Transcriptional Repression of Taurine Transporter Gene (TauT) by p53 in Renal Cells

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SUMMARY

Taurine, an intracellular osmolyte whose body pool size is adaptively regulated by the kidney, is required for normal renal development. Overexpression of the p53 tumor suppressor gene in p53 transgenic mice results in renal malformation, suggesting that altered expression of certain p53 target gene(s) involved in renal development may be responsible. This study shows that the taurine transporter gene (TauT) is a transcriptional target of p53. Expression of TauT was decreased after activation of p53 by doxorubicin, a DNA-damaging drug, in 293 and NRK-52E renal cells. TauT promoter activity was decreased 5-10-fold by cotransfection of a full-length TauT promoter-reporter construct with p53, which was reversed by cotransfection with a mutant p53 (p53-281). Electrophoretic mobility shift assays (EMSAs) using nuclear extracts from p53-expressing (10)1val cells showed a putative p53-binding site in the TauT promoter region, which bound to the p53 in EMSAs. Mutation of this p53 consensus sequence abolished binding of p53. These results demonstrate that TauT may represent a downstream target gene of p53 that could link the roles of p53 in renal development and apoptosis.
INTRODUCTION

The β-amino acid taurine plays an important role in cell volume regulation. The total body pool of taurine is controlled at the brush border surface of the renal proximal tubule by a NaCl-dependent transporter that is up- or down-regulated by the availability of dietary taurine or its precursor amino acids, cysteine and methionine (1). Species in which activity of the enzyme cysteine decarboxlyase is absent (cats) or insufficient (infant mammals, including humans) require supplemental dietary taurine for normal renal and central nervous system development (2, 3). The rat taurine transporter gene (TauT) has been cloned and characterized to help study the mechanism(s) and significance of the regulation of this important osmolyte (4).

The promoter region of the taurine transporter gene contains a consensus binding site for the p53 tumor suppressor gene, which functions as a cell cycle checkpoint, blocking cell division in the G1 phase to allow repair of damaged DNA or even triggering apoptosis in cells that have defective genomes (5). Numerous stimuli trigger increases in the level of p53 expression, including DNA-damaging drugs, ionizing radiation, ultraviolet light, and hypoxia (6-9). Varmus’ group has found that transgenic mice over-expressing p53 undergo progressive renal failure through a novel mechanism by which p53 appears to alter cellular differentiation, rather than by growth arrest or the direct induction of apoptosis (10). These findings suggest that altered expression of certain p53 target gene(s) involved in renal development may be responsible for p53-induced progressive renal failure in p53 transgenic mice.

Interestingly, the progressive renal failure found in p53 transgenic mice is similar to observations made regarding the offspring of taurine-deficient cats, which showed ongoing kidney damage in
addition to abnormal renal and retinal development (2,10), suggesting that the taurine transporter gene may be an important target of p53. Recently, Heller-Stilb et al. created a taut-/- mouse model in which knockout of TauT resulted in severe and progressive retinal degeneration, a small brain, and shrunken kidneys (11). These findings suggest that the TauT gene is required for normal kidney development in mice.

A recent study shows that the Fas (CD95) cell surface receptor is up-regulated by DNA-damaging agents that appear to be p53-dependent (12). Stimulation of Fas receptor with Fas antibody leads to release of cellular taurine, which coincides with cell shrinkage and precedes DNA fragmentation. However, Fas receptor-mediated apoptosis is blunted by increases in extracellular osmolarity (13), suggesting that taurine uptake mediated by the taurine transporter plays a role in the cell volume regulatory mechanism during apoptotic cell death. This hypothesis is strongly supported by observations in taut-/- mice, in which the progressive retinal degeneration was found to be caused by apoptosis (11). Therefore, regulation of TauT by p53 may also be important in Fas-mediated apoptosis.

EXPERIMENTAL PROCEDURES

Construction of the reporter gene-- The promoter region of TauT was identified in previous studies (14), and a p53-binding consensus site was found in the TauT promoter sequence, located at -663 to -695. In this study, approximately 1.1 kb of the TauT promoter region DNA was used as the template for PCR (GenBank accession number AR151716) and the PCR fragment was cloned into the promoterless luciferase vector pGL3-Basic (Promega, Madison, WI) to generate the plasmid p923 for use in transfections and luciferase assays. The conditions
used are 30 cycles of 1 min of denaturation at 94 °C, 1 min of annealing at 58 °C, and 1 min of elongation at 72 °C. The sense primer (5’-GGGGTACCTTACTGAAGGTCACACAGC-3’) designed for PCR contained a unique site for KpnI, and the antisense primer (5’-AAGATCTTGGCACGGGAG-TTCA-3’) contained a unique site for Bgl II. PCR products were digested with KpnI and Bgl II and re-ligated into KpnI and Bgl II sites of pGL3-Basic to generate plasmids containing segments of the TauT promoter sequence extending from the +48 nucleotide corresponding to the transcriptional start site. The constructs were verified by DNA sequencing. The p53-binding site deletion (d pGL-563) and p53 mutation (mt pGL-963) constructs were generated from the p923 plasmid by using sense primers 5’-GGGGTACCGAGTTGGGGAGGGA-3’, and

5’GGGGTACCAGATGAGGAAACCCCCACACAAGGTCTGGGGCTTGCTGATGTCA 3’, respectively. The antisense primer used for these constructs was the same as described above.

Cell culture-- Human embryonic kidney (293), normal rat kidney (NRK-52E), pig kidney (LLC-PK1) and murine fibroblast (10)1 val cells were cultured according to AATC (American Association Tissue Culture) guidelines. Briefly, cells were grown as confluent monolayers in 10 cm diameter tissue culture plates in media specific for each cell line with 10% fetal calf serum at 37°C in the presence of 5% CO₂ in a humidified incubator. Cells were plated 18 h before transfection and fed with fresh medium 4 h before transfection.

Measurement of taurine transport-- Taurine transport studies were performed on confluent monolayers 3 days after seeding cells. Briefly, cells were washed with Earle’s Balanced Salt Solution (EBSS) at 37°C. Uptake was initiated by the addition of uptake buffer (2 mM KCl, 1
mM MgCl₂, 96 mM NaCl, 1.8 mM CaCl₂, 5 mM Hepes, pH 7.6) to which 50 µM unlabeled taurine and 0.5 µCi/ml ¹⁴C-taurine (Perkin Elmer, Boston, MA) were added. After incubation for 30 min at room temperature, uptake was terminated by the removal of uptake buffer followed by three rapid washes with cold EBSS. Cells were solubilized in 1% SDS in 0.2 N NaOH and radioactivity was counted in a Packard 2000-CA Liquid Scintillation Analyzer.

**Stable expression of ts p53 in NRK-52E cells**-- NRK-52E cells were stably transfected with a temperature-sensitive mutant p53 allele (ts p53 val 135), which expresses a mutant p53 product at 37 °C in transfected cells, but wild-type p53 conformation when the cells are cultured at 32 °C. NRK-52E cells were transfected with nsvcl/neo val 135 (a DNA construct containing the neo and ts p53 genes) and CMV neo Bam plasmid DNA (vector control) using a lipofectamine transfection kit (Life Technologies, Inc., Grand Island, NY). Genticin (G418, 1mg/ml, Clonotech, Palo Alto, CA) was used for colony selection. G418-resistant colonies (NRK-52E/ts p53) were screened by Western blot analysis for p53 expression. In order to test the role of wild-type p53 in TauT expression, p53-positive NRK-52E cells were used in our study.

**Transient transfection**-- Plasmid DNA was introduced into cultured mammalian cells using cationic liposomes (LipofectAMINE, Life Technologies). Transfection was carried out for 16-18 h, and then cells were washed twice with phosphate-buffered saline and incubated in fresh medium for 24-48 h before harvesting. pGL-control, which contains a luciferase gene driven by the SV40 early region promoter/enhancer, and empty pGL-Basic vectors were used as positive and negative controls, respectively. To standardize the transfection efficiency, 0.1 µg of pRL-CMV vector (pRL Renilla Luciferase control reporter vector, Promega) was cotransfected in all
experiments. Cells were harvested 48 h after transfection and lysed in 200 µl of reporter lysis buffer (Promega). A luciferase assay was performed using a dual-luciferase assay kit (Promega), and activity was measured with an Optocomp 1 luminometer (MGM Instruments, Inc., Hamden, CT). Promoter activity (mean ± SD of four samples in relative light units) of each construct is represented by relative light output normalized to pRL-CMV control. Graphs represent typical results of four separate experiments. The concentration of protein in the cell extracts was determined using the Bradford method (Bio-Rad, Hercules, CA) (15).

Northern blot analysis-- Thirty µg of total RNA were separated in an agarose gel and transferred to a nylon membrane by overnight capillary blotting in 10 x SSC (sodium chloride/sodium citrate buffer). The Northern blot was hybridized overnight at 42°C with a 32P-labeled riboprobe of the taurine transporter cDNA. The blot was washed successively in standard decreasing concentrations of SSC/0.1% SDS at 65°C for 30 min each and exposed to Kodak film with one intensifying screen at -80°C for 24 h.

Western blot analysis-- Cells were lysed in 50 µl M-PER mammalian protein extraction reagent (Pierce, Inc., Rockford, IL) supplemented with protease inhibitors cocktail for use with mammalian cell and tissues extracts (Sigma, St. Louis, MO). The lysates were cleared by centrifugation at 14,000 x g for 2 min, and the supernatants were transferred to clean tubes. Equal amounts of protein (50 µg) were separated by electrophoresis on a 12% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane (Millipore, Bedford, MA) using a semi-dry electrophoretic transfer system (Bio-Rad, Hercules, CA). Membranes were incubated in 5% nonfat dry milk in Tris base/sodium chloride (TBS) buffer with 0.2% Tween 20
(TBST) at 4 °C overnight. The membranes were incubated with primary antibody for 1 h at room temperature at the following dilutions: p53 Fl-393 (Santa Crux, CA) 1:2000, and 1:5000 of an affinity-purified antibody against the C-terminal peptide sequence of the taurine transporter (16). Blots were washed with TBST and incubated with horseradish peroxidase-linked secondary antibody (Sigma). TauT or p53 protein was detected using a chemiluminescent detection kit (Pierce, Inc.).

Electrophoretic mobility shift assay (EMSA)-- Double-stranded oligonucleotides corresponding to the putative p53-binding site in the TauT promoter region were end-labelled with polynucleotide kinase using [r-32P] ATP. Nuclear extracts were isolated by high salt extraction of nuclei (17). The binding reactions were performed by using a gel shift assay systems following the manufacturer’s instructions (Promega). Briefly, the labelled oligonucleotides and nuclear extracts were incubated in gel shift binding buffer [20% glycerol, 5 mM MgCl₂, 2.5 mM EDTA, 2.5 mM DTT, 250 mM NaCl, 50 mM Tris-HCl (pH 7.5), and 0.25 mg/ml poly (dl-dC)] on ice for 30 min, and then the DNA-protein complexes were run on 10% non-denaturing acrylamide TBE gels (Bio-Rad) in 0.5x TBE buffer at 190 V for 45 min. The gel was dried at 70°C for 30 min and exposed to Kodak film with an intensifying screen for 1 h at -80°C.

Statistics--All experiments were performed in triplicate. Luciferase assays are expressed in units of relative light output. The data represent the mean ± SEM of 3 or 4 experiments. Statistical comparisons were made using one-way ANOVA and Student’s t test to determine significant differences in the means.
RESULTS

*TauT is down-regulated by p53*— Our initial experiments examined whether doxorubicin, a DNA-damaging drug, would induce endogenous p53 expression in 293 and NRK-52E renal cells. A p53-negative cell line, murine fibroblast (10)1, was used as control. Expression of p53 was induced in both 293 and NRK-52E cells after treatment with doxorubicin (500 ng/ml) for 48 h, as compared to untreated cells. As expected, p53 was not detected in (10)1 cells before or after doxorubicin treatment (Fig. 1A). In the renal cells, the increase in the level of p53 was accompanied by a decrease in TauT expression at both the level of mRNA and protein (Fig. 1B & 1C). Doxorubicin had no effect on TauT expression in the p53-negative (10)1 cells.

To examine the effect of endogenous p53 production on taurine transporter function, the three cell lines above were cultured in the presence of doxorubicin (500 ng/ml) for 48 h and then taurine uptake was measured. As expected, doxorubicin significantly decreased taurine uptake in 293 and NRK-52E renal cells (70% of control), but had no effect in p53-negative (10)1 cells (Fig. 1D). These results show that overexpression of endogenous p53 decreases expression of TauT in both 293 and NRK-52E cells, which in turn results in the decrease of taurine uptake by the cells.

To extend this study, a temperature-sensitive p53 DNA (p53 val135) was stably transfected into NRK-52E cells. At 37°C the p53 protein in these cells exists in a mutant conformation, and a temperature shift to 32°C induces a wild-type conformation. Expression of TauT was studied by changing the incubation temperature of the cells. Switching the temperature from 37°C to 32°C for 24 h did not affect expression of TauT in NRK-52E/neo cells (data not shown). NRK-
52E/tsp53 cells expressed a high level of p53, which was not affected by temperature switching (Fig. 2A). However, expression of TauT was decreased in NRK-52E/ts p53 cells cultured at 32°C, whereas the level of the housekeeping gene ß-actin remained unchanged (Fig. 2B & 2C). The reduction of TauT expression led to a decrease in taurine uptake by wild-type p53-expressing NRK-52E cells (Fig. 2D). The p53-induced down-regulation of TauT was also found to be time-dependent (Fig. 3A, 3B, & 3C).

*TauT is transcriptionally repressed by p53*—To determine if down-regulation of the TauT gene by p53 occurs at the transcriptional level, the reporter construct pGL-963, containing a putative p53-binding site from -663 to -695 (Fig. 4A), was transiently transfected into 293, NRK-52E, and (10)1 cells. Regulation of TauT promoter function by p53 was examined in cells treated with either doxorubicin or cotransfection of p53. As shown in Fig. 4B, doxorubicin decreased TauT promoter activity in both 293 and NRK-52E cells, but had no effect in the (10)1 cells. Consistent with these results, transient cotransfection of p53 also significantly repressed TauT promoter activity in a dose-dependent manner (Fig. 4C). Repression of TauT promoter function by p53 was confirmed by using NRK-52E cells expressing ts p53. In this study we demonstrated that the transiently transfected luciferase reporter gene driven by the TauT promoter was markedly down-regulated by wild-type p53 induction (Fig. 4D). Cotransfection of mutant p53 (p53-281) had no effect on TauT promoter activity (Fig. 4E). However, cotransfection of mutant p53 blocked the effect of wild-type p53 on TauT promoter function, demonstrating the dominant-negative effect of mutant p53.

*Effect of p53 induction on adaptive regulation of TauT in LLC-PK1 renal cells*— Previous
studies have shown that expression of TauT in renal cells is adaptively regulated by the availability of taurine (1). To determine if this renal adaptive regulation of TauT is altered by p53, the reporter construct pGL-963, containing the cis-element for adaptive regulation (4), was transiently transfected into LLC-PK1 cells cultured in medium containing 0 µM, 50 µM, or 500 µM taurine for 48 h with or without doxorubicin. As shown in Fig. 5A, TauT promoter activity was adaptively regulated by the external concentration of taurine, i.e., TauT promoter activity was up- or down-regulated when cells were cultured in medium containing 0 µM or 500 µM taurine, as compared to control (50 µM taurine). Activation of p53 by doxorubicin repressed TauT promoter activity, but had no effect on adaptive regulation of TauT promoter function. To confirm these results, the function of the taurine transporter was measured in cells cultured in medium containing 0 µM, 50 µM, or 500 µM taurine for 48 h with or without doxorubicin. As shown in Fig. 5B, taurine uptake by the cells was adaptively regulated by medium taurine concentration. Treatment with doxorubicin reduced uptake of taurine by LLC-PK1 cells, but did not alter the taurine concentration-mediated adaptive regulation of TauT expression.

*p53-binding site is necessary for transcriptional repression of TauT by p53*—To determine whether the putative p53-binding site is necessary for repression of TauT promoter activity, we created two constructs which contained either a deletion or a mutation of the p53-binding site (Fig.6A). The negative effect of p53 on TauT promoter activity was abolished in both constructs (Fig.6B), suggesting that the DNA sequence of the TauT promoter sequence from -663 to -695 is essential for p53-induced down-regulation of TauT expression in renal cells. To define whether p53 binds to this putative p53-binding site in the TauT promoter, we carried out electrophoretic mobility shift assays using a synthetic oligonucleotides corresponding to this site
As shown in Fig. 7B, nuclear extracts from p53-positive (10)1 val cells, which produce wild-type p53, yielded a band (lane 3) that could be competed out by excess unlabeled probe (lanes 4-5). p53-negative (10)1 cells did not yield a band (lane 2). A nonspecific oligonucleotide (lane 6) and a mutated oligonucleotide (lane 7 - the p53-binding core sequence CTTG was mutated to TTTT), were found to be unable to compete for the p53 binding to this putative site. Furthermore, mutation of the core sequence results in the loss of p53 binding to this site (lane 8). A polyclonal antibody to p53 (FL-393, 1 µg) resulted in a minor super-shifted band (lane 9). However, a significant super shift was observed when a higher dose of antibody (Ab-7, 2 µg) was applied to the EMSA (Fig. 7C), suggesting that p53 specifically binds to the putative p53-binding site in the TauT promoter region.

DISCUSSION

Taurine, an osmoregulator and intracellular calcium flux regulator, and the most abundant intracellular free amino acid present in brain and renal cells, has been shown to reduce cell damage associated with the ischemia/reperfusion phenomena (18). Competitive inhibition of taurine transport by β-alanine decreases intracellular taurine content and impairs cell volume regulation, which can lead to abnormal cell size and shape (19). Therefore, factors that affect the taurine transporter gene at either the transcriptional or post-transcriptional level may cause taurine deficiency in certain types of cells. In cats, in utero taurine deficiency results in gait disturbance and abnormal development of the cerebellum, spine, retina, and kidney (19). Of particular interest is that the taurine-deficient cat model displays renal defects that include simplification of the tubular epithelium and renal scarring. Nearly identical renal histopathologic
findings are present in the kidneys of the p53-overexpression mouse (10). Heller-Stilb et al., have generated a line of taurine transporter knockout (taut-/-) mice expressing a truncated transporter protein in the kidney, in which the renal level of taurine is about 20% of normal control (taut+/+ mice) (11). This truncated transporter appears to be partially functioning, since renal cells do not synthesize taurine and the relatively large amount of taurine found in the kidney must be transferred from the liver and/or diet by active uptake. In contrast, the taurine content of heart and skeletal muscle of taut -/- mice was reduced to 1-2% of the taut +/+ mice.

Mutation of TauT results in retinal degeneration and diminished renal size, suggesting that TauT per se plays an important role in normal retinal and renal development. These observations led us to investigate whether TauT is a target of the p53 tumor suppressor gene, and a possible mechanism by which overexpression of p53 induces kidney malformation.

We have identified TauT as a gene related to renal developmental that is negatively regulated by p53 in renal cells. Unlike other p53 target genes, such as survivin, cdc2, cdc25c, or p21waf1, TauT is not a cell cycle-regulated gene (20-23). Expression of TauT has been detected in a variety of tissues, and it is particularly abundant in kidney cells and proliferating lymphocytes. In the kidney, which controls the total body pool of taurine, expression of TauT is adaptively regulated by the availability of dietary taurine. We have shown that the cis-element for TauT adaptive regulation is located immediately downstream of a p53 consensus binding site in the TauT promoter region (14). In the present study we found that activation of p53 by doxorubicin repressed TauT expression, but did not impair renal adaptive regulation of the TauT gene. The importance of this renal adaptive regulation of TauT expression is unknown, but it could be
important in the development of the embryo, since the TauT mutant female mice mentioned above showed a marked fetal wastage (11), as did the taurine-deficient cat model (2).

In this study we show that p53 binds to the TauT promoter at a consensus p53-binding site, raising the possibility that p53 represses TauT by interfering with Sp1-mediated transactivation. We have found that the Sp1 transcription factor is essential for the basal promoter function of TauT by showing that deletion of Sp1 sites located in the TauT basal promoter region abolishes the TauT promoter-driven reporter gene expression (4). Bargonetti, et al., have shown that p53 binds to a region of the SV40 virus which contains GC-box DNA binding sites for the ubiquitously expressed transcription factor Sp1 (24). They further demonstrated that p53 repressed HIV-LTR transcription by influencing Sp1 binding to the Sp1 sites located in the HIV-LTR promoter region, which resulted in a change in the DNA binding capacity of Sp1. It has been shown that Sp1 and p53 form a heterocomplex that can recognize either a p53 or a Sp1 binding site, and the p53-Sp1 complex functions as a repressor for many genes (24, 25).

The p53-binding consensus site located at -663 to -695 in the TauT promoter region contains two p53-binding half-sites separated by 13 base pairs (Fig. 7A). Three mismatches were found in one of the half-sites. El-Deiry et al. defined the consensus p53-binding site as two copies of the sequence 5'-RRR-C(A/T)(T/A)G-YYY-3' (where R=Pu with substitutions of A or G; Y=Py with substitutions of T or C), separated by 0-13 base pairs: functional p53-binding sites can contain up to four mismatches in this consensus, but the C and G residues are invariant (26). In this study we showed that p53 repressed TauT transcription by directly binding to the DNA sequence between -663 and -695 of the TauT promoter. Mutation of this consensus site
abolished the p53 binding to the TauT promoter, and abrogated the effect of p53 on TauT promoter function.

Elevated levels of p53 have been found in the kidneys of animal models of acute renal failure induced by cisplatin administration (27). Negative regulation of the TauT gene expression by p53 may play a role in the action of cytotoxic drugs, such as cisplatin-induced renal failure. Cisplatin accumulates in cells from all nephron segments but is preferentially taken up by the highly susceptible proximal tubule cells within the S3 segment, the site for renal adaptive regulation of TauT, which bear the brunt of the damage (28, 29). A recent study showed that taurine was able to attenuate cisplatin-induced nephrotoxicity and protect renal tubular cells from tubular atrophy and apoptosis (30). One of the prominent features of TauT mutant mice is the severe and progressive retinal degeneration caused by programmed cell death (apoptosis) (11).

Cell shrinkage is a major characteristic of apoptosis. The cell surface receptor Fas (CD95), a member of the tumor necrosis factor (TNF) receptor superfamily, is a known regulator of apoptosis in certain types of cells (14). Stimulation of Fas receptor with Fas antibody leads to release of cellular taurine, which coincides with cell shrinkage and precedes DNA fragmentation. However, Fas receptor-mediated apoptosis is blunted by increases in extracellular osmolarity, suggesting that taurine uptake mediated by the taurine transporter plays a role in the cell volume regulatory mechanism during apoptosis. Taken altogether, these results suggest that TauT plays an important role in the development and maintenance of retinal and renal function and morphology by protecting the cells from stress-induced apoptosis. Whether TauT functions as
an anti-apoptotic gene directly involved in the pathway of Fas- and/or p53-induced apoptosis remains the subject of future study.
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FIGURE LEGENDS

FIG. 1. TauT is down-regulated following induction of endogenous wild-type (wt) p53 by doxorubicin. **A,** Western blot analysis of p53 gene expression in cells treated with or without doxorubicin (DOX) for 48 h. Equal amounts of protein from 293 (lanes 1,2), NRK-52E (lanes 3,4), or (10)1 cells (lanes 5,6) were loaded on an SDS-polyacrylamide gel (12% acrylamide) and p53 protein was detected as described in the methods. The housekeeping gene β-actin was used as loading control. Cells in lanes 1, 3, and 5 were untreated controls. Cells in lanes 2, 4, and 6 were treated with 500 ng/ml doxorubicin for 48 h. **B,** Northern blot analysis of TauT expression in cells treated with or without doxorubicin for 48 h. Cells in lanes 1, 3, and 5 were untreated controls. Cells in lanes 2, 4, and 6 were treated with doxorubicin for 48 h. β-actin was used as an internal control for loading. **C,** Western blot analysis of TauT gene expression in the cells treated with or without doxorubicin for 48 h. The loading sequence was same as described for B. **D,** Measurement of taurine uptake in cells treated with or without doxorubicin for 48 h. The relative levels of intensity of each blot were quantified using a BioRad GS 700 densitometer and expressed as means ± S.E.M. of three independent experiments. Data were normalized to the β-actin densitometric value. *P<0.05 vs control (untreated cells).

FIG. 2. Repression of TauT expression by stable transfection of temperature sensitive (ts) p53 in renal cells. NRK-52E/ts p53 cells were cultured at 37°C or 32°C for 24 h, and then cells were prepared for Northern blot, Western blot, or taurine uptake studies. **A,** Western blot analysis of p53 expression was performed using an antibody against the p53. Lanes 1-2, NRK-52E/ts p53 cells cultured at 37°C; lanes 3-4, NRK-52E/ts p53 cells cultured at 32°C. **B,** Northern blot
analysis was carried out using a specific RNA probe generated from rB16a (rat taurine transporter cDNA) (30). Lanes 1-2, NRK-52E/ts p53 cells cultured at 37°C; lanes 3-4, NRK-52E/ts p53 cells cultured at 32°C. C, Western blot analysis of TauT expression was performed using an antibody against the C-terminal peptide sequence of TauT. Sample loading sequence was the same as described in B. D, taurine uptake by NRK-52E cells with or without wild-type p53 over-expression. The relative levels of intensity of each blot were quantified using a BioRad GS 700 densitometer and expressed as means ± S.E.M. of three independent experiments. Data were normalized to the β-actin densitometric value. * P<0.05 vs control (cells cultured at 37°C).

FIG. 3. Time course of TauT expression negatively regulated by wild type p53. NRK-52E/ts p53 cells were cultured at 32°C, and then cells were prepared for Northern blot (A), Western blot (B), or taurine uptake (C) analysis at the time points indicated. β-actin was used as an internal control for loading. The relative levels of intensity of each blot were quantified using a BioRad GS 700 densitometer and expressed as means ± S.E.M. of three independent experiments. Data were normalized to the β-actin densitometric value. * P<0.05, and ** P<0.01 vs control (0h).

FIG. 4. Down-regulation of TauT promoter activity by p53 in renal cells. A, Sequence of TauT promoter and the construct of pGL-963 reporter gene. A consensus p53-binding site located at -663 to -695 relative to the transcription start site is shown. B, pGL-963 was transiently expressed in 293, NRK-52E, and (10)1 cells treated with or without doxorubicin (Dox) for 48 h. The luciferase assay was performed in the cell lysates. C shows the dose-dependent effects of
p53 on the TauT promoter activity. D, pGL-963 was transiently expressed in NRK-52E/neo and NRK-52E/tsp53 cells, and then cells were cultured at 37°C and 32°C for 24h. E, pGL-963 was cotransfected with a mutant p53 (p53-281), showing the dominant-negative effect of mutant p53 on wt p53-induced repression of TauT promoter activity. To control for transfection efficiency, cells were co-transfected with pRL-CMV vector (0.1 µg), luciferase activity was measured in the same cell lysates by Dual-luciferase reporter assay system. The promoter activity (mean ± S.D. of four samples in relative light units) was represented by relative light output normalized to pRL-CMV control. The graph represents typical results of four separate experiments. * P<0.01 vs control cells.

FIG. 5. p53 represses but does not alter the renal adaptive regulation of TauT. A, reporter gene pGL-963 was transiently transfected into LLC-PK1 cells cultured in medium containing 0µM, 50µM, or 500 µM taurine with (+) or without (-) doxorubicin for 48 h, and then a luciferase assay was performed as described in FIG. 4. B, LLC-PK1 cells were cultured in medium containing 0µM, 50µM, or 500 µM taurine with (+) and without (-) doxorubicin for 48 h, and then taurine uptake was measured. Transport activity is expressed in units of pmol taurine/mg protein and represents the mean of three experiments performed in duplicate. * P<0.05 vs cells cultured in 50 µM taurine medium without doxorubicin. ** P<0.05 vs cells cultured in 50 µM taurine medium with doxorubicin (500 ng/ml).

FIG. 6. p53 binds to a consensus p53-binding site in the TauT promoter region. A, schematic representation of TauT promoter-reporter constructs. B, effect of mutation of the p53 consensus
site in the TauT promoter on repression by wild-type p53 in NRK-52E cells. * P<0.01 vs control (-p53 cells).

FIG. 7. Electrophoretic mobility shift assay using the putative p53-binding site sequence of the TauT promoter. A, sequences of oligonucleotides corresponding to nucleotide positions -663 to -695 relative to the transcription start site in TauT (wt-TauT). Sequences of mutant oligonucleotide (mt-TauT) and a nonspecific oligonucleotide from the TauT promoter region (non-TauT). B, electrophoretic mobility shift assays were done using radiolabeled TauT oligonucleotides with nuclear extracts from (10)1 val cells expressing p53. Lane 1, wt-TauT probe alone; lane 2, wt-TauT probe plus nuclear extracts from p53 null (10)1 cells; lane 3, wt-TauT probe plus nuclear extracts from p53-expressing (10)1 val cells; lanes 4 and 5, probe plus nuclear extracts from p53-expressing (10)1 val cells and unlabeled probe at 1:50 (lane 4) or 1:100 (lane 5); lane 6, wt-TauT probe plus non-TauT oligonucleotide with nuclear extracts from p53-expressing (10)1 val cells; lane 7, wt-TauT probe with nuclear extracts from p53-expressing (10)1 val cells plus unlabeled mt-TauT (1:50); lane 8, mt-TauT probe with nuclear extracts from p53-expressing (10)1 val cells; lane 9, wt-TauT probe with nuclear extracts from p53-expressing (10)1 val cells plus p53 polyclonal antibody (FL-393, 1 µg). C, electrophoretic mobility shift assays were done using radiolabeled TauT oligonucleotides with nuclear extracts from (10)1 val cells expressing p53. Lane 1, wt-TauT probe alone; lane 2, wt-TauT probe plus nuclear extracts from p53 null (10)1 cells; lane 3, wt-TauT probe plus nuclear extracts from p53-expressing (10)1 val cells; lanes 4, wt-TauT probe with nuclear extracts from p53-expressing (10)1 val cells; lanes 5, wt-TauT probe with nuclear extracts from p53-expressing (10)1 val cells plus p53 polyclonal antibody (Ab-7, 2 µg).
Han et al. Figure 1C

C

[Image: Western blot analysis showing bands labeled TauT and β-actin. Bands are labeled 1 to 6.]

[Graph: Bar chart showing relative abundance of TauT in 293 cells, NRK-52E, and (10)1 cells with and without DOX treatment. Asterisks indicate significant differences.]
Han et al. Figure 1D

D

![Graph showing taurine pmol/h/mg protein for 293 cells, NRK-52E, and (10)1 cells with and without DOX treatment. The graph indicates a significant increase in taurine production with DOX treatment in NRK-52E cells compared to 293 cells and (10)1 cells.]
Han et al. Figure 2C

![Image of Western blot showing expression of TauT and β-actin under 37°C and 32°C conditions. The bar graph below compares the relative abundance of the proteins at these temperatures.]
Han et al. Figure 2D
Han et al. Figure 3A

![Image of a Western blot with bands labeled TauT and β-actin, and a bar graph showing relative abundance over time (0h, 8h, 16h, 24h, 36h).](http://www.jbc.org/Downloaded from http://www.jbc.org/)
Han et al. Figure 3B

B

| Time | 0h | 8h | 16h | 24h | 36h |
|------|----|----|-----|-----|-----|
| TauT |    |    |     |     |     |
| β-actin |    |    |     |     |     |

---

**Relative Abundance**

| Time | 0h | 8h | 16h | 24h | 36h |
|------|----|----|-----|-----|-----|
| MRK/4p53 |    |    |     |     |     |
Han et al. Figure 3C

![Graph showing taurine pmoles/h/mg protein over time (0h, 8h, 16h, 24h, 36h) for different conditions.](chart)
A
-751 CCA GAG CAG CCAGA G TGA GTAGTCC CACTATTT TTTCCGT TTTCACAGA
-701 TGA GAAAAAC AAGCAC AAGAGGT CTGGG GAA CTTGC CTGATGTCACA
-651 GCC ACA GCAAAAA GTCAG GGGAGC T TCAA AAAA AGACAG CCTCATG
-601 TCCGA GCTCTG C GCGCGGTGCTGCATGC GTCGTTGCTGTCGTTG TC
-551 TGTGTG TGTGTTGTTGTTGTTGTGTCGTTGTCGTTGTTGTTG GCGTGCCTGACGC
-501 CCA ACCCCGGCCCGTTAGC CGCCCGCCCGGCAAGCTGGTATTTTTCC
-451 CCGCCAAGCA GGA TGAGTGG GTATGCGGAGA GCTGCGTTCAGACA CAAGACAG ACA
-401 CGCGAGGTCAAGGAGAAGGCGGTATTACA CCTCGCTCCTC CGCCGCCGC
-351 CGCCAG

p53 site

TATA

PGL-963

luc

B

Relative light output

-DOX
+DOX

293 cells
NRK
(10)1 cell

*
Han et al. Figure 4C

![Graph showing relative light output for different p53 concentrations (0 μg, 0.5 μg, 1.0 μg, 2.0 μg). The graph compares 293, NRK, and (10)1 cell lines, with error bars indicating variability. Asterisks denote significant differences.](http://www.jbc.org/)
Han et al. Figure 4D&E
Han et al. Figure 5A&B

A

B

Relative output

0 μM 50 μM 500 μM (Taurine)

0 μM 50 μM 500 μM (Taurine)

Taurine/mg protein

0 2 4 6 8 10 12

-DOX +DOX

-DOX +DOX

* **

* **
Han et al. Figure 7A&B

A

wtTauT  TGAGGAACCAAGACACAGAAGGTCTGGGGAACCTTGCTGATG
nonTauT  AGGAGGACATGCTCATATGAAAAAATTGAATGGCACAGA
mtTauT  TGAGGAACCAAGACACAGAAGGTCTGGGGAATTTCCTGATG

B

C

1  2  3  4  5  6  7  8  9

1  2  3  4

\(\uparrow\)
Transcriptional repression of taurine transporter gene (TauT) by p53 in renal cells
Xiaobin Han, A Budreau Patters and Russell W. Chesney

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