Reexpression of Thyroid Peroxidase in a Derivative of an Undifferentiated Thyroid Carcinoma Cell Line by Introduction of Wild-Type p53

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ABSTRACT

Loss of function of p53 is believed to result in transformation through impairment of its properties as a transcription factor, which interferes with the regulation of the cell cycle and under certain conditions, with programmed cell death. We report that stable transfection of clonal undifferentiated thyroid carcinoma cell lines harboring endogenous p53 mutations with a wild-type p53 expression vector not only yields transfectants expressing authentic wild-type p53. Among these, most exhibited an increase in doubling time and an impairment of colony formation in soft agar. Only one clonal wild-type p53-overexpressing derivative of the NPA papillary carcinoma cell line was obtained, and these cells were found to reexpress thyroid peroxidase (TPO). This clone also demonstrated reexpression of the paired box domain transcription factor Pax-8, which specifically activates transcription of TPO. Wild-type p53 did not directly stimulate transcriptional activity of a TPO promoter construct. Although the low frequency of authentic wild-type p53 stable transfectants limits the power of this analysis, these data suggest that in addition to its role in malignant transformation, p53 may be significant in the determination or maintenance of cell differentiation in thyroid neoplasms.

INTRODUCTION

The specialized function of thyocytes is lost in undifferentiated carcinomas of follicular cells (1). This fact has prognostic significance because the survival of patients with thyroid cancer is dependent in part on the extent to which the neoplastic cells retain the ability to trap radioiodine (2). Recently, a high prevalence of mutations of the p53 tumor suppressor gene has been reported in undifferentiated and anaplastic thyroid carcinomas and in thyroid carcinoma cell lines (3–7). p53 is a nuclear phosphoprotein that binds DNA both nonspecifically as well as at specific sequences and controls the transcriptional activity of certain genes (8–11). Induction of wild-type p53 in transformed cells causes growth arrest in the G1 phase of the cell cycle (12, 13) and can evoke apoptosis in myeloid leukemic cells (14). Inactivating mutations of p53 interfere with these critical functions and are thus believed to be major determinants of the phenotype of many forms of cancer.

Point mutations, as well as gene amplification of ras proto-oncogenes, have been implicated in the early stages of thyroid tumorigenesis (15–18). Besides the impact of inappropriate activation of ras on cell growth, transformation, and genomic stability (19, 20), mutant ras oncogenes decrease expression of Tg3 and TPO, two major differentiated gene products of the thyroid gland (21). These effects can be accounted for in part by impairment of the expression and/or action of thyroid-specific transcription factors (21). However, it is clear that many thyroid neoplasms harboring ras mutations retain certain differentiated properties. This suggests that some of the later mutational events leading to progression to the aggressive forms of the disease may have a further negative impact on thyroid differentiation. The association between p53 mutations and complete loss of differentiation in thyroid tumors in vivo prompted us to examine the effect of transfection of wt p53 on the transformed phenotype and on differentiated gene expression of thyroid carcinoma cell lines harboring endogenous p53 mutations.

MATERIALS AND METHODS

Cell Lines. The undifferentiated human thyroid carcinoma cell line (ARO), poorly differentiated papillary carcinoma cell line (NPA), and two follicular carcinoma cell lines (FRO and WRO) (22, 23) were provided by Dr. Guy Juillard (UCLA) and maintained in RPMI 1640 supplemented with 10% FCS, 100 μM nonessential amino acids, and 0.13 mg/ml sodium pyruvate.

Generation of Stable Transfectants. Cells (2.5 × 106) of the indicated cell line were stably transfected with 10 μg of either the wt p53 expression vector pC53SN3, the mutant p53 expression vector pcD273 (codon 273: Arg to His) (24), or pCMV-neo by lipofectamin-mediated gene transfer, according to the manufacturer’s instructions (GIBCO BRL, Gaithersburg, MD). After 24 h, transfected cells were selected in 600 μg/ml geneticin (G418; GIBCO-BRL) for NPA and ARO cells or 400 μg/ml for WRO and FRO cells. G418-resistant colonies were isolated after 3 weeks and propagated. Integration of the transfected DNA was confirmed by Southern blotting and expression of the appropriate product by RT-PCR (see below).

Nucleic Acid Extraction. RNA and DNA were extracted from a cesium chloride ultracentrifugation gradient as described (25). The DNA pellet was rinsed in 80% ethanol and then resuspended in diethylpyrocarbonate-treated H2O. The DNA layer was immediately precipitated in 2.5 volumes of ethanol and recovered by spooling. The DNA pellet was then rinsed in 10 ml of 80% ethanol and recovered by centrifugation at 3000 × g at room temperature.

RT-PCR Assay for p53, TTF-1, and Pax-8 mRNA. To determine expression of wt p53 mRNA in the stably transfected lines derived from WRO, NPA, ARO, or FRO cells, 250 ng of RNA were reverse transcribed at 42°C for 1 h with 200 units/μl Superscript II reverse transcriptase (GIBCO BRL, Grand Island, NY) in the presence of 500 ng/μl oligo(dT) primer, 100 mM DTT, and 0.5 μM ethylene diaminotetraacetic acid (EDTA), and 0.5% SDS. The mixture was then phenol-chloroform extracted. Sodium acetate (final concentration, 0.3 M) was added to the aqueous layer, which was then ethanol precipitated. DNA was pelletized, air-dried, and resuspended in H2O. After quantification by absorption at 260 nm, extracts were stored until assayed (DNA at −70°C; RNA at −20°C).

RT-PCR Assay for p53, TTF-1, and Pax-8 mRNA. To determine expression of wt p53 mRNA in the stably transfected lines derived from WRO, NPA, ARO, or FRO cells, 250 ng of RNA were reverse transcribed at 42°C for 1 h with 200 units/μl Superscript II reverse transcriptase (GIBCO BRL, Grand Island, NY) in the presence of 500 ng/μl oligo(dT) primer, 100 mM DTT, and 250 μM deoxynucleotide triphosphate mixture in the manufacturer’s buffer (50 μl final volume). After denaturation at 95°C for 5 min, a predicted 275-bp product was amplified from a 2-μl aliquot of the reaction in the presence of 1.5 μl PCR reaction mixture (Perkin-Elmer/Cetus, Emeryville, CA) in the manufacturer’s buffer. Additional primers were used for amplification of a 32-bp fragment of the cDNA. A fragment was amplified using a 32-bp primer of the cDNA. A fragment was amplified using a 32-bp fragment of the cDNA. A fragment was amplified using a 32-bp fragment of the cDNA.

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3. The abbreviations used are: Tg, thyroglobulin; wt, wild type; RT-PCR, reverse transcription-PCR; CMV, cytomegalovirus; TPO, thyroid peroxidase; TSH, thyroid; TSH-R, TSH receptor.
To determine expression of Pax-8 and TTF-1 mRNA in parental and wt p53-expressing stable transfectants of human thyroid carcinoma cell lines, total RNA from the indicated samples was reverse transcribed as described above, and aliquots of the cDNA were amplified with the following intron-flanking primers: hPax-8: upstream, 5'-ACCCCAAGGTGTTGGAGAAAGA-3'; downstream, 3'-GAAGTGTCGAGCGCTGCTC-5'; and hTTF-1: upstream, 5'-TTCTCCAGGGCAGCAGTGA-3'; downstream, 3'-TGTTTTACTTTGCGGCCGGC-5'. The PCR products were blot and hybridized with either an Xho-PstI fragment of Pax 8 cDNA (C27BXX22/1) or an ApaI-NotI fragment of human TTF-1 cDNA (RcCMV-THA) (26, 27).

Sequencing. The structural integrity of the transfected wt p53 DNA was confirmed by PCR amplification, cloning, and sequencing of wt p53 from genomic DNA of IN20 cells. PCR products were directly cloned into a pCRRII vector (TA cloning kit; Invitrogen, San Diego, CA), according to the manufacturer's instructions. Transformation of DHa competent cells (Life Technologies, Gaithersburg, MD) was followed by selection in plates containing 30 μg/ml kanamycin and 25 μg/ml X-gal. White colonies were then grown up for restriction analysis, and positive clones were used for sequencing with a Sequenase kit (U.S. Biochem Corp.). The two sets of overlapping primers were used to subclone the integrated pC53SN3 from serially passaged cells: (a) bracketing the CMV 5' untranslated region through nucleotide 958 of wt p53 cDNA (8); upstream, 5'-TGGAGACGCCATCCACG-3'; downstream, 3'-AGGAAA'TFGCGTGTGGAGT-3'; and (b) bracketing nucleotide 909 of wt p53 cDNA and a region complementary to the β-globin polyadenylation sequence of pC53SN3: upstream, 5'-AGGAAAATTTGCGTTGAGT-3'; downstream, 3'-GGAGACGTTTTAATACCC-5'. Purified PCR products were ligated into a TA cloning vector (Invitrogen, San Diego, CA), and the full length wt p53 sequenced using 4 overlapping sets of primers.

Anchorage-independent Growth. To determine the ability of parental and p53-transfected thyroid carcinoma cell lines to form colonies in soft agar, 2 × 104 cells of the indicated clones were seeded in triplicate in multi-well dishes in 0.367% agar. Total colony numbers (>50 cells/colony) were scored after 9 and 13 days.

Northern Blotting of TPO mRNA. Northern blot of 20 μg of total RNA of the indicated cell lines were hybridized with a 3 kb EcoRI insert of human TPO cDNA clone TPO3 (28). To control for uniformity of RNA loading, blots were rehybridized with cyclophilin cDNA (29).

Western Blotting of TPO. Fifty-μg protein extracts of lysates of NPA cells, normal thyroid tissue, and 1N20 cells (extracted in a buffer containing 5 mM EDTA, 10 μg/ml leupeptin, 2 μg phenylmethylsulfon fluoride, 0.5% Triton X-100, and 0.5 mg/ml bacitracin) were electrophoresed in a 7.5% polyacrylamide gel and transferred to nitrocellulose; then the membrane was incubated for 16 h at 4°C with a 1:1000 dilution of a monoclonal antibody to human TPO (30). The membrane was then incubated with horseradish peroxidase-conjugated secondary antibody (goat anti-mouse IgG) for 1 h at room temperature. The protein was detected using the 3,3'-dimethylbenzidine method (Sigma Chemical Co.).

### DETERMINATION OF TPO PROMOTER ACTIVITY

The expression of wt and mutant p53 transfectants was determined in transient transfection experiments. Plasmid DNA was transfected into parental and parental cell lines using the FuGENE transfection reagent from Boehringer Mannheim. The following plasmids were used:

- **p42OTPOL**: a plasmid containing 420 bp of promoter region of the TPO promoter.
- **TPOEM**: a plasmid containing 350 bp of promoter region of the TPO promoter.
- **CL53SN3**: a plasmid containing 530 bp of promoter region of the TPO promoter.
- **pCD273**: a plasmid containing 273 bp of promoter region of the TPO promoter.
- **pCD292**: a plasmid containing 292 bp of promoter region of the TPO promoter.
- **pCMV-neo**: a plasmid containing 292 bp of promoter region of the TPO promoter.

Preliminary experiments revealed that the parental WRO cell line expressed Pax-8 and TTF-1 at a lower level compared to the ARO cell line. Therefore, the ARO cell line was chosen for further experiments. The parental ARO cell line was transfected with the wt p53 expression vector pC53SN3 (24).

**RESULTS**

The following thyroid carcinoma cell lines harbor heterozygous point mutations of p53: NPA (codon 266: Gly to Glu); WRO (223: Pro to Leu); and ARO (273: Arg to His). A fourth follicular thyroid carcinoma cell line, FRO, has no structural defects in exons 5–8 of p53, as determined by single strand conformation polymorphism, yet has a marked decrease in p53 mRNA abundance (4). These cell lines were transfected with the wt p53 expression vector pC53SN3 (24). Transfection efficiency was marked in pC53SN3-transfected cells in all but the ARO cell line, suggesting that overexpression of wt p53 inhibits cell growth or is incompatible with sustained proliferation of the undifferentiated thyroid cell lines (Table 1).

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Experiment</th>
<th>pC53SN3</th>
<th>pCMV-neo</th>
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<tbody>
<tr>
<td>NPA</td>
<td>1</td>
<td>4.2 ± 4.4</td>
<td>23 ± 5.3</td>
</tr>
<tr>
<td>WRO</td>
<td>1</td>
<td>1.2 ± 0.7</td>
<td>17.9 ± 6.3</td>
</tr>
<tr>
<td>FRO</td>
<td>1</td>
<td>2.3 ± 0.8</td>
<td>12.8 ± 3.5</td>
</tr>
<tr>
<td>ARO</td>
<td>1</td>
<td>100 ± 0</td>
<td>25 ± 7.1</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>50 ± 14.2</td>
<td>19.1 ± 6.3</td>
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</table>

### Table 1 Transfection efficiency of thyroid carcinoma cell lines

<table>
<thead>
<tr>
<th>Cells (4 × 10⁴) were transfected with 5 μg of either pC53SN3 or pCMV-neo. Data represent X ± SD of geneticin-resistant colonies of triplicatedishes.</th>
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<tbody>
<tr>
<td>NPA</td>
<td>1</td>
<td>100 ± 0</td>
<td>23 ± 5.3</td>
</tr>
<tr>
<td>WRO</td>
<td>1</td>
<td>1.2 ± 0.7</td>
<td>17.9 ± 6.3</td>
</tr>
<tr>
<td>FRO</td>
<td>1</td>
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<td>12.8 ± 3.5</td>
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<td>ARO</td>
<td>1</td>
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<td>2</td>
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<td>19.1 ± 6.3</td>
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</tbody>
</table>

Cell line Experiment pC53SN3 pCMV-neo
NPA 1 4.2 ± 4.4 23 ± 5.3
WRO 1 1.2 ± 0.7 17.9 ± 6.3
FRO 1 2.3 ± 0.8 12.8 ± 3.5
ARO 1 100 ± 0 25 ± 7.1
2 50 ± 14.2 19.1 ± 6.3

WILD-TYPE p53 AND REEXPRESSION OF TPO

**Fig. 1. RT-PCR of pC53SN3 mRNA in transfected human thyroid carcinoma cell lines.** Total RNA obtained from cell lines derived from WRO, NPA, ARO, or FRO cells was reverse transcribed with Superscript II. A predicted 275-bp product was then amplified using the following intron-flanking primers bracketing the CMV 5' untranslated region through nucleotide 163 of wt p53: upstream, 5'-CTGCAAGCCCATCAGG-3'; downstream, 3'-GCCACGGTGCGAAGCGAC-5'. Lanes 1, 3, 5, 7, and 9, subjected to PCR with the reverse transcriptase having been omitted; Lanes 2, 4, 6, and 8, treated with reverse transcriptase. A: Lanes 1 and 2, normal thyroid; Lanes 3 and 4, parental WRO; and the following pC53SN3-transfected cells derived from WRO: Lanes 5 and 6 (1W2); Lanes 7 and 8 (1W4). B: derived from NPA: Lanes 1 and 2 (1N4); Lanes 3 and 4 (1N20). C: derived from ARO: Lanes 1 and 2 (1A1); Lanes 3 and 4 (1A5). D: derived from FRO: Lanes 1 and 2 (1F1); Lanes 3 and 4 (1F3). Extreme right lanes in all panels correspond to amplified pC53SN3 using identical primers. A product of 970-bp includes intronic sequences. Blots were hybridized with a 32P-labeled, 1.8-kb BamHI insert of pC53SN3.
ysis of cell lines derived from stable transfection with pC53N3 showed that only a minority expressed vector-derived wt p53 mRNA, particularly in the NPA transfectants (3 of 8 WRO; 1 of 16 NPA; 1 of 2 FRO; and 11 of 13 ARO), as assessed with RT-PCR (Fig. 1), providing further evidence for negative selection by expression of the foreign gene. In about one-third of the clones, the exogenous p53 gene was rearranged as determined by Southern blotting, and in several derivatives, aberrant-sized p53 mRNA transcripts were detected by Northern blotting. Again, the only exception to this paradigm was the wt p53-expressing ARO cells, from which vector-derived wt p53-expressing cells were readily obtainable, suggesting that growth was not as easily repressed by wt p53 in this undifferentiated carcinoma cell line. Nevertheless, doubling time (Fig. 2) was prolonged in all wt p53-expressing clones tested. These cells also displayed loss of transforming properties as determined by colony formation in soft agar (Table 2). The modest decrease in soft agar colony formation in NPA and ARO cells may reflect the impairment in cell growth. This was not the case in FRO cells, in which colony formation was entirely abrogated in wt p53 transfectants. Of note is the fact that FRO cells transfected with the neomycin-resistant gene also exhibited a modest impairment in colony formation in soft agar, probably due to insertional effects of the transfected DNA fragment. There were no discernible changes in the phenotypic appearance by light microscopy of any of the wt p53-overexpressing cell lines.

Table 2. Soft agar colony formation of parental and wt p53-expressing thyroid carcinoma cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>No. of soft agar colonies scored at 9 days</th>
<th>No. of soft agar colonies scored at 13 days</th>
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<tbody>
<tr>
<td>ARO</td>
<td>112 ± 9</td>
<td>173 ± 13</td>
</tr>
<tr>
<td>ARO (neo)</td>
<td>137 ± 41</td>
<td>186 ± 17</td>
</tr>
<tr>
<td>ARO (1A5) (wt p53/neo)</td>
<td>85 ± 5</td>
<td>74 ± 24</td>
</tr>
<tr>
<td>FRO</td>
<td>150 ± 11</td>
<td>244 ± 12</td>
</tr>
<tr>
<td>FRO (neo)</td>
<td>61 ± 21</td>
<td>74 ± 2</td>
</tr>
<tr>
<td>FRO (1F3) (wt p53/neo)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NPA</td>
<td>0</td>
<td>108 ± 6</td>
</tr>
<tr>
<td>NPA (neo)</td>
<td>0</td>
<td>186 ± 17</td>
</tr>
<tr>
<td>NPA (1N20) (wt p53/neo)</td>
<td>0</td>
<td>67 ± 10</td>
</tr>
</tbody>
</table>

NPA, parental cell line. •, wt p53 transfected. Doubling times were derived from lines fitted to log cell numbers.
overexpression of Pax-8 markedly induced transcription directed by the TPO promoter construct in both NPA and 1N20 cells in a concentration-dependent manner. These effects of Pax-8 were blunted when cells were cotransfected with TPOPM, a promoter fragment containing an 8-bp substitution that preferentially interferes with binding of Pax-8 to the TPO promoter (26). In contrast, transient expression of Pax-8 powerfully activated transcription through TPO-EM, in which a 10-nucleotide substitution of a region determining binding to V/FF-1 but not Pax-8 has been introduced (data not shown). In contrast, transient transfection with wt (pCS3SN3) or mutant p53 (pCD273) did not significantly increase promoter activity of p420TPOL (Fig. 5). Interference with Pax-8-induced TPO promoter activity was less obvious in cotransfection experiments with the mutant p53 expression vector pCD 273.

**DISCUSSION**

The disruption of the normal checks and balances controlling cell proliferation introduced by transforming oncogenes or tumor suppressor genes is often accompanied by an impairment of cell differentiation. This process is imbued with clinical significance in tumors of thyroid follicular cells because impairment of radioiodine uptake in advanced cancers removes the best therapeutic alternative in patients with metastatic disease. Mutations affecting several cancer genes (*i.e.*, ras, G_s, TSH receptor, ret/PTC, trk, and p53) have been observed in

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*Fig. 3. A. expression of TPO mRNA in wt p53-expressing NPA thyroid carcinoma cells. Northern blot of 20 µg of total RNA of NPA cells (NPA (UT)) and NPA R cell lines stably transfected with pC53SN3 (Lanes 1–8). Lanes 1–7, cell lines that have integrated pC53SN3 but do not express the transfected wt p53 mRNA. Only 1N2O cells (Lane 8), which express the authentic transfected wt p53 cDNA, also express TPO mRNA, as shown after hybridization with a 3-kb EcoRI insert of human TPO cDNA clone TPO3 (Ref. 21; top). Lower panel, rehybridization with cyclophilin cDNA (22). B. expression of immunoreactive TPO in 1N2O cells. Fifty-µg protein extracts of lysates of NPA cells, normal thyroid tissue (NT), and 1N20 cells were electrophoresed in a 7.5% polyacrylamide gel and transferred to nitrocellulose; then the membrane was incubated with a monoclonal antibody to human TPO (23). Arrow, M, 107.000 (107 KD) band in NT and NPA cells compatible with predicted size of TPO.*
human thyroid neoplasms, and a sequence of genetic events has been proposed based on the relative prevalence of these defects in the various tumor phenotypes (reviewed in Ref. 33). Many, if not all, of these structurally defective genes also impact on thyroid differentiation.

Thyroid cells normally express Tg and TPO, are TSH dependent for growth, and concentrate iodide. The expression of Tg, TPO, and TSH-R is controlled by the interaction of a complement of thyroid-specific transcription factors with the respective promoters of these genes. TTF-1 and Pax-8 are expressed in the thyroid at the onset of organogenesis (34). Whereas TTF-1 is the main regulator of the Tg promoter, Pax-8, although able to bind to specific elements on both promoters, appears to preferentially activate transcription of TPO (26). The ability of cancer genes to disrupt cell differentiation is ultimately dependent on the manner in which they may interfere with the transcription of various genes. In K-ras-transformed rat thyroid FRTL-5 cells, TTF-1 mRNA is markedly reduced, and activation of a Tg promoter can be restored by overexpression of TTF-1 (31). However, using a temperature-sensitive variant of K-ras, Avvedimento et al. (35) demonstrated that an impairment in TTF-1 binding to its specific site on the Tg promoter appears to be an early dedifferentiating event. H-ras transformation is associated with impairment in Tg expression without changes in the abundance of TTF-1 (31). These effects of mutant ras may be secondary to dephosphorylation of TTF-1 (31). Little is known about the manner in which activating rearrangements of the tyrosine kinase receptor genes ret and trk may impact on thyroid cell differentiation.

Several investigators have found mutations of p53 to be prevalent in thyroid carcinomas (3—7, 36), predominantly those with an undifferentiated phenotype (3—7). Thyroid carcinoma cell lines also exhibit a high frequency of p53 mutations (4, 37). As found in other cell lineages (24, 38), stable transfection of thyroid cancer cell lines with a wt p53 expression vector was associated with a 5—10-fold impairment in transfection efficiency, consistent with a marked growth-inhibitory effect imparted by expression of the foreign gene product. Indeed, the majority of the geneticin-resistant clones obtained did not express the transfected wt p53, consistent with the findings of Baker et al. (24) using identical vectors in colorectal carcinoma cell lines. ARO cells represent an exception to this paradigm because a paradoxical increase in transfection efficiency was observed. Furthermore, almost all ARO transfectants expressed vector-derived wt p53 mRNA. Although the cause of this unknown, the relative resistance of ARO cells to the effects of wt p53 may be due to additional oncogenic events affecting target genes subject to transcriptional regulation by the tumor suppressor. Mechanisms accounting for resistance to p53-mediated G1 arrest have been described and include the overexpression of products such as B-myb subject to transcriptional repression by p53 (39).

Only one transfectant colony, 1N20, expressing the authentic wt p53 product was obtained from the papillary thyroid carcinoma cell line NPA. These cells reexpressed TPO mRNA and protein. The redifferentiating effects were partial because 1N20 cells had no detectable Tg or TSH-R mRNA. The likelihood that the observed changes in TPO expression are simply due to clonal variation is small because none of the 25 clones obtained after stable transfection with either pCMVneo alone (n = 5), pCD273 (n = 5), or pCS3N3 but (that did not express authentic p53; n = 15) expressed TPO mRNA. Furthermore, one wt p53-transfected FRO cell clone (1F3) had a faint but detectable increase in TPO mRNA abundance. Nevertheless, the low frequency of generation of authentic exogenous wtp53-expressing transfectants are a limitation to the ability to reproduce these findings in several independently generated clones.

Expression of TPO in 1N20 cells was accompanied by reexpression of the paired-box developmental control gene Pax-8. Pax-8 is expressed in the hindbrain and neural tube of the embryo, as well as in the developing kidney and thyroid (40). The ability of transiently expressed Pax-8 to transactivate the TPO promoter in NPA cells strongly implicates this factor in the differentiation-promoting effects of wt p53. These effects were due to a specific interaction of Pax-8 with the TPO promoter because they were markedly blunted when cells were transfected with a promoter lacking the major Pax-8 bind-

Fig. 4. Expression of Pax-8 and TTF-1 mRNA in parental and a wt p53-expressing stable transfectant of the human thyroid carcinoma cell line NPA. Total RNA from the indicated samples was reverse transcribed, and aliquots of the cDNA were amplified with the following intron-flanking primers: hPax-8: upstream, 5'-ACCCCCAAGGTGGTGAGC'-3'; downstream, 3'-GAAGTCGGTGGTGAGC'FC-S'; and hTI'F-l : upstream, 5'-TTCTCGAGCCGAGGTGTA-3'; downstream, 3'-TG'TTACTTCGCGGGTCCG-5'. The PCR products were blotted and hybridized with either an Xho-PstI fragment of Pax 8 cDNA (C2IBXX22/1) or an Apal-NotI fragment of human TTF-1 cDNA (RCMV-THA). NT, normal thyroid tissue. BL, blank (no template). The identity of the 450-bp Pax-8 and 158 TFF-l PCR products was confirmed by direct sequencing.
indirect or mediated through interactions with DNA elements not contained within the 420-bp TPO promoter fragment. In support of these observations (41), we report elsewhere that transfection of well-differentiated PC13 thyroid cells with the mutant p53 construct pC53-SCX3 (codon 143: Val to Ala) is associated with a selective decrease in Pax-8 mRNA, whereas TTF-1 mRNA remains fairly stable. These transformants displayed a complete loss of TPO mRNA and a marked decrease in Tg mRNA abundance. Thus, the dedifferentiating effects of mutant p53 in thyroid cells are reciprocal to the actions of wt p53 in the IN20 cell line and mirror the findings reported in this paper.

Although wt p53 had no demonstrable direct trans-activating properties on the promoter, it did lead to a dose-dependent impairment of Pax-8-induced p42OTPO-luciferase activity. It is unclear whether the paradoxical interference of wt p53 with Pax-8-induced transcriptional activation is due to protein-protein interactions (42–46), competition for similar or adjacent DNA binding sites, or through indirect mechanisms. Alternatively, p53 may exert these effects through nonspecific impairment of transcription of TPO (11).

Although several properties of p53 have been described (24, 38, 47–50), a role in the control or maintenance of differentiated gene expression is not clearly established. The fact that mice homozygous for a null-p53 allele appear to be phenotypically normal at birth has been taken as evidence that p53 does not play a key role in development or that its function can be compensated for by redundant pathways (51). However, in various forms of malignancy, mutations of p53 are associated with loss of differentiation. The acute phase chronic myelogenous leukemia cell line K562 is induced to express up to 50-fold more hemoglobin after introduction of normal p53 (52, 53). We propose that in thyroid cancer, in which the transcriptional machinery controlling differentiated gene expression is already compromised by earlier genetic defects (21, 33, 54–57), p53 may be a differentiation factor of last resort. Loss of function of p53 may lead to a selective decrease of TPO, primarily through abrogation of Pax-8 gene expression, an effect that can be reversed by stable overexpression of wt p53. Whether Pax-8 transcription is directly activated by p53 is presently being explored.

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