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Modulation of the transcriptional activity of thyroid hormone receptors by the tumor suppressor p53

(gene regulation/transcription factors/protein–protein interaction)

NIDA YAP, CHIA-LIN YU, AND SHEUE-YANN CHENG*

Gene Regulation Section, Laboratory of Molecular Biology, Division of Basic Sciences, National Cancer Institute, National Institutes of Health, Building 37, Room 2D24, 37 Convent Drive, MSC 4255, Bethesda, MD 20892-4255

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ABSTRACT **Thyroid hormone nuclear receptors (TRs) are ligand-dependent transcriptional factors that regulate growth, differentiation, and development. The molecular mechanisms by which TRs mediate these effects are unclear. One prevailing hypothesis suggests that TRs may cooperate with other transcriptional factors to mediate their biological effects. In this study, we tested this hypothesis by examining whether the activity of TRs is modulated by the tumor suppressor p53. p53 is a nuclear protein that regulates gene expression via sequence-specific DNA binding and/or direct protein–protein interaction. We found that the human TR subtype β1 (h-TRβ1) physically interacted with p53 via its DNA binding domain. As a result of this physical interaction, binding of h-TRβ1 to its hormone response elements either as homodimer or as a heterodimer with the retinoic X receptor was inhibited by p53 in a concentration-dependent manner. In transfected cells, wild-type p53 repressed the hormone-dependent transcriptional activation of h-TRβ1. In contrast, mutant p53 either had no effect or activated the transcriptional activity of h-TRβ1 depending on the type of hormone response elements. These results indicate the gene regulating activity of TRs was modulated by p53, suggesting that the cross talk between these two transcriptional factors may play an important role in the biology of normal and cancer cells.**

The thyroid hormone 3,3′,5-triiodo-L-thyronine (T_3) promotes growth, induces differentiation, and regulates metabolic functions. Recent studies (1–3) indicate that these effects are mediated by the interaction of T_3 with the thyroid hormone nuclear receptors (TRs), which are members of the steroid hormone/retinoic acid receptor superfamily. Two TR genes, TRα and TRβ, located on chromosomes 17 and 3, respectively, have been isolated. Three TRs (β1, β2, and α1) are generated from these two genes by alternative splicing (1–3). The gene regulating activity of TRs depends not only on T_3 but also on the specific DNA sequences in the promoter regions of T_3 target genes, known as the thyroid hormone response elements (TREs). TREs consist of consensus DNA sequences with the half-site binding motifs as a palindrome (Pal), as a direct repeat separated by four nucleotides (DR+4), and as an everted repeat separated by six nucleotides (Lys). However, these TREs confer different degree of hormonal responses in a cell-type dependent manner (4), suggesting that the gene regulating activity of TRs could potentially be modulated by other cellular factors. Indeed, TRs have been shown to heterodimerize with all subtypes of the retinoid X receptors (RXRs), the retinoic acid receptors, the vitamin D receptor, and the fatty acid/peroxisome proliferator-activated receptor (1, 5–10). Several transient transfection systems further demonstrated that transactivation activity of TRs is modulated by the RXRs (8, 11–13).

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Based on the above observations, one emerging hypothesis suggests that TRs could mediate their diverse biological effects via cooperation with different transcription factors/receptors. We therefore tested this hypothesis by examining whether the activity of TRs is modulated by the tumor suppressor p53. p53 is a transcription factor that regulates gene expression by binding to specific DNA sequences termed p53 response element. When these sites are near a promoter, p53 stimulates transcription in a dose-dependent manner (14, 15). However, p53 can also repress expression of many genes that lack p53 response elements, including c-*fos*, c-*jun*, retinoblastoma, interleukin 6, and several endogeneous cellular genes that regulate cellular proliferation (16–19). Since the mechanism by which TRs mediate the cell growth is unknown and since both TRs and p53 play a crucial role in cell cycle regulation (20–24), we explored the possibility that TR activity could be regulated by p53. Here we show that human TR subtype β1 (h-TRβ1) physically interacted with p53, resulting in the modulation of the transcriptional activation of h-TRβ1.

MATERIALS AND METHODS

D-threo-[*dichloroacetyl*-1-^{14}C]Chloramphenicol [2.04 GBq/mmol; 55 mCi/mmol (1 Ci = 37 GBq)] and [α-^{32}P]dCTP were obtained from Amersham. Glutathione *S*-transferase (GST)-Sepharose was purchased from Pharmacia. TNT Coupled Reticulocyte Lysate System was from Promega. Monkey CV1 cells and human HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% (vol/vol) fetal calf serum.

Plasmids. The plasmids for expressing h-TRβ1 under the control of cytomegalovirus promoter, pCLC51, were prepared as described (25). The T7 expression plasmids of h-TRβ1 (pCJ3) were constructed as described (26). The T7 expression plasmids of p53 (pCD53, 273H, and 175H) were gifts from C. Miller (Cedars-Sinai Medical Center, University of California at Los Angeles School of Medicine); the expression plasmids of wild-type p53 (p53wt), mutant p53 (175H, 248W, and 273H), pG13-CAT and pMG15-CAT were gifts from B. Vogelstein (Johns Hopkins Oncology Center, Baltimore); pGST–p53wt was a gift from T. Shenk; pGST-TRβ1 was a gift from C. Glass (University of California at San Diego, La Jolla); pTK-LysCAT and pTK-MECAT were gifts from G. Brent (West Los Angeles Veterans Administration Medical Center); and pTK28m was a gift from D. Moore (Harvard Medical School, Boston). pCH110, which is an expression vector for

Abbreviations: T_3, 3,3′,5-triiodo-L-thyronine; TR, thyroid hormone nuclear receptor; h-TRβ1, human TR subtype β1; TRE, thyroid hormone response element; Pal, a palindromic TRE; DR+4, direct repeat of the half-site binding motifs separated by 4 nt; Lys, the TRE of the chicken lysozyme gene that is an everted repeat of the half-site binding motifs separated by 6 nt; CAT, chloramphenicol acetyltransferase; GSH, glutathione; GST, glutathione *S*-transferase; RXR, retinoid X receptor; wt, wild type.

*To whom reprint requests should be addressed.

β-galactosidase, was from Pharmacia, and pCMV-Neo was purchased from Invitrogen.

Analysis of the Interaction of h-TRβ1 with p53. GST/glutathione (GSH) binding system was used to assess the physical interaction of h-TRβ1 and p53. Binding of ^{35}S-labeled h-TRβ1 to GST–p53 was carried out as described with modifications (27). *Escherichia coli* expressed GST (4 μg) or GST–p53 (2 μg) noncovalently bound to GSH-Sepharose beads was preincubated in 500 μl buffer (20 mM Tris·HCl, pH 7.5/100 mM NaCl/2 mM EDTA/0.1% Lubrol/2 mM dithiothreitol/0.05% bovine serum albumin/5% glycerol) at 4°C for 1 hr. Untreated sepharose bead suspension was added to normalize the volume of Sepharose beads per samples. *In vitro* translated ^{35}S-labeled h-TRβ1 (10 μl) synthesized by using the TNT kit was added to the above pretreated beads in 500 μl of the same buffer. The mixture was incubated at 4°C for 2 h with constant shaking. The beads were washed five times with 1 ml ice-cold buffer (2.5% sucrose/2.5 mM Tris·HCl, pH 7.4/2.5 mM EDTA/0.25 M NaCl/1% Lubrol) and boiled in SDS/sample buffer. The proteins were analyzed by SDS/PAGE. The gel was dried and autoradiographed. Conversely, ^{35}S-labeled p53wt (20 μl) was incubated with GST (4 μg) or GST–h-TRβ1 (2 μg) bound to GSH-Sepharose beads. The beads were processed as above.

Electrophoretic Mobility-Shift Assay. Two complimentary oligonucleotides containing F2, DR+4, or Pal sequences (28) were annealed and the recessed 3′ end was filled in with [α-^{32}P]dCTP (100 μCi) using DNA polymerase (Klenow fragment). The labeled probes were purified from a 12% polyacrylamide gel and electrophoretic mobility-shift assay was carried out as described (28). The intensities of retarded bands and free probes were quantified by PhosphorImager (Molecular Dynamics).

Transient Transfection Assay. CV1 cells (4.5×10^5 per 60-mm dish) were transfected with chloramphenicol acetyltransferase (CAT) reporter plasmid containing TRE [pTK-LysCAT, pTK28m(Pal), or pTK-MECAT; 2.0 μg]; h-TRβ1 expression plasmid, pCLC51 (0.5 μg) and pC53wt (at increasing concentrations of 0.5, 2.5, 5.0 μg); and β-galactosidase expression plasmid, pCH110 (1.0 μg). pCMV-Neo was used to adjust total DNA to 8.5 μg per dish. Transfection was carried out using Lipofectamine (GIBCO/BRL). T_3 (100 nM) was added 12–14 hr before the cells were lysed. The lysates were normalized for protein concentrations and analyzed for CAT activity (29). Based on β-galactosidase activity, the variations between duplicates were less than 5%.

RESULTS

p53 Protein Binds Specifically to h-TRβ1. We used the GST/GSH binding system to assess whether h-TRβ1 physically interacted with p53wt. GST alone was used a negative control. Fig. 1*A* shows that ^{35}S-labeled h-TRβ1 bound to GST–p53wt (lane 3) but not GST (lane 2). Under the experimental conditions, 2–3% of the total added h-TRβ1 was bound. This specific interaction was further illustrated by the dose-dependent increased binding of ^{35}S-labeled h-TRβ1 to p53wt shown in Fig. 1*B* and up to ≈10% of total added ^{35}S-labeled h-TRβ1 was bound to p53wt. The nonspecific background as determined by GST alone remained low. The specific physical interaction between h-TRβ1 and p53wt was confirmed in a reverse manner in that ^{35}S-labeled p53wt was shown to bind to GST–h-TRβ1 (lane 6 vs. lane 5 of Fig. 1*A*).

It is known that the biochemical and biological activities of the p53wt are different from the mutant p53 (23, 24). We therefore characterized the interaction of h-TRβ1 with several most frequently observed p53 mutants found in human cancers to see if p53 mutants act differently from the wild-type p53. Using the GST/GSH binding system, we found that p53 mutants also bound to h-TRβ1 (Fig. 1*A*, lanes 8, 10, and 12 vs. the negative controls in lanes 7, 9, and 11). However, the avidity of binding varies with the mutants. Among the three mutants, 141Y bound to h-TRβ1 with the highest avidity (lane 8 vs. lanes 10 and 12). Furthermore, mutant 141Y and 175H more actively bound to h-TRβ1 (Fig. 1*A*, lanes 8 and 10 vs. lane 6), whereas 273H less actively bound to h-TRβ1 than did the p53wt.

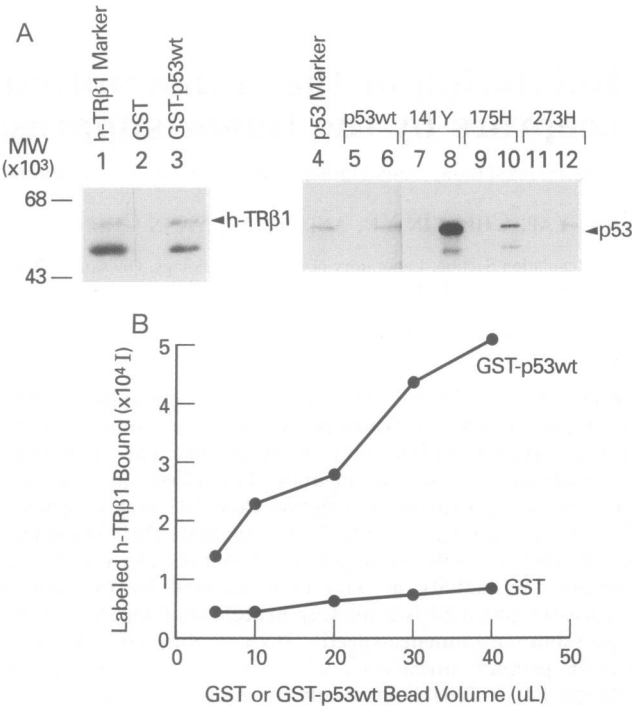

FIG. 1. p53 protein binds specifically to h-TRβ1. (*A*) The binding of ^{35}S-labeled h-TRβ1 to GST–p53 was carried out as described in *Materials and Methods. E. coli* expressed GST (4 μg) or GST–p53 (2 μg) noncovalently bound to GSH-Sepharose beads was incubated with *in vitro* translated ^{35}S-labeled h-TRβ1 synthesized by using the TNT kit. The bound proteins were analyzed by SDS/PAGE. Lane 1, h-TRβ1 marker (0.3 μl); lanes 2 and 3, GST and GST–p53 incubated with ^{35}S-labeled h-TRβ1 (10 μl); lane 4, p53wt marker (0.6 μl). For lanes 5–12, an equal amount of the p53wt and p53 mutant proteins was used. The synthesized proteins were quantified by measuring the intensity of the radioactive bands using PhosphorImager after SDS/PAGE. Lanes 5 and 6, GST and GST-h-TRβ1 incubated with ^{35}S-labeled p53wt (20 μl); lanes 7 and 8, GST and GST-h-TRβ1 incubated with ^{35}S-labeled p53–141Y (20 μl); lanes 9 and 10, with ^{35}S-labeled p53–175H (20 μl); lanes 11 and 12, with ^{35}S-labeled p53–273H (25 μl), respectively. (*B*) Following a similar protocol as above, ^{35}S-labeled h-TRβ1 (10 μl) was incubated with the increasing amounts of GST (2, 4, 8, 12, 16 μg; 0.4 μg/μl bead suspension) or GST–p53 (1, 2, 4, 6, 8 μg; 0.2 μg/μl bead suspension).

To map the region of h-TRβ1 to which p53wt bound, we prepared ^{35}S-labeled truncated h-TRβ1 in which domains were systematically deleted (Fig. 2*A*): ED41, deletion of domain A/B; MD32, deletion of domains A/B and C; KD25, deletion of domains A/B, C, and D (25). We compared the binding of these truncated h-TRβ1 with the intact h-TRβ1 using the GST/GSH binding assay. Lane 6 of Fig. 2*B* shows that deletion of domain A/B had no effect on the binding h-TRβ1 to p53wt. In fact, the binding was enhanced ≈10-fold upon deletion of domain A/B. However, when the DNA binding domain was deleted from h-TRβ1 (MD32 and KD25), binding to p53wt was lost (lanes 9 and 12 vs. lanes 3 and 6), indicating that the DNA binding domain (domain C) of h-TRβ1 was the binding region for p53wt. To further support this conclusion, we prepared the DNA binding domain of h-TRβ1 (LK11; Fig. 2*A*) and carried out similar binding assays. Lane 14 of Fig. 2*B* shows that LK11 (the DNA binding domain of h-TRβ1) bound

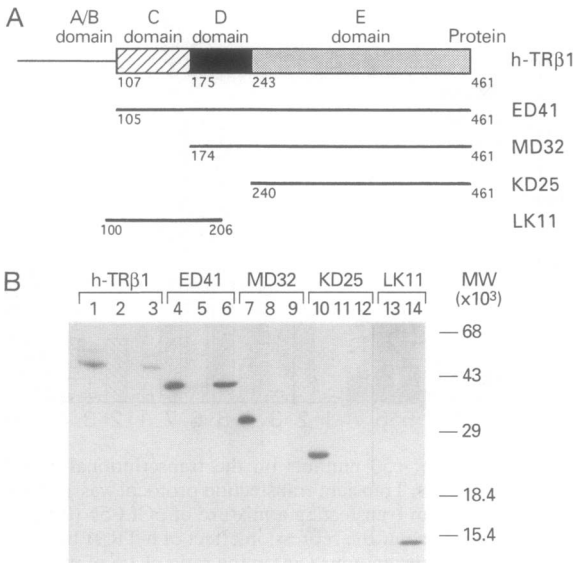

FIG. 2. DNA binding domain of h-TRβ1 is the interaction region for p53. (*A*) Schematic representation of h-TRβ1 deletion mutants (30). The full-length h-TRβ1 has 461 aa. The amino acid sequences from 1–106, 107–174, 175–242, and 243–461 are designated A/B, C, D, and E domains, respectively, based on Green and Chambon (31) and Beck-Peccoz *et al.* (32). The length of each coding region in the expression plasmids is shown with the names of the corresponding intact or truncated proteins. (*B*) Following a similar protocol as in Fig. 1*A*, an equivalent amount of ^{35}S-labeled intact or the truncated h-TRβ1 proteins was incubated separately with an equal amount of GST (4 μg; lanes 2, 5, 8, 11, and 13) or GST–p53wt (2 μg; lanes 3, 6, 9, 12, and 14) bead suspension. An equivalent amount of each ^{35}S-labeled protein synthesized by *in vitro* transcription/translation was immunoprecipitated with monoclonal antibody C3, an antibody directed to the E domain of h-TRβ1 (33), to serve as respective control protein markers (lanes 1, 4, 7, and 10). Lanes 1–3, intact h-TRβ1; lanes 4–6, ED41; lanes 7–9, MD32; lanes 10–12, KD25; and lanes 13 and 14, LK11.

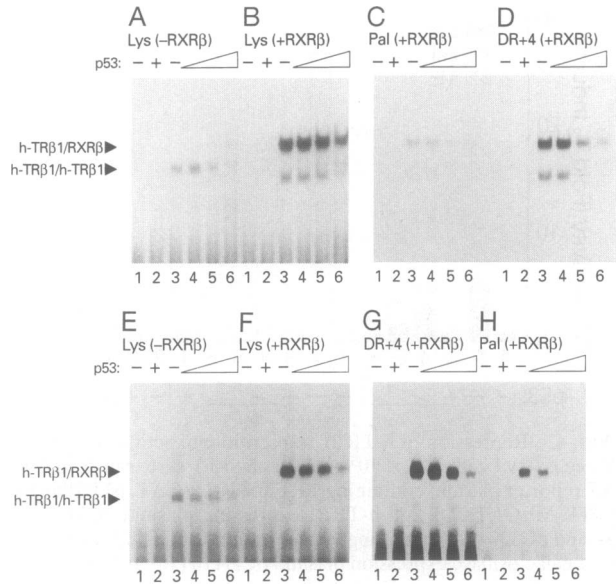

FIG. 3. Wild-type and mutant p53 proteins both block h-TRβ1 binding to TREs. Binding of h-TRβ1 to TREs was assayed by the electrophoretic mobility-shift assay method (28). (*A* and *B*) The effect of p53wt on h-TRβ1 binding to ^{32}P-labeled Lys in the absence or presence of RXRβ, respectively. (*C* and *D*) Effect of p53wt on h-TRβ1 binding to ^{32}P-labeled Pal or DR+4 in the presence of RXRβ, respectively. (*E* and *F*) Effect of p53–175H on h-TRβ1 binding to ^{32}P-labeled Lys, in the absence or presence of RXRβ, respectively. (*G* and *H*) Effect of p53–273H on h-TRβ1 binding to ^{32}P-labeled DR+4 or Pal with RXRβ, respectively. The synthesized p53 and h-TRβ1 proteins were quantified similarly as in Fig. 1 and an equal amount of corresponding proteins was used. Lane 1, unprogrammed rabbit reticulocyte lysate as a negative control (5.5 μl); lane 2, *in vitro* translated p53wt or p53mt (p53–175H or p53–273H) (5.0 μl); lane 3, h-TRβ1 (0.5 μl); lanes 4–6, increasing volume (μl) ratio of p53wt or p53mt to h-TRβ1 lysates (0.5/0.5, 2.5/0.5, 5.0/0.5). Total lysate volume per sample was standardized to 5.5 μl by addition of unprogrammed reticulocyte lysate before incubation. RXRβ protein was prepared from recombinant baculovirus infected Sf9 insect cells (6, 30).

specifically to p53wt as compared with lane 13, which shows the background binding when GST alone was used. The identification of domain C of h-TRβ1 as the region to which p53wt bound could account for the increased binding of p53wt to ED41 in that the DNA binding domain was more accessible to p53wt due to the deletion of the A/B domain.

Binding of h-TRβ1 to TREs Is Inhibited by p53wt. The functional significance of the physical interaction of these two transcription factors was first assessed by the effect of p53wt on the *in vitro* binding of h-TRβ1 to TREs. h-TRβ1 is known to bind to three types of TRE as a homodimer. Its binding to TREs is enhanced by heterodimerization with the RXRs (1, 5–8). Lanes 4–6 (vs. lane 3) of Fig. 3*A* show that binding of h-TRβ1 to Lys as a homodimer was inhibited by p53wt in a concentration-dependent manner. Likewise, binding of h-TRβ1 to Lys as a heterodimer with RXRβ (upper bands in lanes 4–6 of Fig. 3*B*) was similarly inhibited by p53wt. The inhibition of h-TRβ1 binding to p53wt was not limited to Lys. Binding of h-TRβ1 to Pal and DR+4 as a heterodimer were also inhibited in a concentration-dependent manner (Fig. 3 *C* and *D*; lanes 4–6 vs. lane 3, respectively). It is important to point out that the inhibition was not due to the competition of p53wt with h-TRβ1 for binding to TREs because p53wt itself did not recognize any of the three TREs as its DNA binding element (Fig. 3 *A–D*, lanes 2). Furthermore, the observations that the position of TRE-bound h-TRβ1 on the gel did not change in the presence of p53wt (Fig. 3 *A–D*, lanes 3 vs. lanes 4–6), and that the addition of anti-p53wt monoclonal antibodies did not cause the TRE-bound h-TRβ1 to supershift as did the anti-h-TRβ1 monoclonal antibodies (data not shown; ref. 13), indicate that the physical interaction of h-TRβ1 with p53wt may have rendered h-TRβ1 incapable of binding to TREs.

TRE-Dependent Repression of h-TRβ1 Transcriptional Activity by p53wt. The above results prompted us to further examine the effect of p53wt on the transcriptional activity of h-TRβ1 as measured by the TRE-driven CAT in monkey CV1 cell line. Fig. 4 shows that T_3-dependent transcriptional activity of h-TRβ1 varied with TREs. The fold of T_3-induction was 4.9 ± 0.9, 27.3 ± 1.2, and 10.3 ± 0.5 (mean ± SD, n = 7) for Lys, Pal, and ME (the TRE for malic enzyme, a DR+4-like TRE), respectively. However, in all TREs, cotransfection of p53wt expression plasmids led to a repression of the transcriptional activity of h-TRβ1 in a concentration-dependent manner. Similar repression was also observed in human HeLa cells using Lys (data not shown). This repression is not due to the competition of p53wt with h-TRβ1 for binding to TRE because p53wt could not transactivate via TRE-CAT (Fig. 4, see bars 3 for all TREs). Furthermore, the effect of p53wt on the silencing function of the unliganded h-TRβ1 on three TREs was also evaluated and no effect was found (data not shown).

However, the sensitivity of repression of h-TRβ1 by p53wt was TRE-dependent. When an equal amount of h-TRβ1 and p53wt plasmids was co-transfected into CV1 cells, the transcriptional activity mediated by ME was the most sensitive to the repression by p53wt in that 82% of the activity was lost as compared with the 40% seen for Lys and Pal. Among the three TREs, the transcriptional activity mediated by Pal was the least sensitive to the repression effect of p53wt. When 10 times more

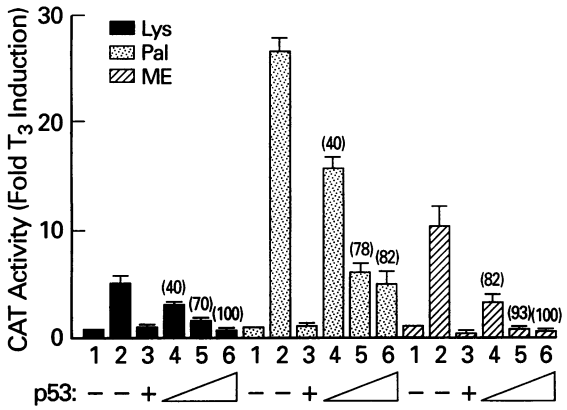

FIG. 4. Repression of h-TRβ1 transcriptional activity by p53wt in CV1 cells. CV1 cells (4.5×10^5 per 60-mm dish) were transfected with CAT reporter plasmid containing TRE [pTK-LysCAT, pTK28m(Pal), or pTK-MECAT; 2.0 μg], h-TRβ1 expression plasmid pCLC51 (0.5 μg), and pC53wt (at increasing concentrations of 0.5, 2.5, and 5.0 μg) and β-galactosidase expression plasmid pCH110 (1.0 μg). pCMV-Neo was used to adjust total DNA to 8.5 μg per dish. Transfection and analyses of CAT activity were carried out as described in *Materials and Methods*. Bars 1 and 2, pCLC51 (0.5 μg) without and with T_3; bar 3, pC53wt (5.0 μg) with T_3; bars 4–6, mixtures of pCLC51 (0.5 mg) and pC53wt (0.5, 2.5, 5.0 μg) with T_3, respectively. The numbers in parentheses (bars 4–6) represent percent repression as compared with the control in bars 2. The error bars are mean ± SD (n = 7).

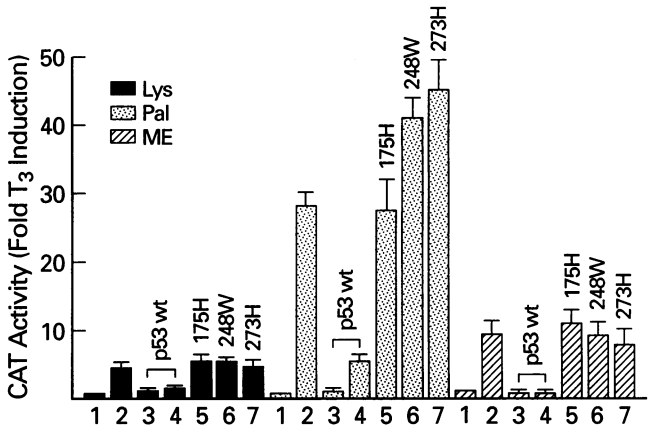

FIG. 5. Effect of p53 mutants on the transcriptional activity of h-TRβ1 in CV1 cells. Transient transfection protocol was as described in Fig. 4. Aside from transfecting a mixture of pCLC51 (0.5 μg) and pC53wt (2.5 μg) to show the repression effect of h-TRβ1 by p53wt for comparison, the same amounts (μg) in the ratio of 1:5 of pCLC51 and pC53–175H, pC53–248W, or pC53–273H mixtures were also transfected separately into CV1 cells. Bars 1 and 2, pCLC51 (0.5 μg) without and with T_3; bars 3, pC53wt only with T_3; bars 4–7, pCLC51 (0.5 μg) with pC53wt, pC53–175H, pC53–248W, or pC53–273H (each 2.5 μg), respectively, with T_3.

of p53wt than h-TRβ1 plasmid was transfected, while the transcriptional activity mediated by Lys or ME was completely repressed, ≈18% of the activity was still remained with Pal.

p53 Mutants Cannot Repress the Transcriptional Activity of h-TRβ1. The observations that mutant p53s bound to h-TRβ1 led us to evaluate whether p53 mutants also affected the binding of h-TRβ1 to TREs. The physical interaction of p53 mutants with h-TRβ1 also led to the inhibition of binding h-TRβ1 to TREs as shown in the representative results (Fig. 3 *E–H*). Lanes 4–6 of Fig. 3 *E* and *F* show that p53–175H inhibited the binding of h-TRβ1 to Lys as a homodimer and as a heterodimer, respectively, in a concentration-dependent manner. Likewise, binding of h-TRβ1 to DR+4 (Fig. 3*G*, lanes 4–6) or Pal (Fig. 3*H*, lanes 4–6) as a heterodimer was inhibited by p53–273H in a concentration-dependent manner.

However, a totally different picture emerged when the effect of p53 mutants on the h-TRβ1-mediated TRE-dependent transcriptional activity was assessed. As shown in Fig. 5, in contrast to p53wt (bars 4 of Fig. 5; also see Fig. 4), p53 mutants could not repress the transcriptional activity of h-TRβ1 mediated by F2 or ME for all three mutants (Fig. 5, bars 5–7). Surprisingly, a ≈1.5-fold activation of the transcriptional activity mediated by Pal was observed for mutant 248W and 273H, but not for mutant 175H (bars 6 and 7 vs. 5). The differences among the three mutants in the effect on the transcriptional activity mediated by Pal was not due to the differences in the expression of mutant p53 genes because cellular protein levels of these three mutants are similar and were 6- to 8-fold higher than that of p53wt (data not shown).

DISCUSSION

The present study identified p53 as a new member of TR-interacting proteins that acted to modulate the transcriptional activity of h-TRβ1. p53wt repressed transcriptional activity of h-TRβ1, whereas p53 mutants either had no effect or activated the transcriptional activity depending on the type of TRE. At the present time, the detailed molecular mechanism for the repression effect of p53wt is unclear. However, our data indicate that this repression was not mediated by the competition of p53wt with h-TRβ1 for binding to the TREs because p53wt neither bound to nor transactivated via TREs. Furthermore, this repression was not due to the effect of p53wt on the corepressor(s) because p53wt did not affect the silencing function of the unliganded h-TRβ1. Our studies demonstrate that the repression process most likely involved h-TRβ1/p53wt interaction as one of the key steps. p53wt bound to the DNA binding domain of h-TRβ1. The interaction of these two proteins probably resulted in conformational changes of h-TRβ1 in that it could no longer bind to TREs and thus it was released from the DNA binding sites (see Fig. 3). However, it was clear that in addition to the obligatory step of h-TRβ1/p53wt interaction, other not yet identified steps were involved in this repression.

p53 mutants were also capable of binding to h-TRβ1 and similarly blocked the binding of h-TRβ1 to its TREs as the p53wt. Yet, p53 mutants not only could not repress the transcriptional activity of h-TRβ1 as the p53wt, but on Pal, a ≈1.5-fold of activation for mutant 248W and 273H was seen. These observations were consistent with the previous findings that the wild-type and mutant p53 can repress or activate in a promoter- and cell-type specific manner (34, 35). It is possible that in CV1 cells, p53 mutants fail to bind to a factor that could be critical for the repression effect of p53wt. The delineation of molecular basis for differential effects of the wild-type and mutant p53 on the activity of h-TRβ1 would await future studies.

The demonstration that p53wt repressed the transcriptional activity of TR has important implications in the biology of normal and cancer cells. For example, TRs are known to stimulate cell growth by shortening the duration of the G_1 phase (20–22), whereas p53wt has anti-proliferative effect and arrests the progression of cells at the G_1 phase (36). This raises the possibility that one of the mechanisms by which p53wt exerts its anti-proliferative effect could be mediated by blocking the effect of TRs. This action, however, is lost in p53 mutants. Our preliminary findings indicate that the transcriptional activity of p53wt was also repressed by TRs (unpublished results). This could explain the observations that x-irradiation-induced neoplastic transformation of cells *in vitro* is enhanced by thyroid hormones (37) as the action of G_1 arrest by p53wt could be abrogated by TRs.

At the present time, it is unknown whether p53 also interacts with other members of the receptor superfamily. However, the findings that the DNA domain of TRs was the interaction site

for p53 suggest that p53 could also interact with other members of the receptor superfamily because the DNA binding domains of the superfamily are highly conserved. This could open the possibility for p53 to cross talk with a large net work of nuclear receptor superfamily to modulate cellular functions.

In spite of recent advances, the mechanisms by which TRs mediate the pleiotropic effects of T_3 remain unclear. One of the fundamental questions is whether the diversity of the pleiotropic effects of T_3 are achieved by the obligatory networking of TRs with other transcription factors/receptors. Increasing evidence has suggested this is a plausible mechanism. Earlier, it has been shown that TRs interact with other members of the nuclear receptor superfamily: the retinoic acid receptors, the RXRs, the vitamin D receptor, and the fatty acid/peroxisome proliferator-activated receptor (5–10). These interactions lead to changes in hormone sensitivity and modulation of the activity of TRs (8, 9, 11–13). TRs have also been shown to interact with the AP1 and the cell-type specific transcriptional activator Pit-1 resulting in antagonistic and synergistic effects, respectively (38, 39). More recently, using the yeast two-hybrid system, additional TR-interacting proteins were identified. Their functions have been suggested to modulate the gene regulating activity of TRs via a T_3-dependent or independent pathway (40, 41). The identification of p53 as one of the TR-interacting proteins in the present study further strengthens the hypothesis that mediation of the pleiotropic effects of T_3 requires the cooperation of TRs with a large network transcription factors/receptors.

We thank Dr. Carl W. Miller for the T7 expression plasmids of p53 (pCD53, 273H, 141Y, and 175H); Dr. B. Vogelstein for the expression plasmids of p53wt and mutant p53 (143A, 175H, 248W, and 273H), pG13–CAT, and pMG15–CAT; Dr. Thomas Shenk for pGST–p53wt; Dr. Christopher Glass for pGST–TRβ1; Dr. David Moore for pTK28m, and Dr. Gregory Brent for pTK–LysCAT and pTK–MECAT.

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