mix was incubated at 25 $^{\circ}\text{C}$ for 45 min followed by incubation at 4 $^{\circ}\text{C}$ for 15 min. A polyclonal antibody (1 μg) from Roche Molecular Biochemicals was included where indicated in the binding reaction.

Reporter Plasmids—The promoter region of human caspase-1 gene from nucleotide position -182 to $+42$ relative to the transcription start site was cloned in pCAT-Basic vector (Promega) and designated as pCAT-ICE-wt (15). Mutated promoter-reporter plasmid named as pCAT-ICE-mt was constructed using primers mut-1, 5'-GGGAAAAGAAA-TAAAGAAATTCATATGAATTCACAGTGAGTATTTCC-3', and mut-2, 5'-GGAAATACTCACTGTGAATTCATATGAATTTCTTTATTTCTTTTCCC-3', by PCR-based site-directed mutagenesis using overlap extension PCR (16). The nucleotide sequence of the mutant, as well as wild-type promoter, in these constructs was confirmed by automated sequencing.

RESULTS AND DISCUSSION

The level of caspase-1 mRNA was determined by RT-PCR analysis in response to transient overexpression of human wild-type p53 in MCF-7 cells. Caspase-1 mRNA level increased severalfold by overexpression of p53 as compared with the control-transfected cells or untransfected cells (Fig. 1A). This increase in the caspase-1 mRNA level was not the result of

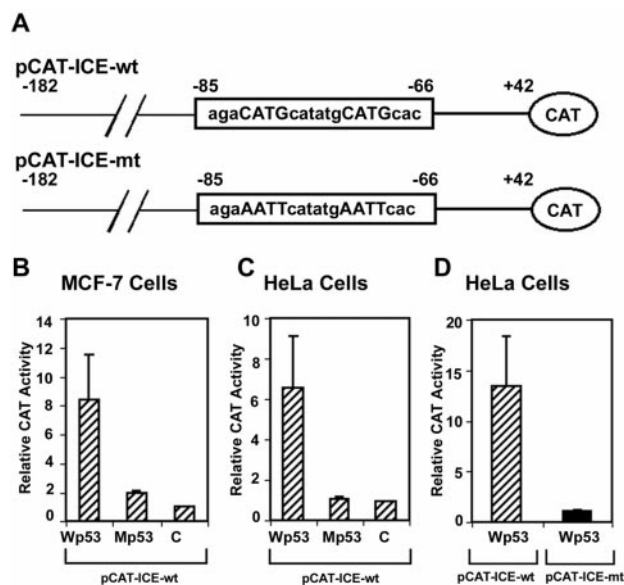


FIG. 2. Activation of transcription from caspase-1 promoter by wild-type p53. *Panel A*, schematic representation of caspase-1 promoter-reporter constructs, pCAT-ICE-wt and pCAT-ICE-mt. The nucleotide sequence of human caspase-1 promoter from position -85 to -66 relative to the transcription start site is shown. *Panels B and C*, activation of caspase-1 promoter by wild-type p53. pCAT-ICE-wt and pCMV-SPORT- β GAL were cotransfected, along with wild-type p53 (*Wp53*) or mutant p53 (*Mp53*) or control plasmids (*C*) in MCF-7 (*B*) and HeLa (*C*) cells. CAT activities relative to control are shown ($n = 4$). The ratio of p53 to reporter plasmid was 2:1 in *panel B* and 1:1 in *panel C*. *Panel D*, effect of mutation of the p53-binding site in caspase-1 promoter on transactivation by wild-type p53. The ratio of p53 to reporter plasmid was 2:1 ($n = 3$).

induction of apoptosis by p53, because treatment of MCF-7 cells with some apoptosis-inducing agents, staurosporine, and cycloheximide did not increase the caspase-1 mRNA level (Fig. 1B). Treatment with staurosporine in fact decreased the level of caspase-1 mRNA. There are five isoforms of caspase-1 mRNA (17). Using another set of primers, we found that the α form, which is proapoptotic, was induced by p53 (Fig. 1, C and D), and β , γ , and δ forms were not induced. By using appropriate primers we found that the ϵ -isoform was also not induced (data not shown).

Examination of the nucleotide sequence of human caspase-1 promoter (18) showed a sequence homologous to the consensus p53-binding site at nucleotide position -85 to -66 relative to the transcriptional start site (Fig. 2A). A caspase-1 promoter-reporter construct (pCAT-ICE-wt) containing this region (nucleotide position -182 to $+42$) was activated over 6–8-fold by cotransfection with normal p53 in MCF-7, as well as HeLa, cells (Fig. 2, B and C). In these experiments the ratio of p53 to reporter plasmid was 1:1 with HeLa cells (Fig. 2C) and 2:1 with MCF-7 cells (Fig. 2B). At a higher ratio (2:1) of p53 to reporter plasmid in HeLa cells there was an over 12-fold increase in activation of transcription from this promoter (Fig. 2D). Mutant p53 (His²⁷³) did not activate this transcription in p53-negative HeLa cells, but in MCF-7 cells, which are p53-positive, it gave a small (less than 2-fold) increase in activity (Fig. 2, B and C). The control plasmid (pCAT-Basic) gave much lower activity and did not show any activation by p53 (data not shown). Mutation of the putative p53-binding site in caspase-1 promoter completely abolished transactivation by p53 (Fig. 2D). These observations suggest that there is only one functional p53-responsive site in this region (-182 to $+42$) of caspase-1 promoter.

To determine whether p53 binds to the putative p53-binding site in human caspase-1 promoter, we carried out electro-

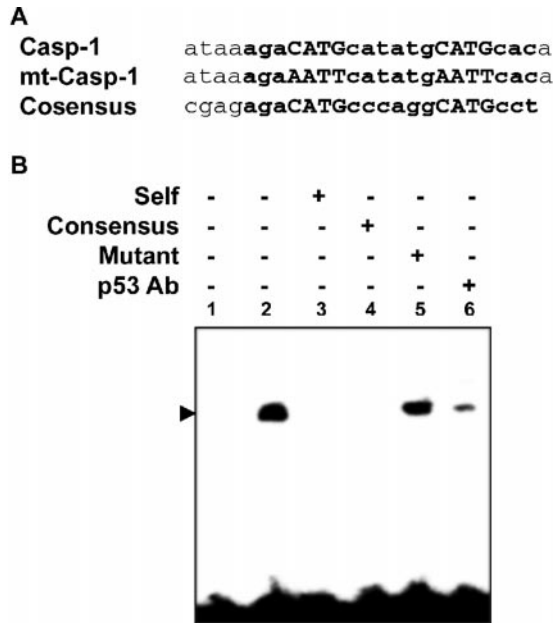


FIG. 3. Electrophoretic mobility shift assay using the putative p53-binding site sequence of caspase-1 promoter. Panel A, sequences of oligonucleotides corresponding to nucleotide positions -89 to -65 relative to the transcription start site in Casp-1. Sequences of mutant oligonucleotide (*mt-Casp-1*) and p53-binding consensus oligonucleotide (*Consensus*) are also shown. Panel B, electrophoretic mobility shift assays were done using radiolabeled Casp-1 oligonucleotide with nuclear extracts from MCF-7 cells treated with 500 ng ml⁻¹ of doxorubicin. Lane 1 is binding without nuclear extract. The arrow shows the p53-specific band that was competed out by a 50-fold excess of unlabeled Casp-1 oligonucleotide (lane 3) and consensus oligonucleotide (lane 4) but not by *mt-Casp-1* oligonucleotide (lane 5). The addition of p53 polyclonal antibody (*p53 Ab*; 1 μ g) immunodepleted the shifted band (lane 6).

phoretic mobility shift assays using a synthetic oligonucleotide corresponding to this site (Fig. 3A). Binding to this oligonucleotide was seen with nuclear extract prepared from MCF-7 cells treated with doxorubicin, which is known to increase the p53 protein level (Fig. 3B, lane 2). This binding was competed out with a 50-fold excess of unlabeled self-oligonucleotide and also with a consensus p53-binding oligonucleotide but not with a mutated oligonucleotide in which the p53-binding core sequence CATG was mutated to AATT (Fig. 3, A and B, lanes 3-5). A polyclonal antibody to p53 immunodepleted the shifted band (Fig. 3B, lane 6). These results suggest that the binding to this oligonucleotide corresponding to the putative p53-binding site in caspase-1 promoter is specific and dependent on p53.

To address the role of endogenous p53 in regulating endogenous caspase-1 gene expression, MCF-7 cells were treated with doxorubicin, which increases the level of p53 protein. Treatment of MCF-7 cells with doxorubicin enhanced the caspase-1 mRNA level 4-5-fold (Fig. 4). Similar treatment of MCF-7-mp53, a clone of MCF-7 cells expressing mutant p53 (His²⁷³) or p53-negative HeLa cells, did not increase the caspase-1 mRNA level (Fig. 4). The basal level of caspase-1 mRNA was higher in MCF-7-mp53 that decreased upon treatment with doxorubicin. The MCF-7-mp53 cell line was obtained by transfection of MCF-7 cells with the His²⁷³ mutant of p53 followed by selection with G418. This mutant of p53 is known to function as a dominant inhibitor of wild-type p53 function (19). Treatment of A549 cells (which have normal p53) with doxorubicin also resulted in an increase in the caspase-1 mRNA level (Fig. 4). These results showed that endogenous p53 can regulate expression of the endogenous caspase-1 gene.

To determine the role of endogenous p53 in regulating caspase-1 promoter, MCF-7, MCF-7-mp53, and HeLa cells

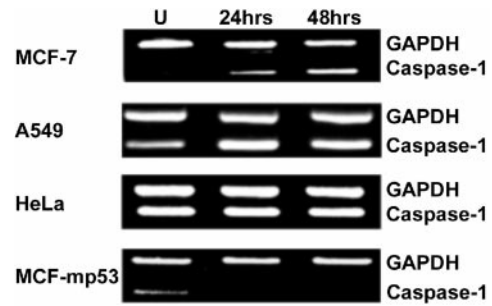


FIG. 4. Regulation of caspase-1 gene expression by endogenous p53. Indicated cells were treated with 500 ng ml⁻¹ doxorubicin for 24 and 48 h. After RNA isolation caspase-1 mRNA levels were analyzed by RT-PCR. U, untreated.

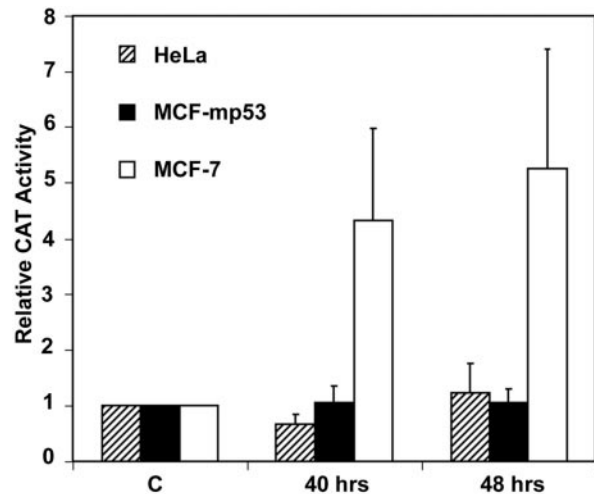


FIG. 5. Transactivation of caspase-1 promoter by up-regulating endogenous p53. The indicated cells were cotransfected with pCAT-ICE-wt and pCMV-SPORT- β GAL, and 24 h post-transfection they were treated with 500 ng ml⁻¹ doxorubicin for 40 and 48 h. CAT activities relative to untreated control are shown ($n = 3$).

were transfected with caspase-1 promoter-reporter plasmid, and after 24 h they were treated with doxorubicin for 40 or 48 h. Doxorubicin treatment resulted in a 4-5-fold increase in caspase-1 promoter activity in MCF-7 cells but not in MCF-7-mp53 or HeLa cells (Fig. 5). These results showed that endogenous wild-type p53 can also activate transcription from the caspase-1 promoter, which is inhibited by the His²⁷³ mutant of p53.

Ectopic expression of caspase-1 is known to induce apoptosis (17, 20). The wild-type p53-induced apoptosis in MCF-7 cells was partially inhibited (50% inhibition) by YVAD-cmk (which preferentially inhibits caspase-1) but not by the caspase-3 family inhibitor DEVD-cmk (data not shown). Doxorubicin-induced apoptosis in MCF-7 cells was also partially inhibited (45% inhibition) by YVAD-cmk and not by DEVD-cmk (data not shown). These observations suggest that caspase-1 contributes in part to p53-mediated apoptosis. Apoptotic pathways are cell type- and stimulus-specific, and it is likely that caspase-1, along with other transcriptional targets, may play a role in p53-mediated apoptosis at least in some cells.

The primary role of caspase-1 is in the production of proinflammatory cytokines interleukin-1 β , interleukin-16, and interleukin-18 (5-7). Wild-type p53 is overexpressed in several inflammatory diseases (reviewed in Ref. 4), but its potential role in inflammation is not understood. Our results, showing that caspase-1 is transcriptionally activated by p53, suggest that p53 has a role in inflammation. Mutational inactivation of

p53 in human tumors would, therefore, lead to reduced inflammatory response, in addition to resistance to apoptosis.

Acknowledgment—S. G. gratefully acknowledges the Council of Scientific and Industrial Research, Government of India, for a senior research fellowship.

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