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Identification and Functional Characterization of the Human *Glutathione S-Transferase P1* Gene as a Novel Transcriptional Target of the *p53* Tumor Suppressor Gene

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Abstract

The glutathione *S*-transferase P1 (GSTP1) is involved in multiple cellular functions, including phase II metabolism, stress response, signaling, and apoptosis. The mechanisms underlying the significantly high GSTP1 expression in many human tumors are, however, currently not well understood. We report here that the *GSTP1* gene is a heretofore unrecognized downstream transcriptional target of the tumor suppressor p53. We identified a p53-binding motif comprising two consecutive half-sites located in intron 4 of the *GSTP1* gene and is highly homologous to consensus p53-binding motifs in other p53-responsive genes. Using a combination of electrophoretic mobility shift assay and DNase I footprinting analyses, we showed that wild-type p53 protein binds to the GSTP1 p53 motif and luciferase reporter assays showed the motif to be transcriptionally functional in human tumor cells. In a temperature-sensitive p53-mutant cells, levels of both p21/WAF1 and GSTP1 gene transcripts increased time dependently when cells were switched from the inactive mutant state to the wild-type p53 state. Small interfering RNA — mediated reduction of p53 expression resulted in a specific decrease in GSTP1 expression and in tumor cells with mutated p53; adenovirally mediated expression of wild-type p53 increased GSTP1 expression significantly. In a panel of early-passage brain tumor cultures from patients, high levels of GSTP1 transcripts and protein were associated with wild-type p53 and, conversely, low GSTP1 levels with mutant p53. p53 expression knockdown by small interfering RNA increased cisplatin sensitivity. The ability of wild-type p53 to transcriptionally activate the human *GSTP1* gene defines a novel mechanism of protecting the genome and, potentially, of tumor drug resistance.

Introduction

The human glutathione *S*-transferase P1 (GSTP1) protein plays several critical roles in both normal and neoplastic cells. Notably, it functions in cellular phase II metabolism by catalyzing the conjugation of the tripeptide glutathione with a wide variety of electrophilic compounds, including genotoxins, carcinogens, and chemotherapeutic agents (1,2). GSTP1 also regulates cellular signaling through binding to a variety of important signaling proteins, including c-Jun NH$_2$-terminal kinase, ASK1, and TRAF2, thereby regulating their downstream signaling

functions (3,4). In most normal tissues, GSTP1 expression is very low but increases significantly in tumors arising from these tissues, including brain tumors, leukemias, lymphomas, sarcomas, and many carcinomas (5-7). The high GSTP1 protein expression and its nuclear localization have been shown to correlate not only with drug resistance but also with increased tumor grade and poor patient survival (6) and to enhance survival of tumor cells under hypoxic condition.

The p53 tumor suppressor, similar to the *GSTP1* gene, is involved in multiple important cellular and molecular events, including cellular proliferation and differentiation, DNA damage/repair, and apoptosis (8,9). Mutations in the *p53* gene are among the most frequent somatic genetic changes observed in human cancers, and consequently, p53 mutational status has been extensively studied for its correlation with tumor grade, patient survival, and treatment outcome in many cancers (10). The results of these studies have, however, been inconsistent with some showing a positive correlation of p53 mutations with tumor growth, progression, and drug resistance (11-14), whereas others showed the mutant p53 status to be deleterious to survival and to favor drug sensitivity (15-17). The findings indicate a high level of complexity in the role of p53 in human cancer and suggest that multiple upstream and downstream pathways of p53 may interact to mediate its effects in this disease.

Despite the important role that GSTP1 plays in oncogenesis (18) and therapeutic outcome (19) in many human tumors, currently, the molecular mechanisms and pathways regulating its expression in tumors are not well understood. Because GSTP1 and the transcription factor p53 are both independently involved in regulating cell growth, cell death, and survival and in cellular protection against genotoxins, we examined, in this study, the possibility that a cross-talk between these two genes could contribute to the regulation of GSTP1 expression in tumor cells. Using both primary tumors and cell lines of human malignant gliomas, we discovered that an intronic p53 consensus binding site present in the *GSTP1* gene was transcriptionally active and responsive to wild-type but not mutant p53. In glioma cells, the GSTP1 gene expression was significantly affected by the p53 mutational status and p53-mediated GSTP1 gene activation may contribute to cisplatin resistance.

Results

Identification of an Intronic p53-Binding Motif in the Human GSTP1 Gene

We conducted a search for putative p53-binding sites in the human *GSTP1* gene using a web-based search engine[3] and identified a putative p53-binding motif located in intron 4 of the *GSTP1* gene and spanning nucleotides +983 and +1,002. The motif comprised two half-sites with no spacer nucleotide between them (Fig. 1A) and sequence wise was highly homologous to the consensus p53-binding sites in several wellknown p53 target genes, such as *p21/WAF1, MDM2, GADD45*, and *ribosomal gene cluster* (*RGC*; Fig. 1A; refs. 20-24). The absence of a nucleotide spacer between the two half-sites is consistent with the 0- to 13-nucleotide spacer requirement in functional p53 motifs and similar to that of the p53 motifs found in p53-responsive genes, such as *MDM2, GADD45*, and *RGC*.

Wild-Type p53 Binds to the GSTP1 p53 Motif

The results of the analysis of the binding affinity and specificity of wild-type p53 protein to the GSTP1 p53 motif, done by electrophoretic mobility shift assay, are summarized in Fig. 1B and show significant binding of proteins from wild-type p53-expressing Sk-Ala cells (*lane 2*) to the ^{32}P-end— labeled GSTP1 p53-binding motif (*a*). The binding was outcompeted by cold GSTP1 p53 motif (*lane 3*) and progressively competed out by increasing amounts of cold

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[3]http://www.cbrc.jp/research/db/TFSEARCH.html

consensus p53-binding site (*lanes 4-6*), indicating specificity of the binding. Fig. 1B (*b*) shows a supershift (*arrow*) of the p53 protein/p53-binding motif complex on addition of an anti—p53-specific antibody.

The results of the DNase I footprinting analysis (Fig. 1C) show the Maxam-Gilbert sequencing (adenine and guanine) of the footprint probe (*lane 1*) and the standard footprint pattern of the DNase I—digested GSTP1 intron 4 probe (*lane 2*). Fig. 1C (*lanes 3* and *4*) shows increased protection of the GSTP1 p53-binding sites in the presence of increasing amounts of protein from the wild-type p53-expressing Sk-Ala cells. The results of the gel shift/supershift and DNase footprinting analysis, taken together, show the specificity of the binding of wild-type p53 protein to the GSTP1 p53-binding sites.

Wild-Type p53 Transactivates Luciferase Gene Expression via the GSTP1 p53 Motif

The structures of the four vectors used in these studies are shown in Fig. 2. The results summarized in Table 1 show that untransfected and pFR-Luc—transfected tumor cells express low levels of luciferase at both temperatures 32°C and 38°C. In contrast, luciferase expression increased significantly in pRGC-p53BS-Luc—transfected cells maintained at 32°C for 24 and 48 h relative to cells at 38°C. In cells transfected with pGSTP1-p53BS(3X)-Luc and pGSTP1-p53BS(4X)-Luc containing three and four GSTP1 p53 motifs, there was a marked increase in luciferase expression, consistent with wild-type p53-mediated transactivation of the *GSTP1* gene via the GSTP1 p53-binding sites.

GSTP1 Gene Expression Is Induced by Wild-Type p53 in Tumor Cells

The results of the Northern blot analysis (Fig. 3A) show a significant increase in GSTP1 gene transcripts in Sk-Ala cells maintained at 32°C (wild-type p53) compared with those at 38°C (mutant p53). On switching from the mutant (38°C) to the wild-type (32°C) p53 state, the levels of GSTP1 gene transcripts in the cells increased time dependently to 4.3-, 12-, 18-, and 29-fold of controls after 6, 12, 24, and 48 h, respectively. In contrast, no change in GSTP1 gene transcript levels was observed in the parental SKLMS-1 cells on switching from 38°C to 32°C. Transcripts of *p21/WAF1*, a p53 responsive gene, also increased significantly on switching of Sk-Ala cells from the mutant p53 state at 38°C to the wild-type p53 state at 32°C for 48 h (Fig. 3B). On switching back to 38°C for 72 h, p21/WAF1 transcripts were undetectable. The increase in GSTP1 and p21/WAF1 gene transcripts at 32°C and decline to the basal level at 38°C indicate that both genes are under the control of the functional wild-type p53. The Western blot analysis (Fig. 3C) also shows higher levels of GSTP1 protein in Sk-Ala cells expressing wild-type p53 (32°C) than those with the mutant p53 (38°C). Consistent with these observations, tumor cells infected with the adenoviral vector Ad/CMV-p53 expressed higher levels of GSTP1 protein than the controls (Fig. 3D). Small interfering RNA (siRNA)-mediated down-regulation of wild-type p53 in U87MG cells was associated with a reduction of GSTP1 protein expression (Fig. 3E) and a decrease of luciferase expression in cells carrying the pGSTP1-p53BS(3X)-Luc construct compared with cells transduced with nonspecific control siRNA (Fig. 3F). Taken together, these results show that wild-type p53, but not mutant p53, transcriptionally activates the human *GSTP1* gene.

Relationship between p53 Mutational Status and GSTP1 Expression in Brain Tumor Cells

The relationship between p53 and GSTP1 was examined in seven human gliomas in early-passage culture and the results are shown in Table 2. DNA sequencing showed three of the cell lines (MGR3, UW28, and UW281) to harbor the wild-type *p53* gene, whereas UW78 cells had a G→A silent mutation at codon 36. MGR2 contained a G→A transition at codon 273, resulting in a substitution of arginine to histidine. MGR1 and UW228 both had C→A transversions at codon 155, changing the encoded amino acid from threonine to asparagine. *GSTP1* gene transcript levels were determined by densitometry of the Northern blots and normalized against

glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The results (Fig. 4A) showed that wild-type p53-containing cell lines contained 2- to 5-fold higher levels of GSTP1 gene transcripts compared with those with mutant p53. GSTP1 protein expression, determined by Western blotting (Fig. 4B), also showed GSTP1 to be consistently elevated in tumors with the wild-type p53 compared with those with the mutant forms.

Furthermore, we hypothesized that p53-mediated GSTP1 gene activation may play a role in the resistance of glioblastomas to cisplatin given the ability of GSTP1 to inactivate cisplatin. If our hypothesis is correct, p53 expression knockdown by siRNA is expected to lead to reduced GSTP1 protein levels and increased cisplatin sensitivity. We used human glioblastoma U87MG cells in these studies as they express the wild-type p53 (25) and are highly resistant to cisplatin with an IC_{50} of 134 µmol/L. U87MG cells were ideal for these studies as they can be transfected with high efficiency, which was critical for sufficient p53 knockdown by siRNA. Moreover, U87MG cells transfected with nonspecific siRNA were highly resistant to cisplatin, whereas those with the p53 siRNA showed significantly increased sensitivity ($P = 0.027$; Table 3). Collectively, these data indicate that wild-type p53 expression correlates with high GSTP1 gene activity in brain tumors and that high levels of wild-type p53 contribute to cisplatin resistance of glioblastoma cells, in part, due to p53-mediated GSTP1 transcriptional up-regulation.

Discussion

The tumor suppressor p53 is a transcriptional factor, the biological effects of which are mediated largely through the downstream target genes that it transactivates. This study reports that the human *GSTP1* gene is a heretofore unrecognized downstream target of p53 and is transcriptionally activated by p53 through binding of p53 to a p53-binding motif present in the *GSTP1* gene, located in intron 4 of the *GSTP1* gene at nucleotides +983 to +1,002 relative to the transcription start site. The motif 5′-GGGCAGGCATGGGCAAGCCT-3′ is homologous in sequence to the p53 motifs in other p53 responsive genes, including *p21/WAF1, MDM2, GADD45*, and *RGC* (20-24), and comprises two half-sites. One of the half-sites is a perfect match to the consensus p53 sequence 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′ (24), whereas the other half-site contains two mismatches from the consensus motif. No spacer nucleotides separate the p53 half-sites in the *GSTP1* gene. Such a spacerless p53-binding site has also been previously observed in *MDM2* (21), *GADD45* (22), and *RGC* (23) genes. Molecular and functional analysis showed that the wild-type p53 protein binds to the p53-binding site in the human *GSTP1* gene and activates its transcription.

The p53 tumor suppressor has been extensively investigated for its role in oncogenesis and tumor progression and in therapeutic outcome in many human cancers (10). Based on the high frequency of p53 mutations in human tumors, efforts have been made to treat tumors by restoring the wild-type p53 function (26-28). Despite the initial promise, clinical trials of wild-type p53 gene therapy, alone or in combination with chemotherapy, have shown little clinical efficacy (29,30). Simultaneously, the results of studies of p53 mutational status as a correlate of patient response to therapy have also yielded mixed results. Thus, in gliomas, the presence of wild-type p53 results in higher resistance to cisplatin than when the gene is mutated and/or functionally inactive and that abrogation of the wild-type p53 (e.g., via antisense oligonucleotides) sensitizes tumor cells to cisplatin (31). Similar observations of wild-type p53 conferring drug resistance or decreased drug sensitivity have also been made in other cancer types, such as breast, bladder, and ovarian carcinomas (15-17). In contrast, several other reports showed that p53 mutations are associated with drug resistance and that restoration of p53 function reverses such resistance (11-14).

Although several transcriptional mechanisms, such as those mediated by Sp1 (32), AP1 (33), retinoic acid response element (34), and PKA/CREB1 (35), have been shown to regulate GSTP1 gene expression, the best-characterized regulating mechanism of GSTP1 expression in tumors is that mediated by the differential methylation of the GSTP1 promoter (36,37). Our results in this study indicate that the cross-talk between the *p53* tumor suppressor and the *GSTP1* gene is a novel, heretofore, unrecognized mechanism by which *GSTP1* gene expression can be regulated in tumor cells. This interaction between the *p53* and *GSTP1* genes could shed light on the complex role that these two genes and their encoded proteins play in protecting the cellular genome against genotoxic compounds and in regulating cellular response to DNA damage, oxidative stress, and other stimuli (1,3,4) and as a determinant of cancer risk and outcome of cancer therapy. Our findings that the *GSTP1* gene contains a p53-binding site that is functionally active and can be activated by wild-type p53 are thus highly significant. The results with the cells from seven gliomas suggest that tumors with wild-type p53 express high levels of GSTP1 and, thus, are likely to be more resistant to alkylating agents. This is supported by a large body of evidence indicating that high levels of GSTP1 expression are associated with increased resistance to chemotherapy in many cancers (5,38-40).

Although the mechanisms underlying the association of wild-type p53 with drug resistance is likely to be complex and to include effects on the cell cycle and altered apoptotic response (41), our data suggest that, in GSTP1-expressing tumors, the ability of p53 to increase GSTP1 expression will be an important part of this mechanism of drug resistance and stress response.

Together, our findings define a novel mechanism by which wild-type p53 regulates GSTP1 expression and protects the genome from alkylating and free radical—generating compounds. In tumors, the transcriptional activation of the *GSTP1* gene by p53 will lead to increased survival and increased drug resistance, whereas in normal cells this could lead to protection against genotoxins. Further studies are necessary to define the effect and significance of these findings in these and other important cellular GSTP1-mediated processes and in outcome of cancer therapy.

Materials and Methods

Cell Lines and Cell Culture

The SKLMS-1 is a human leiomyosarcoma cell line with a codon 245 mutation (glycine to serine) in the p53 protein and is routinely maintained in DMEM with 10% FCS and antibiotics. Sk-Ala cells are stable transfectants generated from SKLMS-1 cells that harbor a temperature-sensitive p53 with a V143A mutation. At 38°C, Sk-Ala cells express a *p53* gene in the mutant conformation/function but, at 32°C, assume the wild-type p53 conformation/function. The human anaplastic astrocytoma (MGR1), the human glioblastoma (MGR2, MGR3, UW28, UW281, and UW78), and the human medulloblastoma (UW228) cell lines were all early-passage cultures established from primary specimens in the laboratory of Dr. Francis Ali-Osman (5). U87MG human glioblastoma cells were obtained from the American Type Culture Collection and express the wild-type p53 (25). All cells were maintained in DMEM supplemented with 10% FCS.

Reagents and Chemicals

p53 siRNA and nonspecific control siRNA were purchased form Millipore/Upstate. p53 and control viruses, Ad/CMV-p53 and Ad/CMV, respectively, were obtained from the Institutional Vector Core at The University of Texas M. D. Anderson Cancer Center. Unless otherwise stated, all biochemicals and chemicals were purchased from Sigma.

Identification of p53-Binding Motifs in the GSTP1 Gene

A web-based search engine[3] was used to search the human *GSTP1* gene for sequences matching the p53 consensus motif 5′-PuPuPuC(A/T)(T/A)GPyPyPy-3′. The target sequence analyzed covered nucleotides —2,000 to +2,975 and spanned the entire *GSTP1* gene, including all seven exons and six introns (34).

Electrophoretic Mobility Shift Assay

As part of its functional characterization, we examined the ability of wild-type p53 protein to bind to the GSTP1 putative p53-binding motif by electrophoretic mobility shift assay, as previously described (35). Oligonucleotides corresponding to the GSTP1 p53-binding motif and control consensus p53-binding sites (24), namely, 5′-CCAGGGCAGGCATGGGCAAGCCTCTG-3′ and 5′-TAGGCATGTCTAGGCATGTCTA-3′, respectively, were ^{32}P labeled and used as binding probes. For competition experiments, the p53-containing protein extracts were preincubated with 50-fold excess of unlabeled GSTP1 p53 motif (Fig. 1B, *lane 3*) or with 50-, 100-, and 200-fold excess (Fig. 1B, *lanes 4-6*) of unlabeled consensus p53 motif probe for 15 min before the addition of the ^{32}P-labeled probes. For the gel supershift assays, 1 μg of an anti-p53 monoclonal antibody (DO-1; Santa Cruz Biotechnology) was added to the p53-containing protein extracts that had been preincubated with ^{32}P-labeled GSTP1 p53 motif for 30 min at room temperature.

DNase I Footprinting Assays

DNase I footprinting was done as previously described (35) to determine the direct binding of the p53 protein to p53-binding motif. The *GSTP1* intron 4 probe containing the putative p53-binding sites was generated by PCR using the primers 5′-GTGAGTCTTGAACCTCCAAGT-3′ (forward) and 5′-CTGGGGTTGGGTGGAGGGGAG-3′ (reverse) and genomic DNA from the MGR3 glioblastoma cell line as template. An aliquot of the end-labeled probe was subjected to Maxam-Gilbert DNA sequencing (42).

Plasmid Constructs, Transfection, and Luciferase Reporter Assay

To study the functionality of the GSTP1 p53-binding motifs further, we examined its ability to activate gene expression p53 dependently using a luciferase reporter vector, pFR-Luc (Stratagene), containing the firefly luciferase cDNA. DNA fragments containing three and four tandem repeats of the GSTP1 p53-binding motif, 5′-AGGCAGGCATGGGCAAGCCT-3′, were synthesized by PCR and cloned into the vector at a site 36 nucleotides upstream of the luciferase start codon. The resulting constructs, each containing a TATA box, control promoter, and the firefly luciferase cDNA, were designated pGSTP1-p53BS(3X)-Luc and pGSTP1-p53BS(4X)-Luc, respectively. The pRGC-p53BS-Luc plasmid (Stratagene) is consisted of 15 tandem repeats of the RGC p53-binding site, 5′-TGCCTGGACTTGCCTGG-3′ (23). A *Renilla* luciferase expression vector, pRL-CMV (Promega), was used as a transfection efficiency control. Transfections were done using MBS Mammalian Transfection kit (Stratagene), Lipofectamine LTX (Invitrogen), and FuGENE HD (Roche). The cells were then lysed and luciferase activity was measured in the supernatant using the Dual-Luciferase Assay kit (Promega). Relative luciferase activity was compared following normalization of the firefly luciferase against that of the *Renilla* luciferase.

Gene Expression Analyses by Northern Blotting

Tumor cells treated as per experimental protocol were harvested and total RNA was extracted using the acid guanidinium thiocyanate-phenol-chloroform (34,43). Northern blotting was done using *GSTP1* and *p53* cDNA generated by PCR as we had described previously (34,

44). The human p21/WAF1 cDNA probe was generated by PCR using primers with sequences 5′-TAAGAGGAGGCGCCATGTCAGAAC-3′ (forward) and 5′-TCCTGTGGGCGGATTAGGGCTTCCT-3′ (reverse). GAPDH cDNA probe was purchased from Clontech Laboratories. Following autoradiography, the bands were quantitated by densitometry and target gene transcripts were normalized against GAPDH.

Western Blot Analysis

Tumor cells in exponential growth were treated as per experimental protocol and lysed by sonication, and particle-free supernatants were prepared by centrifugation at 15,000 rpm for 20 min. The protein extracts were subjected to Western blot analysis, as previously described (45), using the following antibodies: anti-GSTP1 rabbit polyclonal antibody (Stressgen), anti-p53 mouse monoclonal antibody (DO-1), and anti-β-actin mouse monoclonal antibody (Sigma).

Analysis of p53 Mutational Status in Brain Tumor Cell Lines

Total genomic DNA was isolated from the eight brain tumor cell lines, MGR1, MGR2, MGR3, UW28, UW281, UW78, and UW228, using the phenol-chloroform-isoamyl alcohol procedure. The extracted DNA was used to amplify each of exons 2 to 8 of the *p53* gene. Each PCR product was cloned into the pCR2.1 using the TA cloning kit (Invitrogen). Positive clones were subjected to DNA sequencing, as we previously described (46).

Statistical Analysis

Statistical analysis was done using the Student's *t* test. Statisca (StatSoft) and Microsoft Excel were used to analyze the data. Statistical significance was set at *P* < 0.05.

Acknowledgements

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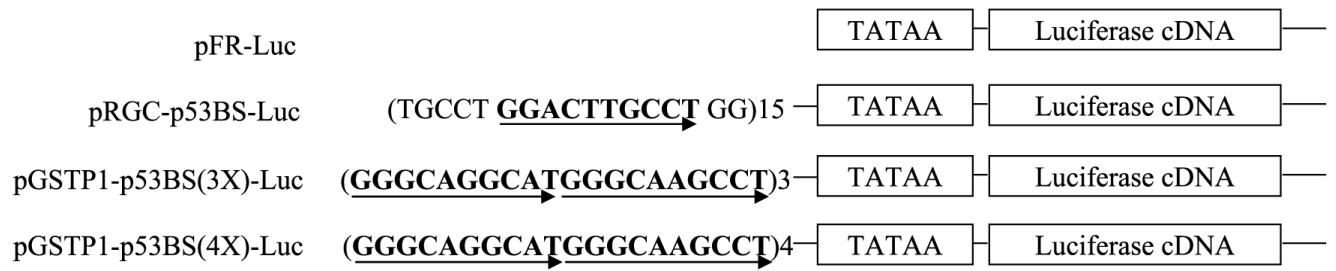
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A

Consensus p53 motif: PuPuPuC(A/T)(T/A)GPyPyPy-0 to13 nt-PuPuPuC(A/T)(T/A)GPyPyPy

GSTP1:   GGGCAgGCaT--0 nt--GGGCAAGCCT

p21:   GAACATGTCC--0 nt--cAACATGTTg

MDM2:   GGtCAAGTTC-18 nt-AGACAcGTTC

GADD45: GAACATGTCT--0 nt--AAGCATGCTg

RGC: AtcCTTGCCT--0 nt--GGACTTGCCT

B   **a. Gel shift**   **C**
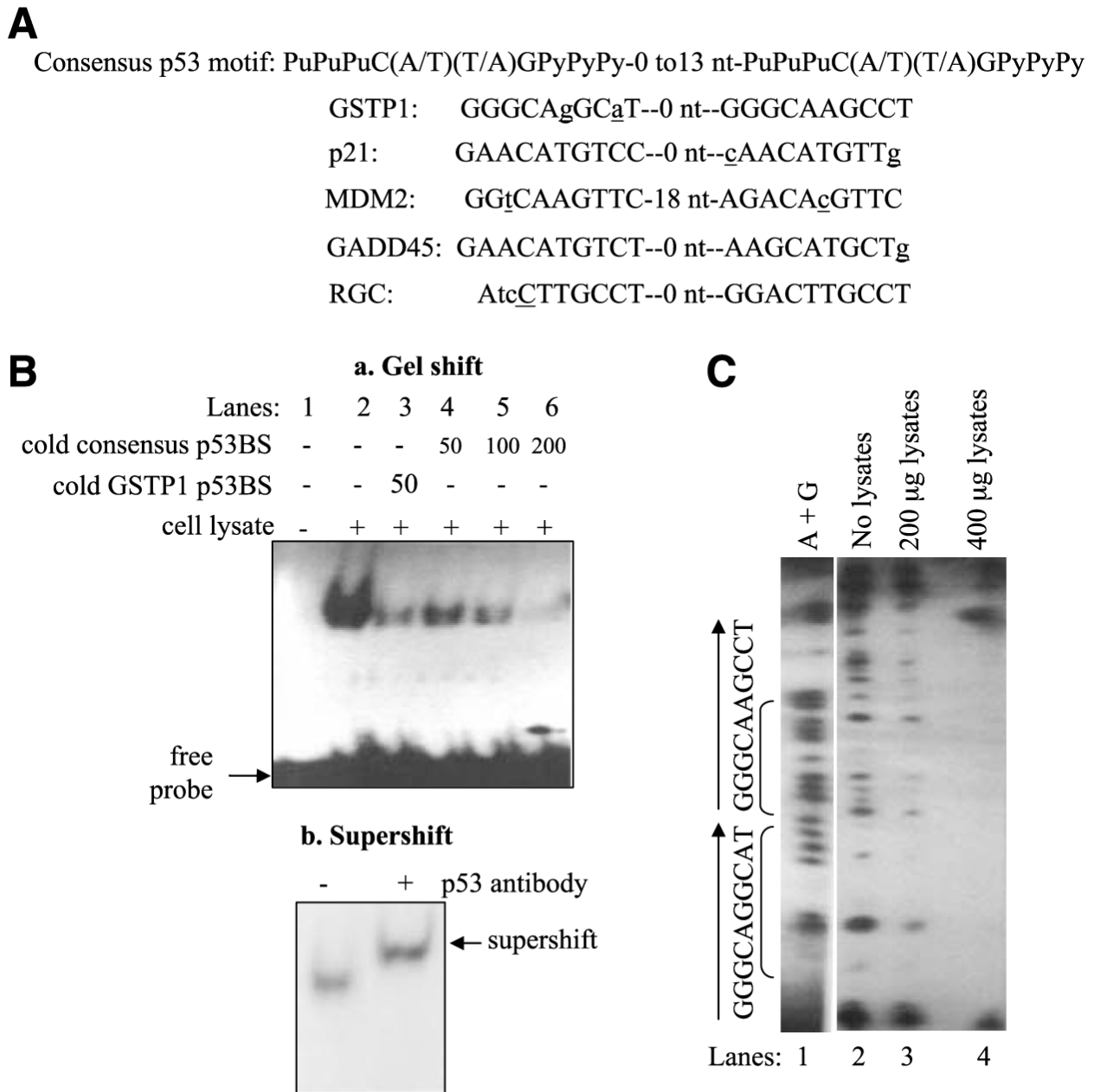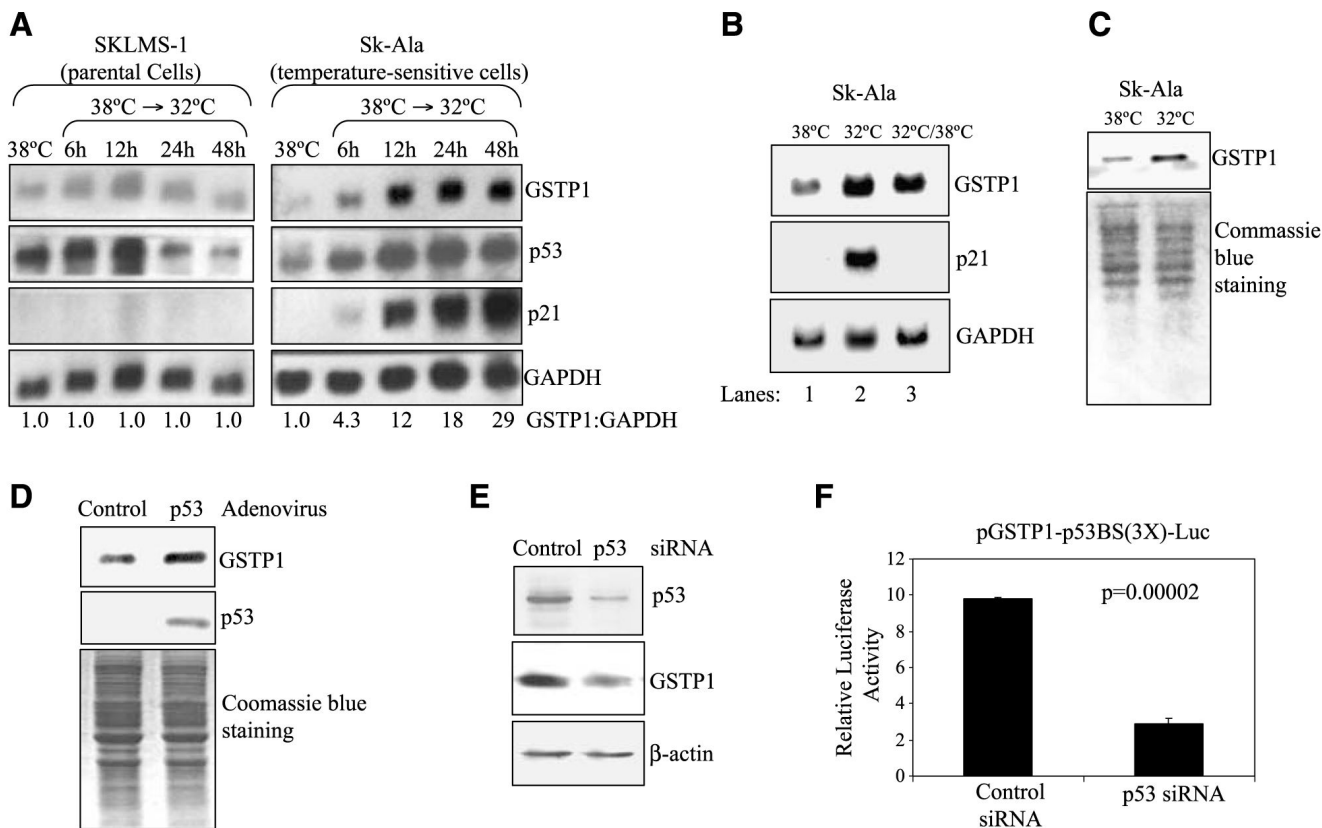

FIGURE 1.
Identification and functional characterization of the GSTP1 p53-binding sites. **A.** Comparison of the p53-binding motif in intron 4 of the *GSTP1* gene with consensus p53-binding site (24) and those in known p53-responsive genes. Spacer nucleotides between two p53 half-sites are indicated. Nucleotides that do not match the consensus p53-binding motifs are in lower case and underlined. **B.** Binding of wild-type p53 protein to the GSTP1 p53-binding motif. Electrophoretic mobility shift assay was done with oligonucleotides corresponding to the GSTP1 p53-binding motif and protein extracts from Sk-Ala cells containing wild-type p53. a, lane 1, ^{32}P-labeled GSTP1 p53 motif with no protein extract; lane 2, ^{32}P-labeled probe and p53-containing protein extracts; lane 3, competition with 50-fold excess of unlabeled GSTP1

p53 motif probe; lanes 4 to 6, competition experiments with 50-, 100-, and 200-fold excess of unlabeled (cold) consensus binding sites (24). b, supershift of p53 protein bound to GSTP1-binding motif. The p53 monoclonal antibody DO-1 was added to protein extract from Sk-Ala cells preincubated with the ^{32}P-end—labeled probe. Arrow, supershifted band. **C.** DNase I footprinting analysis of wild-type p53 protein binding to the GSTP1 p53 motif. Wild-type p53 protein extracts from Sk-Ala cells were used in these studies. Lane 1, Maxam-Gilbert sequencing of the nucleotide sequence (A+G) of the footprint probe; lane 2, sequence of the GSTP1 p53 motif without protein extract; lanes 3 and 4, contained 200 and 400 μg of p53-containing protein from Sk-Ala cells, respectively. Brackets, the sequence of the protected region corresponds to the GSTP1 p53-binding motif.

pFR-Luc		TATAA ⊢ Luciferase cDNA ⊢
pRGC-p53BS-Luc	(TGCCT **GGACTTGCCT** GG)15 ⊣	TATAA ⊢ Luciferase cDNA ⊢
pGSTP1-p53BS(3X)-Luc	(**GGGCAGGCATGGGCAAGCCT**)3 ⊣	TATAA ⊢ Luciferase cDNA ⊢
pGSTP1-p53BS(4X)-Luc	(**GGGCAGGCATGGGCAAGCCT**)4 ⊣	TATAA ⊢ Luciferase cDNA ⊢

FIGURE 2.
Wild-type p53 activates luciferase reporters driven by the GSTP1 p53-binding motif. Structure
of GSTP1 p53-binding motif-containing vectors.

FIGURE 3.
GSTP1 gene expression is induced by wild-type p53 in tumor cells. Sk-Ala cells stably express wild-type p53 at 38°C and inactive mutant at 32°C. SKLMS-1 expresses inactive p53 at both temperatures. **A.** *GSTP1* gene transcript was examined by Northern blotting in both cell lines maintained at 38°C for 48 h and then switched to 32°C for 6, 12, 24, and 48 h. The membranes were blotted for GSTP1, p53, p21/WAF1, and the loading control GAPDH. Band signals were quantified densitometrically and used to compute GSTP1 to GAPDH ratios. **B.** *GSTP1* gene is transcriptionally activated in cells with wild-type. Sk-Ala cells were maintained at 38°C (mutant p53; *lane 1*), switched to 32°C (wild-type p53) for 48 h (*lane 2*), returned to 38°C (mutant p53) for 72 h (*lane 3*), harvested, and subjected to Northern blot analyses for transcripts of the *GSTP1, p21/WAF1*, and *GAPDH* genes. **C.** p53 protein with the wild-type conformation/ function increases GSTP1 protein expression. Sk-Ala cells were maintained at 38°C and switched to 32°C for 48 h. Equal loading is indicated by Coomassie blue staining. **D.** Forced expression of the wild-type p53 increased GSTP1 expression. MGR1 cells were infected with the adenovirus carrying wild-type p53, Ad/CMV-p53, and the control virus Ad/CMV. After 48 h, infected cells were harvested and subjected to Western blotting to determine expression of GSTP1 and p53. Equal loading is indicated by Coomassie blue staining. **E** and **F.** p53 knockdown suppresses GSTP1 expression. In **E**, U87MG cells were transfected with nonspecific control siRNA and p53 siRNA for 48 h and lysed and total protein was extracted. Western blot analyses were then done to determine levels of p53, GSTP1, and β-actin. In **F**, U87MG cells were transfected with pGSTP1-p53BS(3X)-Luc/pRL-CMV and control siRNA or p53 siRNA and luciferase activity was determined 48 h after transfection, as described earlier. Columns, mean of three independent experiments; bars, SD. Student's *t* test was subsequently carried.
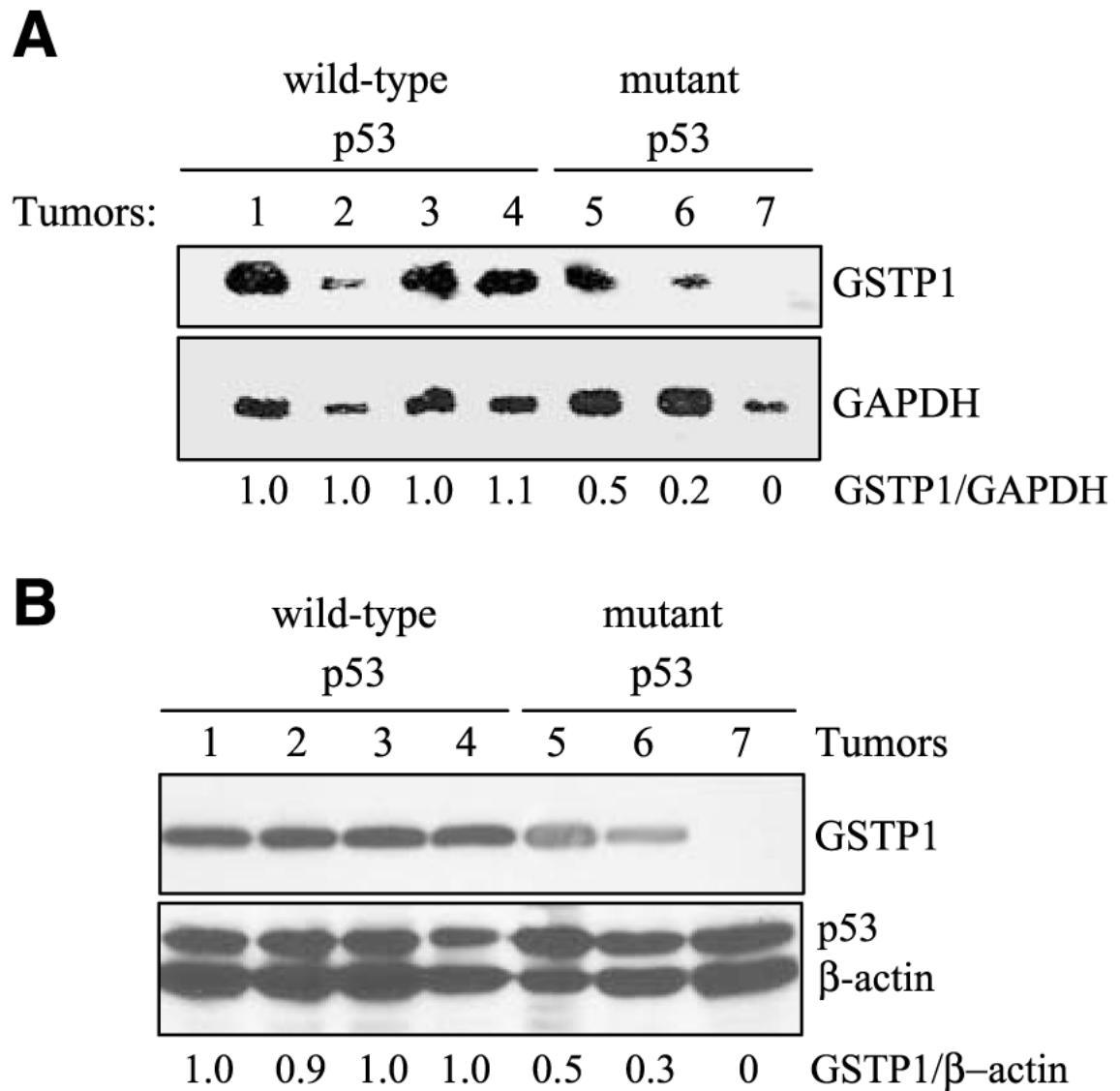
FIGURE 4.
Relationship between p53 mutational status and GSTP1 expression in brain tumor cells. Seven early-passage cultures of human gliomas were used in these studies. **A.** Higher GSTP1 transcript levels in brain tumor cells with wild-type p53. GSTP1 gene transcript levels were examined by Northern blotting and relative GSTP1 expression (GSTP1/ GAPDH) was determined using MGR3 as standard. **B.** Increased GSTP1 protein expression in brain tumor cells correlates with the presence of wild-type p53. Levels of GSTP1 and p53 proteins were determined by Western blotting. Relative GSTP1 levels are expressed as GSTP1/β-actin with MGR3 as expression standard.

TABLE 1

Functional p53 Time Dependently Activates Promoters Containing GSTP1 p53 Motif

Vectors	38°C				32°C			
	24 h	48 h			24 h	48 h		
None	1.86 ± 0.44	1.53 ± 0.14			1.48 ± 2.57	0.24 ± 0.20		
pFR-Luc	0.14 ± 0.18	0.87 ± 0.66			0.14 ± 0.00	3.89 ± 2.15		
pRGC-p53BS-Luc	2.00 ± 0.10	0.91 ± 0.91			3,755.05 ± 112.61	6,095.77 ± 190.09		
pGSTP1-p53BS(3X)-Luc	2.11 ± 0.43	1.81 ± 0.47			393.72 ± 27.36	1,734.02 ± 83.67		
pGSTP1-p53BS(4X)-Luc	0.47 ± 0.81	1.26 ± 1.09			2,592.93 ± 245.43	8,846.25 ± 69.83		

NOTE: Sk-Ala cells were transfected with various constructs and maintained at 38°C or 32°C for 24 and 48 h before luciferase assays. Relative luciferase activity was computed as described earlier and SDs from three independent experiments.

TABLE 2

Mutational Status of *p53* Gene in Brain Tumor Cells

Brain Tumor Cell Line	p53 Status	Mutated Exon	Mutated Codon	Nucleotide Changes	Amino Acid Substitution
MGR3	Wild-type	-	-	-	-
UW28	Wild-type	-	-	-	-
UW281	Wild-type	-	-	-	-
UW78	Wild-type	4	36	CCG→CCA	None
MGR2	Mutant	8	273	CGT→CAT	Arg→His
MGR1	Mutant	5	155	ACC→AAC	Thr→Asn
UW228	Mutant	5	155	ACC→AAC	Thr→Asn

TABLE 3

Reduced Expression of Both p53 and GSTP1 Rendered Resistant Glioblastoma Cells to Cisplatin Treatments

	Control	CDDP
Nonspecific control siRNA	100%	$72.5 \pm 6.6\%$
p53 siRNA	100%	$45.8 \pm 4.5\%$

NOTE: U87MG cells in 96-well culture plates were transfected with the nonspecific control siRNA or p53 siRNA, and 24 h after transfection, cells were treated with and without 200 µmol/L cisplatin for 24 h and harvested and cell viability was determined using the CellTiter Blue Assay kit. All data represent means and SDs from three independent experiments. Student's *t* test was conducted to determine whether a significant difference existed in the survival rate between the nonspecific control siRNA — transfected and p53 siRNA — transfected cells. *P* value was subsequently determined to be 0.027.