

Thyroid Hormone Receptor Is a Negative Regulator in p53-Mediated Signaling Pathways

GONZALO BARRERA-HERNANDEZ,¹ QIMIN ZHAN,² ROSEMARY WONG,³ and SHEUE-YANN CHENG¹

ABSTRACT

Thyroid hormone nuclear receptors (TRs) are ligand-dependent transcription factors which regulate growth, differentiation, and development. The molecular mechanism by which TRs mediated these effects remains unclear. A prevailing hypothesis is that TRs exert their biological effects by cooperating with other transcription factors. We have recently shown that the human TR subtype $\beta 1$ (hTR $\beta 1$) interacts with the tumor suppressor p53, which plays a critical role in cell-cycle regulation and tumorigenesis. This interaction of hTR $\beta 1$ with p53 leads to an impairment of TR function. The present study examined whether hTR $\beta 1$ could modulate the function of p53. Mapping of the domains of p53 responsible for the interaction with hTR $\beta 1$ indicated that the regions involved resided in the DNA-binding domain and carboxy terminus of p53. In agreement with this finding, hTR $\beta 1$ increased the binding of p53 to p53 DNA-binding elements. This increase in DNA binding, however, resulted in repression of p53-dependent transcription activation in transfected cells. Furthermore, hTR $\beta 1$ led to an inhibition of the p53-mediated induction of *bax* and *gadd45* expression. In contrast, the p53-induced expression of *p21* was not affected by hTR $\beta 1$, suggesting that the expression of p53-regulated genes is differentially modulated by hTR $\beta 1$. Because the expressions of *bax*, *gadd45*, and *p21* are directly regulated by p53, these results indicate that hTR $\beta 1$ can modulate p53-regulated gene expression and support the hypothesis that there is cross-talk between these two regulatory pathways. The cross-talk between these two transcription factors could play an important role in the biology of normal and cancer cells.

INTRODUCTION

THYROID HORMONE RECEPTORS (TRs) are ligand-dependent transcription factors which regulate growth, differentiation, and development (Lazar, 1993; Cheng, 1995; Oppenheimer *et al.*, 1996). The TRs are members of the steroid hormone/retinoic acid receptor superfamily. Two TR genes, TR α and TR β , located on chromosome 17 and 3, respectively, give rise by alternative splicing to four TR isoforms: $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$. The TRs mediate the biological activity of the thyroid hormone, T₃, by binding to specific DNA sequences known as thyroid hormone response elements (TREs) in the promoter region of T₃ target genes (Lazar, 1993; Cheng, 1995; Oppenheimer *et al.*, 1996). Although T₃ has been shown to induce cell proliferation by stimulating progression through

the G₁ phase of the cell cycle (DeFesi and Surks, 1981; DeFesi *et al.*, 1984, 1985), the mechanism by which this is accomplished remains unknown.

We have recently reported that human thyroid hormone receptor $\beta 1$ (TR $\beta 1$) specifically interacts with p53, and that this interaction inhibits the transcriptional activation of hTR $\beta 1$ (Yap *et al.*, 1996) and leads to impairment of TR function (Bhat *et al.*, 1997). These results indicate that there is cross-talk between the TR- and p53-mediated pathways. The former leads to a growth-stimulatory effect, and the latter is involved in growth inhibition (Perry and Levine, 1993; Selter and Montenarh, 1994; Maxwell and Roth, 1994; Leonard *et al.*, 1995).

The importance of the p53 gene in cell growth and tumorigenesis is exemplified by the fact that more than 50% of human cancers examined contain mutations in this gene (Zambetti

¹Laboratory of Molecular Biology and ²Laboratory of Molecular Pharmacology, Division of Basic Sciences, National Cancer Institute, Bethesda, MD 20892-4255.

³Molecular Hematology Branch, National Heart, Lung and Blood Institute, National Institutes of Health, Bethesda, MD 20892-4255.

and Levine, 1993; Hostein *et al.*, 1994). The product of this gene, the p53 protein, enhances the transcription rate of its target genes by specifically binding to p53 response elements in their promoter sequences (Selter and Montenarh, 1994; Zambetti and Levine, 1993; Prives and Manfredi, 1993; Selivanova and Wiman, 1995). For example, the expression of genes such as *gadd45*, *mdm2*, *bax*, and *p21* is induced by wildtype (wt) p53 (Zambetti and Levine, 1993; Selivanova and Wiman, 1995; Levine, 1997). In addition to activating gene transcription by direct interaction with its DNA-binding sites, p53 can also repress transcription of many genes that lack p53 response elements. This p53-mediated repression of gene transcription is believed to occur by protein–protein interaction (Selter and Montenarh, 1994; Selivanova and Wiman, 1995; Levine, 1997). The identification of new p53-associated proteins has been the focus of major research, in the hope that these proteins will provide a better understanding of p53 function as a regulator of the cell cycle and tumorigenesis. A variety of cellular and viral proteins have been shown to interact with p53, resulting in changes in their biological activity, function, or both (Prives and Manfredi, 1993; Pietenpol and Vogelstein, 1993; Selter and Montenarh, 1994; Leonard *et al.*, 1995; Selivanova and Weiman, 1995; Levine, 1997). The interaction between hTR β 1 and p53 shown previously (Yap *et al.*, 1996; Bhat *et al.*, 1997) prompted us to examine whether hTR β 1, a transcription factor, could modulate the functions of p53.

In the present study, we found that the interaction between hTR β 1 and p53 led to changes in both the DNA-binding and transactivation potential of p53. More importantly, we show that this interaction led to changes in the expression of p53 downstream target genes such as *gadd45* and *bax*. The finding that the expression of *p21* was not affected by this interaction suggests that hTR β 1 selectively affected the expression of some, but not all, p53-regulated genes. Our findings indicate that hTR β 1 can modulate p53-mediated signaling pathways.

MATERIALS AND METHODS

Plasmids

The following cDNA clones were used for RNA analysis: pHuIB2, a nearly full-length human *gadd45* clone (Papathanasiou *et al.*, 1991); pZL-WAF1, a full-length human *p21^{CIP1/WAF1}* clone (El-Deiry *et al.*, 1993), referred to as *waf1*, which was provided by B. Vogelstein (Johns Hopkins Oncology Center, MD); and N7p18, a human *bax* cDNA clone, provided by S. Korsemyer (Oltvai *et al.*, 1993). The plasmids containing wildtype (wt) p53 DNA-binding sites (PG₁₃-CAT) and mutated p53 DNA-binding sites (MG₁₅-CAT) were provided by B. Vogelstein. The PG-259, a 259-bp DNA fragment containing 13 tandem repeats of a wt p53 binding site, was obtained by restriction digestion of PG₁₃-CAT. Restriction digestion of MG₁₅-CAT released MG-15, a 285-bp DNA fragment containing 15 tandem repeats of a mutated binding site (El-Deiry *et al.*, 1992). The pGST-TR β 1 and pTK28m were gifts from C. Glass (University of California at San Diego) and D. Moore (Baylor College, Houston, TX), respectively. The pCH110, an expression vector for β -galactosidase, was from Pharmacia. The hTR β 1 expression plasmid pCLC51 and the

expression plasmid for human papilloma virus type 16 E6 (pCMV-E6) are as previously described (Lin *et al.*, 1992, and Kessis *et al.*, 1993, respectively).

Preparation of truncated p53 expression plasmids using T7 promoter

A plasmid containing the cDNA for wt p53 was used to construct the expression vectors for the N-terminus (N-), DNA-binding domain (D-), and C-terminus (C-) of p53. The N-, D-, and C- regions of p53 were amplified by polymerase chain reaction (PCR) with primers which include an *Nde*I site in the sense primer and an *Eco*RI site in the antisense primer to facilitate subsequent screening of clones. For the N-terminus of p53: sense = 5'-atagtacatATGGAGGAGCCGCAGTCAGAT-3'; antisense = 5'-tgtactgaattctcaCTGGGAAGGGACAGAA-GATGA-3'. For the D-domain of p53: sense = 5'-atagtacatatgAAAACCTACCAGGGCAGATAC-3'; antisense = 5'-tgtactgaattctcaGGGCAGCTCGTGGTGAGGCTC-3'. For the C-terminus of p53: sense = 5'-atagtacatatgCCAGGGAGCACTA-AGCGAGCA-3'; antisense = 5'-tgtactgaattctcaTCAGTCTG-AGTCAGGCCCTCTGT-3'. The PCR reactions were carried out in a total volume of 100 μ l containing 100 ng of the p53 plasmid, 100 pmoles of each sense and antisense primer, 0.15 mM dNTPs (Boehringer-Mannheim), 2 units of Vent polymerase (NEB), and 1 \times Thermopol buffer (NEB). Cycling conditions were as follows: 94°C for 5 min, then 25 cycles of 94°C for 1 min, 55°C for 1.5 min, and 72°C for 1 min. The expected sizes of 299, 599, and 281 bp of the N-, D-, and C-termini, respectively, obtained by PCR were purified (SpinBind columns, FMC Products), and each fragment was ligated into the *Nde*I/*Eco*RI site of a pCJ T7 expression vector.

Cell culture

Human breast carcinoma MCF-7 cells were maintained in 1 \times RPMI consisting of RPMI-1640 (Quality Biological Inc., Gaithersburg, MD) supplemented with 1 \times penicillin-streptomycin (GIBCO-BRL, Grand Island, NY), 2 mM L-glutamine, and 10% fetal bovine serum (FBS; GIBCO-BRL) at 37°C/5% CO₂. For counting, cells were released by brief trypsinization, collected, and counted electronically in a Coulter Z1 instrument (Coulter Electronics Limited, England).

Interaction of hTR β 1 with p53

The physical interaction between hTR β 1 and N-, D-, and C-p53 was assessed by the GST/glutathione (GSH) binding assay. The binding of GST-hTR β 1 to either ³²S-labeled N-, D-, C-, or wt p53 was performed as described previously for wt p53 (Yap *et al.*, 1996). *In vitro*-translated ³²S-labeled N-, D-, C-, and wt p53 were synthesized with the TNT Coupled Reticulocyte Lysate kit (Promega, Madison, WI).

Transient transfection assay

Human lung carcinoma H1299 cells were seeded at a density of 3.6 \times 10⁵ cells/60-mm dish in RPMI containing 10% FBS as described above. The cells were transfected by the lipofectamine (GIBCO-BRL) method with chloramphenicol acetyltransferase (CAT) reporter plasmids containing Pal TRE (pTK28m-CAT; 1 μ g) or wt p53 DNA binding sites (pG₁₃-

CAT; 1 μ g), pC53wt (0.2 μ g), β -galactosidase expression plasmid (pCH110, Pharmacia; 1 μ g), a hTR β 1 expression vector (pCLC51; at increasing concentrations of 0.2, 1.0, or 2.0 μ g), and pCMV-E6 (1 μ g) as indicated. pBSK was used to adjust the total DNA to 3.7 μ g. The T₃ (100 nM) was added 24 h prior to harvesting of the cells. β -Galactosidase was used to control for transfection efficiency, and the lysates were normalized for protein concentrations.

Viral infection

For viral infection, MCF-7 cells were seeded at a density of 3×10^6 /150-mm dishes and cultured overnight for attachment. Cells were washed with phosphate-buffered saline and the medium changed to Opti-MEM I serum-free medium (GIBCO-BRL). Ten plaque-forming units/cell of a replication-deficient recombinant adenovirus containing a cDNA for hTR β 1 (hTR β 1-virus; Hayashi *et al.*, 1996), or no insert as a control (Null-virus; Katayose *et al.*, 1995), was added, and the cells were incubated for 2 h at 37°C/5% CO₂ with gentle rocking. An equal volume of 2X RPMI was added, and the cells were incubated for 24 h at 37°C/5% CO₂ before subjecting them to γ -irradiation at room temperature using a Mark I Shepherd Model 68 with a ¹³⁷Cs source delivering 5 Gy/min.

RNA isolation and analysis

The cells that had been infected with hTR β 1-virus or Null Ad-virus as described above were lysed in 4 M guanidinium thiocyanate, and total cellular RNA was isolated by the Chomczynski method (Chomczynski and Sacchi, 1987). Poly(A) RNA was prepared as described previously (Hollander and Fornace, 1989). For quantitative dot-blot analysis, 0.8 μ g of poly(A) RNA was directly blotted onto Nytran membranes at eight 2-fold dilutions for each sample. The blots were hybridized with cDNA probes, and the relative poly(A) content of each RNA sample in the dot-blot analysis was estimated using a labeled polythymidylic acid probe as described previously (Hollander and Fornace, 1989; 1990). At least four dot-blot determinations for each point were included in the analysis. Standard curves for control samples at various dilutions were generated using a computer program, which then determined the relative increase for experimental samples (Hollander and Fornace, 1989; 1990).

Protein-DNA binding assay

The binding assay procedure was carried out essentially as described by El-Deiry *et al.* (1992). Briefly, the PG-259 or MG-287 DNA fragments were labeled by Klenow fill-in and incubated with ~200 ng of baculovirus-expressed wt p53 in the presence or absence of *E. coli*-expressed hTR β 1 (Bhat *et al.*, 1993), as indicated. The total protein content in each reaction was kept constant by addition of bovine serum albumin, fraction V (Pierce, Rockford, IL). The DNA fragments bound to p53 were complexed to monoclonal anti-p53 specific antibodies (400 ng each of OPO9 and OPO3; Oncogene Science, Inc., Cambridge, MA) and the protein-DNA complexes immunoprecipitated with Staphylococcal A protein (IgGsorb, Malden, MA). The immunoprecipitate was washed, and bound DNA was purified by treatment with SDS and proteinase K for 30 min at 48°C, followed by phenol-chloroform extraction and ethanol

precipitation. The precipitated DNA was analyzed by nondenaturing 5.4% polyacrylamide gel electrophoresis (PAGE) and detected by autoradiography.

RESULTS

The C-terminus and the DNA-binding domain of p53 interact physically with hTR β 1

We have previously shown that p53 binds specifically to the DNA-binding domain of hTR β 1 (Yap *et al.*, 1996), but the domain(s) of p53 which interacts with hTR β 1 is unknown. We therefore mapped the region(s) of p53 responsible for physically interacting with hTR β 1. We prepared ³⁵S-labeled truncated p53 containing either the DNA-binding domain (D-), the C-terminus (C-), or the N-terminus (N-) of wt p53. The binding of these truncated p53 proteins to hTR β 1 was assessed by the GST/GSH binding system (Fig. 1). As a negative control, GST alone was used. As previously reported, wt p53 specifically interacted with hTR β 1 (Fig. 1; lane 1 compared with lane 2, which shows the background binding when GST alone was used). Furthermore, the interaction with hTR β 1 was preserved when only the D-domain of p53 was used in the assay (compare lane 3 with the negative control in lane 4). A weak inter-

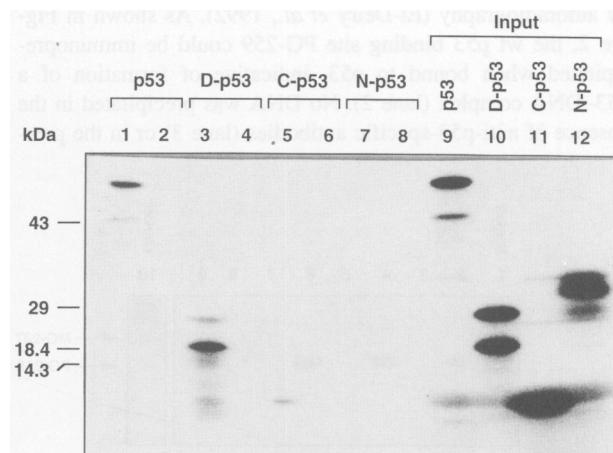


FIG. 1. The DNA binding domain and C terminus of p53 bind to hTR β 1. The binding of GST-hTR β 1 to ³⁵S-labeled DNA-binding domain (D-p53), C-terminus (C-p53), N-terminus (N-p53), or wt p53 was performed as described in Materials and Methods. *E. coli*-expressed GST or GST-hTR β 1 noncovalently bound to GSH-Sepharose beads was incubated with *in vitro*-translated ³⁵S-labeled D-p53, C-p53, N-p53, or wt p53 synthesized using the TNT kit. Lanes 2, 4, 6, and 8 correspond to GST incubated with ³⁵S-labeled wt p53, D-p53, C-p53, or N-p53, respectively, as a negative control. Lanes 1, 3, 5, and 7 correspond to GST-hTR β 1 incubated with ³⁵S-labeled wt p53, D-p53, C-p53, or N-p53, respectively. Lanes 9 through 12 are markers for the migration of ³⁵S-labeled wt p53, D-p53, C-p53, or N-p53, as indicated above each lane. The bottom band in lane 10 corresponds to the ~20-kDa band expected for ³⁵S-labeled D-p53. One tenth of the amount of ³⁵S-labeled wt p53 used for the GST-binding assay was loaded in lane 9, 1/20 of ³⁵S-labeled D-p53 in lane 10, and 1/5 of ³⁵S-labeled C-p53 or N-p53 in lanes 11 and 12, respectively.

action with the C-domain of p53 could also be detected (lane 5 compared with lane 6). In contrast, no specific binding of N-p53 to hTR β 1 could be detected (lane 7) with this system. Lanes 9, 10, 11, and 12 show the positions of wt p53, D-p53 (lower band), C-p53, and N-p53, respectively, with the lysates loaded directly on the gel. These results indicate that the DNA-binding domain of p53, and probably also the C terminus, mediate its interaction with hTR β 1.

Specific binding of p53 to DNA is enhanced by hTR β 1

Previously, we have shown that the physical interaction of p53 and hTR β 1 leads to inhibition of the binding of hTR β 1 to TREs (Yap *et al.*, 1996). The finding that the DNA-binding domain of p53 is one of the interaction sites with hTR β 1 prompted us to investigate whether the binding of p53 to its DNA-binding element was affected by hTR β 1. To examine the effect of hTR β 1 on the p53-binding activity, we performed a widely used p53 DNA-binding assay developed by El-Deiry *et al.* (1992). The ability of p53 to bind to a multimerized p53 consensus DNA-binding site was examined in the presence or absence of increasing amounts of hTR β 1 (Fig. 2). p53 was incubated with its 32 P-labeled DNA-binding element, the p53-DNA complexes were immunoprecipitated with anti-p53 antibodies, and the DNA specifically bound to p53 was analyzed by non-denaturing PAGE. Using this approach, only the DNA specifically bound to p53 could be immunoprecipitated and detected by autoradiography (El-Deiry *et al.*, 1992). As shown in Figure 2, the wt p53 binding site PG-259 could be immunoprecipitated when bound to p53, indicative of formation of a p53-DNA complex (lane 2). No DNA was precipitated in the absence of anti-p53-specific antibodies (lane 3) or in the pres-

ence of nonspecific antibodies as a negative control (data not shown). When hTR β 1 was included at a 1:3 ratio of p53:hTR β 1, there was an increase in the binding of p53 to its consensus DNA-binding site PG-259, as evidenced by an increase in the immunoprecipitated DNA (compare lane 4 with lane 2). Furthermore, this effect of hTR β 1 was dose dependent, as there was a further increase in the binding of p53 to PG-259 when a 1:6 ratio of p53:hTR β 1 was used (compare lane 6 with lanes 4 and 2). In contrast, no DNA was precipitated in the absence of anti-p53 antibodies (lanes 5 and 7) or in the presence of nonspecific antibodies as a negative control (data not shown). To confirm that the binding of p53 to the DNA-binding element PG-259 was specific, we performed the binding assay with MG-287, a DNA fragment containing mutated p53-binding sites (El-Deiry *et al.*, 1992). No MG-287 DNA could be detected in the precipitated material in either the presence or absence of anti-p53 antibodies (lanes 8 and 9, respectively), indicating that p53 could not bind efficiently to this mutated p53-binding element. Together, these results indicate that the interaction of hTR β 1 with p53 led to an increase in the specific DNA binding of p53.

hTR β 1 inhibits p53-dependent transcriptional activity

The above results prompted us to determine whether the increase in the DNA binding of p53 by hTR β 1 led to changes in p53-mediated transcriptional activity. We examined the p53-mediated transcriptional activation in human lung carcinoma H1299 cells, which lack endogenous hTR β 1 and p53, as measured by the PG-287-driven CAT activity (PG₁₃-CAT containing the same wt p53-binding sites used in the DNA-binding studies). The results of this experiment are shown in Figure 3. The lack of endogenous hTR β 1 activity in H1299 cells is shown in lanes 1 and 2. When these cells were transfected with a CAT reporter plasmid containing two palindromic thyroid hormone response elements (TRE-Pal) in tandem (pTK28M-CAT), only background activity was detected (bar 1). Addition of T₃ did not enhance activity, indicating the lack of endogenous TRs. The lack of endogenous hTR β 1 in these cells was confirmed by Western blot analysis (data not shown). Cotransfection of hTR β 1 in the presence of T₃ led to a significant hormone-induced transcriptional activation (compare bar 4 with bar 3). These results demonstrate that transcriptionally active hTR β 1 can be expressed in H1299 cells. Cotransfection of PG₁₃-CAT (containing wt p53-binding sites) and p53 into these cells led to p53-dependent transcriptional activation (bar 5). In contrast, cotransfection of p53 and a promoter containing the mutated p53-binding sites (MG₁₅CAT) did not lead to transcriptional activation in either the presence or absence of hTR β 1, confirming the specificity of the p53-mediated activation of transcription (data not shown). This result is in agreement with the lack of binding of p53 to MG-287 observed in Figure 2. When an equal amount of the p53 and hTR β 1 plasmids was cotransfected into H1299 cells, there was no significant change in p53-mediated transcriptional activation, either in the presence (bar 6 compared with bar 5) or absence (bar 7 compared with bar 5) of T₃. However, when the hTR β 1 expression vector was cotransfected at a 5- or 10-fold excess relative to that of the p53 expression vector, there was a significant and dose-dependent reduction in p53-mediated transcriptional activation (bars 8 and

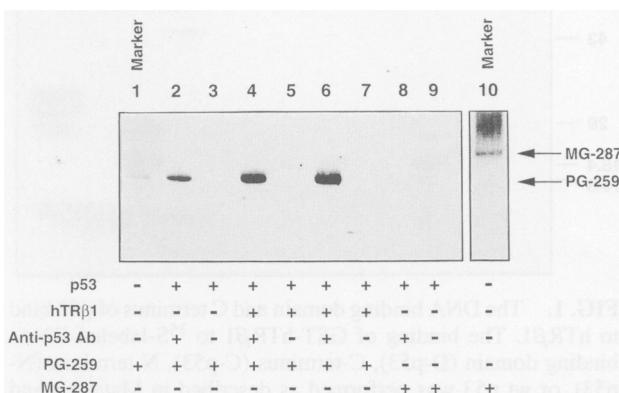


FIG. 2. Effect of hTR β 1 on the DNA binding of p53. 32 P-Labeled DNA fragments containing either wt (PG-259) or mutated (MG-287) p53 binding sites were incubated with wt p53 in the presence or absence of hTR β 1, and the protein-DNA complexes were immunoprecipitated as described in Materials and Methods. The DNA in the immunoprecipitate was purified, analyzed by non-denaturing PAGE, and detected by autoradiography. Lanes 1 and 10, 32 P-labeled PG-259 or MG-287, respectively, loaded as a control for the migration of the DNA fragments after immunoprecipitation. Lanes 2–9, 32 P-labeled DNA fragments purified from the DNA-protein immunoprecipitates in the presence (lanes 4, 5, 6, and 7) or absence (lanes 2, 3, 8, and 9) of hTR β 1. A 1:3 and 1:6 ratio of p53 to hTR β 1 was used in lanes 4 and 6, respectively.

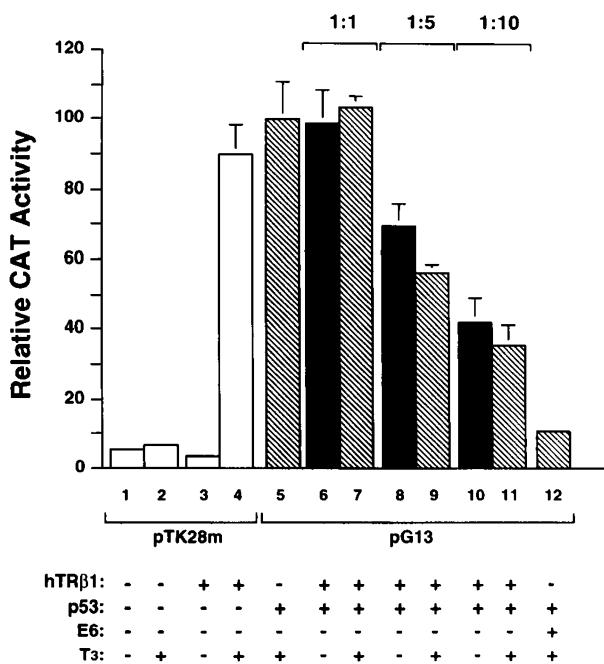


FIG. 3. Effect of hTR β 1 on the transcriptional activation of p53 in H1299 cells. Cells (3.6×10^5 per 60-mm dish) were transfected with CAT reporter plasmids containing Pal TRE (pTK28m-CAT; 1 μ g) or wt p53 DNA-binding sites (PG₁₃-CAT; 1 μ g), pC53wt (0.2 μ g), β -galactosidase expression plasmid (pCH110; 0.5 μ g), a hTR β 1 expression vector (pCLC51; at increasing concentrations of 0.2, 1.0, or 2.0 μ g), and an expression plasmid for the human papilloma virus type 16 E6 protein (pCMV-E6; 1 μ g) were indicated. Bars 1 and 2, pTK28m-CAT without and with T₃, respectively. Bars 3 and 4, pTK28m-CAT in the presence of pCLC51 (1 μ g) without and with T₃, respectively. Bar 5, PG₁₃-CAT (1 μ g) in the presence of pC53wt (0.2 μ g) and T₃ as a positive control for p53 transcriptional activation. Bars 6–11, PG₁₃-CAT (1 μ g) in the presence of pC53wt (0.2 μ g) and increasing amounts of pCLC51 (0.2, 1.0, or 2.0 μ g). The ratio of pC53wt:pCLC51 in the absence or presence of T₃, as indicated, is as follows: bars 6 and 7, 1:1 ratio; bars 8 and 9, 1:5 ratio; bars 10 and 11, 1:10 ratio. Bar 12, pC53wt (0.2 μ g) in the presence of pCMV-E6 (1 μ g) as a positive control for inhibition of p53 transcriptional activation. Transfection and analysis of CAT activity was as described in Materials and Methods. Results shown are representative of three independent experiments.

10 compared with bar 6). This reduction in p53-mediated transcriptional activation was slightly, although not significantly, enhanced in the presence of T₃ (compare bar 9 with 8 and bar 11 with 10). The inhibition of p53 transcriptional activity by hTR β 1 was not caused by simple squelching because the transcriptional activity of an SV40-lacZ reporter was not decreased in the cells with exogenous hTR β 1 expression (data not shown). An expression plasmid for the human papilloma virus type 16 E6 protein was cotransfected with p53 as a control for inhibition of p53-mediated transcriptional activation. As expected, expression of the E6 protein, which leads to degradation of p53 (Leonard *et al.*, 1995), resulted in reduced p53-induced transcriptional activation (bar 12 compared with bar 5).

hTR β 1 affects expression of p53-regulated genes

Because the expression of hTR β 1 resulted in inhibition of p53-mediated transcriptional activation, we then examined whether hTR β 1 affected the expression of genes known to be directly regulated by p53 at the transcriptional level. We used human breast carcinoma MCF-7 cells, which lack endogenous hTR β 1 (lane 1 of the top panel in Fig. 4A). We therefore expressed hTR β 1 in MCF-7 cells via replication-deficient adenovirus (Bhat *et al.*, 1997) to evaluate the effect of hTR β 1 on the gene activation activity of the endogenous p53. Lane 2 of the top panel of Figure 4A shows that hTR β 1 could be expressed in MCF-7 cells by using a hTR β 1-containing adenoviral vector. Lane 3 shows the marker hTR β 1 as a control. The bottom panel of Figure 4A shows that the endogenous p53 protein levels can be induced by γ -irradiation (lane 2 vs. lane 1; Fan *et al.*, 1995) and the induction of p53 protein caused by γ -irradiation was not affected by the expression of hTR β 1 (Fig. 4A, below; compare lanes 1 and 2 with lanes 3 and 4).

We examined the mRNA levels of three p53 target genes: *gadd45*, which has growth-suppressing properties and is involved in DNA repair; *bax*, which promotes apoptosis; and *p21*, a cyclin-dependent kinase inhibitor whose induction can lead to cell-cycle arrest. The relative mRNA levels were determined by dot blot hybridization, and the quantitation is shown in Figure 4B–D. As previously reported (Zhan *et al.*, 1995), p53 induction by γ -irradiation of these cells resulted in an increase in *gadd45* mRNA (Fig. 4B; compare bar 2 with 1). Expression of hTR β 1 by viral infection (above in Fig. 4A) decreased the p53-mediated induction of *gadd45* mRNA in these cells (Fig. 4B; compare bar 6 with 5). Because the induction of p53 protein by γ -irradiation was not affected by the expression of hTR β 1 (below in Fig. 4A), these results indicate that the decrease in *gadd45* mRNA was not attributable to a decrease in p53 protein levels caused by the expression of hTR β 1. As a control, MCF-7 cells were infected with a null virus containing no cDNA insert. In contrast to the effect of hTR β 1, infection of these cells with the null virus did not significantly affect either the basal or p53-induced levels of *gadd45* mRNA (Fig. 4B, bars 3 and 4 compared with bars 1 and 2). Similar to the effect on *gadd45* mRNA, although to a lesser extent, expression of hTR β 1 resulted in impaired induction of *bax* mRNA levels (Fig. 4C, bars 5 and 6 compared with bars 1 and 2). Infection with the null virus did not significantly affect *bax* mRNA levels (Fig. 4C, bars 3 and 4 compared with bars 1 and 2). The induction of *p21* mRNA levels by γ -irradiation, in contrast to that seen with *gadd45* and *bax*, was not affected by expression of hTR β 1 in these cells (Fig. 4D). These results suggest that hTR β 1 affects the expression of some, but not all, p53-regulated genes.

DISCUSSION

We previously reported that p53 could cross-talk with hTR β 1 in its signaling pathways to modulate gene regulatory functions (Bhat *et al.*, 1997). Both the DNA-binding and transcriptional activity of hTR β 1 are inhibited by p53, and this inhibition leads to downregulation of growth hormone gene expression, which is under positive regulation by TRs (Bhat *et al.*, 1997). In the present study, we examined whether the interaction between

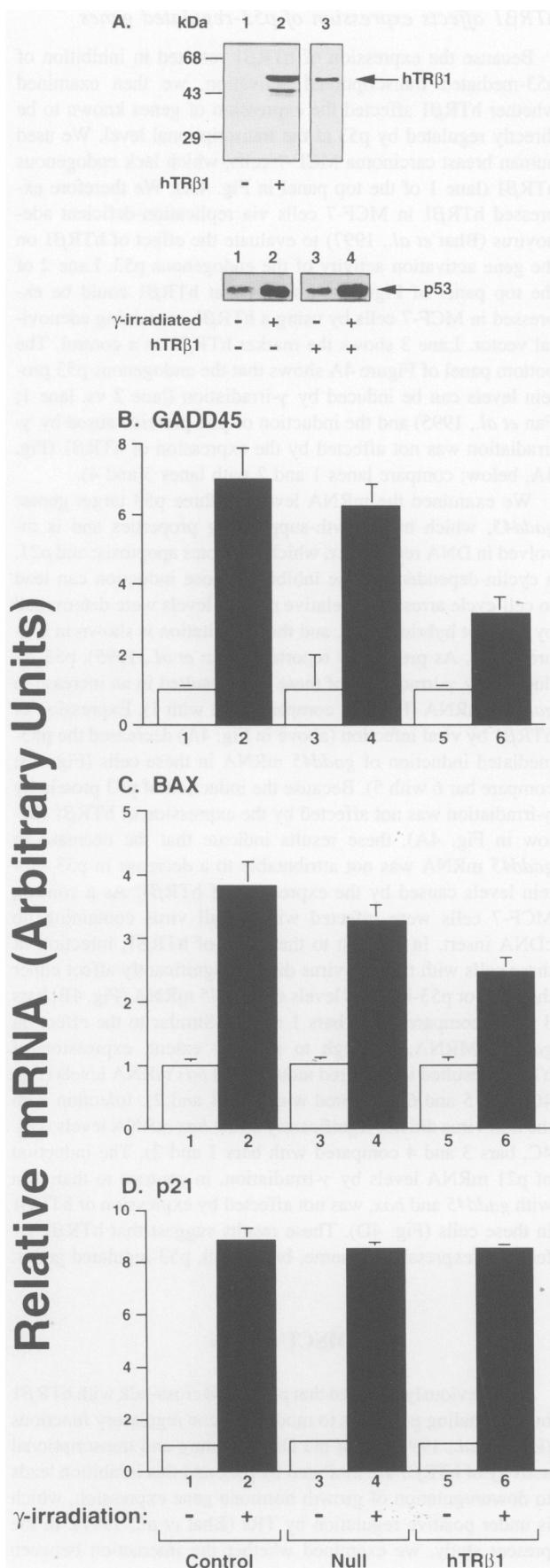


FIG. 4. Effect of TR on the induction of p21^{CIP1/WAF1}, GADD45, and BAX mRNAs in MCF-7 cells after DNA damage. Cells were seeded at a density of 3×10^6 /150-mm dishes and infected with 10 pfu of a replication-deficient recombinant adenovirus containing a cDNA for hTR β 1 or no insert (Null virus) as a control, as indicated in Materials and Methods. **A.** Western blot analysis of hTR β 1 (top) and p53 (bottom). Cells were infected with a viral construct containing the hTR β 1 cDNA or an empty vector as a negative control, as indicated below the figures. Where indicated, cells were γ -irradiated after viral infection as described in Materials and Methods. **E. coli**-expressed hTR β 1 was used as a positive control (above, lane 3). Protein extracts from these cells were separated by SDS-PAGE, transferred to nitrocellulose, and probed for hTR β 1 (top) or p53 (bottom) as previously described (Zhu *et al.*, 1997, and Zhan *et al.*, 1996, respectively). **B, C, D.** Relative abundance of GADD45, BAX, and p21 mRNAs, respectively. The cells were exposed to γ -irradiation (1260 rads) as indicated below the figure and harvested 4 h later. Poly(A) RNA was isolated, and the abundance of the indicated transcripts was determined by quantitative dot-blot hybridization of cellular poly(A) RNA as described in Materials and Methods.

hTR β 1 and p53, in addition to affecting hTR β 1 activity, affected the function of p53. Our results indicate that the interaction between these two proteins led to an increase in the specific binding of p53 to a p53 DNA-binding element. In spite of this increase in the DNA-binding activity of p53, the interaction with hTR β 1 resulted in a decrease in its transcriptional activation and a reduction in the expression of p53-induced genes such as *gadd45* and *bax*. In contrast, the p53-mediated induction of p21 was not affected by hTR β 1 expression. These results suggest that hTR β 1 does not negatively affect the expression of all p53-regulated genes but rather selectively modulates the expression of certain of these genes. In support of our finding that hTR β 1 selectively affects certain functions of p53, it has recently been reported that the interaction of the glucocorticoid receptor (GR) with p53 inhibits p53-dependent transactivation but without affecting its transrepression functions (Maiyar *et al.*, 1997). This observation indicates that the function of p53 can also be modulated by other members of the steroid hormone/retinoic acid receptor superfamily and lends support to the notion that this effect is selective for certain functions of p53. Clearly, additional studies would be required to examine the mechanism by which hTR β 1 selectively affects p53-induced gene expression.

We have mapped the domains of p53 involved in its interaction with hTR β 1 to the central DNA-binding domain and the C terminus of the protein (see Fig. 1). Both of these regions are known to mediate the binding of p53 to DNA. The DNA-binding domain of p53 is responsible for the sequence-specific binding properties of the protein, and mutations in this region greatly reduce its specific DNA-binding activity. The C terminus of p53 is responsible for a relatively sequence-independent binding to DNA. However, this region is clearly involved in regulating the ability of p53 to bind to specific DNA sequences through the central domain (Bargoniotti *et al.*, 1993; Halazonetis and Kandil, 1993; Pavletich *et al.*, 1993; Selivanova and Wiman, 1995; Levine 1997; Anderson *et al.*, 1997). The find-

ing that hTR β 1 targeted the domains of p53 primarily involved in DNA binding and not the N terminus, which has been implicated in transcriptional activation (Picksley and Lane, 1994; Selivanova and Wiman, 1995; Levine 1997), suggests that the repression of p53 transcriptional activation by hTR β 1 reported here is attributable to an indirect effect and not to direct interference with the basal transcriptional machinery.

Consistent with an interaction of hTR β 1 with regions of p53 important for DNA binding, we found that hTR β 1 led to an increase in the sequence-specific DNA binding of p53 (see Fig. 2). The hTR β 1-induced changes in the DNA-binding activity of p53 prompted us to determine whether this effect was accompanied by changes in p53-mediated transcriptional activity. We found that cotransfection of hTR β 1 and p53 in transient transfection assays could lead to decreased p53-mediated transcriptional activation. Furthermore, an increase in the amount of cotransfected hTR β 1 expression vector led to a further repression of transcriptional activation (see Fig. 3), suggesting that the effect of hTR β 1 was dose dependent. In addition, the unliganded receptor by itself repressed p53 transcriptional activation. Therefore, there is a hormone-independent inhibition of p53 transcriptional activation by hTR β 1. The finding that the transcriptional activation of p53 was repressed by hTR β 1 in spite of an increase in its DNA-binding activity supports the notion that hTR β 1 might be acting indirectly, either by preventing the interaction of p53 with other factors or by altering the effect of these factors as modulators of p53-dependent transactivation. In support of this possibility, both p53 and TR have been reported to interact with CBP/p300 (Chakravarti *et al.*, 1996; Gu *et al.*, 1997; Lill *et al.*, 1997; Avantaggiati *et al.*, 1997), a CREB-binding protein with histone acetyltransferase activity. This interaction with CBP/p300 leads to stimulation of the transcriptional activity of both TR and p53. It is possible, therefore, that the binding of hTR β 1 to p53 impairs the ability of CBP/p300 to act as a p53 coactivator to potentiate its transcriptional activity. However, CBP/p300 binds to the N-terminal transcriptional activation domain of p53 (Lill *et al.*, 1997), a region to which we could not detect any binding of hTR β 1 in our GST-binding assay (see Fig. 1, lane 7), suggesting that hTR β 1 might not directly inhibit the binding of CBP/p300 to p53. This issue was not addressed in the present study, and additional studies would be required to examine this possibility.

The finding that hTR β 1 can cross-talk with p53 signaling pathways is of importance to our understanding of cell-cycle control and tumorigenesis because it raises the possibility that T₃ might be involved in modulating some of the effects of p53. In agreement with this possibility, T₃ has been reported to enhance X-ray-induced neoplastic transformation *in vitro* (Guernsey and Fisher, 1990). In addition, because p53 has antiproliferative properties and may lead to cell-cycle arrest in G₁ (Maxwell and Roth, 1994; Leonard *et al.*, 1995; Selivanova and Wiman, 1995; Levine, 1997), whereas T₃ promotes cell growth by shortening the G₁ phase (DeFesi and Surks, 1981; DeFesi *et al.*, 1984, 1985), it is possible that the growth-stimulatory effect of hTR β 1 is secondary, at least in part, to modulation of p53 functions. The finding that two members of the steroid hormone/retinoic acid receptor superfamily can modulate p53 function (hTR β 1: this study; Maiyar *et al.*, 1997) suggests that other members of the superfamily may also participate in the cross-talk between these regulatory pathways.

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Address reprint requests to:

*Dr. Sheue-Yann Cheng
Building 37, Rm. 2D-24
37 Convent Drive MSC 4255
National Cancer Institute
Bethesda, MD 20892-4255*

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