A Novel PTEN/Mutant p53/c-Myc/Bcl-XL Axis Mediates Context-Dependent Oncogenic Effects of PTEN with Implications for Cancer Prognosis and Therapy[1,2]

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Abstract

Phosphatase and tensin homolog located on chromosome 10 (PTEN) is one of the most frequently mutated tumor suppressors in human cancer including in glioblastoma. Here, we show that PTEN exerts unconventional oncogenic effects in glioblastoma through a novel PTEN/mutant p53/c-Myc/Bcl-XL molecular and functional axis. Using a wide array of molecular, genetic, and functional approaches, we demonstrate that PTEN enhances a transcriptional complex containing gain-of-function mutant p53, CBP, and NFY in human glioblastoma cells and tumor tissues. The mutant p53/CBP/NFY complex transcriptionally activates the oncogenes *c-Myc* and *Bcl-XL*, leading to increased cell proliferation, survival, invasion, and clonogenicity. Disruption of the mutant p53/c-Myc/Bcl-XL axis or mutant p53/CBP/NFY complex reverses the transcriptional and oncogenic effects of PTEN and unmasks its tumor-suppressive function. Consistent with these data, we find that PTEN expression is associated with worse patient survival than PTEN loss in tumors harboring mutant p53 and that a small molecule modulator of p53 exerts greater antitumor effects in PTEN-expressing cancer cells. Altogether, our study describes a new signaling pathway that mediates context-dependent oncogenic/tumor-suppressive role of PTEN. The data also indicate that the combined mutational status of PTEN and p53 influences cancer prognosis and anticancer therapies that target PTEN and p53.

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Introduction

Since its discovery, the importance and variety of functions of *phosphatase and tensin homolog located on chromosome 10* (*PTEN*) in cancer has surpassed all predictions and qualified this gene as one of the most versatile and commonly altered tumor suppressors in human cancer [1]. PTEN is mostly known for its negative regulatory effects on the phosphatidylinositide 3-kinase (PI3K) pathway, which are mediated by its lipid phosphatase activity [2–4]. However, numerous studies also describe other activities that are believed to be primarily exerted by nuclear PTEN [5,6]. Among tumor suppressors, PTEN is unique because subtle changes of expression can have profound effects on tumorigenesis [1,2,7]. Most human tumors retain some expression of PTEN, and a subset of human tumors displays levels of PTEN only slightly below the average levels of normal tissues [1,7]. Despite the generally accepted tumor-suppressive functions of PTEN, a few recent reports have described data that are seemingly inconsistent with such functions. Although PTEN loss is frequently associated with metastasis, recent reports demonstrated that there was PTEN gain from primary breast carcinoma to metastasis [8]. Moreover, PTEN was shown to promote early renal tumorigenesis through induction of hypoxia-inducible factor 2alpha (HIF-2α) in von Hippel Lindau null renal cell

carcinoma [9]. Remarkably, we recently found that PTEN exerted oncogenic effects in glioblastoma cells harboring *p53* mutations (mut-p53) [10]. However, the underpinning mechanisms of the PTEN oncogenic effects are not known.

p53 is an important tumor suppressor that maintains genetic stability in mammals by its multiple regulatory roles on cell cycle, apoptosis, senescence, and differentiation [11–13]. *p53* is mutated in approximately 50% of all human cancers [14]. The vast majority of *p53* mutations in cancer are point mutations that are associated with high mutant protein expression [15]. *p53* mutations in cancer have three, not mutually exclusive, types of outcome [15,16]: 1) mutations that result in abrogation of tumor suppressor function of the affected TP53 allele; 2) mutations that exert dominant-negative effects over co-expressed wild-type p53 (wt-p53); 3) mutants that acquire new activities that contribute to various stages of tumor progression and to increased resistance to anticancer treatments. The latter are referred to as gain-of-function mutants and comprise many of the hotspot *p53* mutations [16,17]. The modes of action of gain-of-function mut-p53 are not well understood but are believed to involve direct or indirect transcriptional activation or inhibition of gene sets other than those regulated by wt-p53 as well as interaction with p63 and p73 [18,19]. Among other gain-of-function p53 mutants were shown to transcriptionally regulate *CDC25C, c-Myc, Bcl-XL, vascular endothelial growth factor A, Id2,* and *FAS* [20–22].

Recent work demonstrated that PTEN and wt-p53 enhance each other's tumor-suppressive functions [23–25]. However, to our best knowledge, nothing is known about the mechanistic interactions between PTEN and mut-p53 in human cancer. In the present study, we show that PTEN exerts context-dependent oncogenic effects through a novel PTEN/mut-p53/c-Myc/Bcl-XL axis. We show that PTEN enhances a transcriptional complex containing mut-p53, CBP, and NFY. The mut-p53/CBP/NFY complex binds to the promoter of the oncogenes *c-Myc* and *Bcl-XL* and induces their transcription. *c-Myc* and *Bcl-XL* induction leads to increased cell proliferation, survival, invasion, and clonogenicity. Knockdown of any component of the novel mut-p53/c-Myc/Bcl-XL axis and complex reversed the oncogenic effects of PTEN. Consistent with the unexpected oncogenic effects of PTEN in mut-p53 cells, we find that PTEN expression is associated with worse survival than PTEN loss in mut-p53 glioblastoma tumors. We also show that a small molecule modulator of p53, PRIMA-1, has greater antitumor effects when PTEN is expressed in cancer cells. Our study therefore supports the novel idea of a dual role of PTEN in cancer and uncovers novel mechanisms of PTEN oncogenic effects in mut-p53 cancer cells and demonstrates their implications for prognosis and therapy.

Materials and Methods

Cells and Reagents

U373 glioblastoma cells were grown in Dulbecco's modified Eagle's medium (DMEM; 1 g/l glucose with L-glutamine) supplemented with Hepes buffer and 10% FBS. SNB19 glioblastoma cells were grown in DMEM/F12 (1:1, L-glutamine, 15 mM Hepes) supplemented with 10% FBS. U87 glioblastoma cells were grown in MEM supplemented with sodium pyruvate, sodium bicarbonate, and 10% FBS. LNZ308 glioblastoma cells and 293T cells were cultured in DMEM (4.5 g/l glucose, L-glutamine, and sodium pyruvate) supplemented with 10% FBS. GBM6 primary glioblastoma cells were grown in DMEM (4.5 g/l glucose, L-glutamine, and sodium pyruvate) supplemented with 2.5% FBS.

All p53 exons were sequenced for all cells as described below. U373, SNB19, and GBM6 harbored homozygous p53 R273H gain-of-function mutation. LNZ308 was p53-null. The PTEN status was also determined for all cells. U373, SNB19, U87, and LNZ308 were PTEN-null, and GBM6 was PTEN-positive. Antibodies for immunoblot analysis, immunoprecipitation (IP), immunodepletion (ID), and native polyacrylamide gel electrophoresis (PAGE) were anti-CBP (Cell Signaling Technology, Danvers, MA), anti-NFYA (Santa Cruz Biotechnology, Santa Cruz, CA), anti-p53 (Santa Cruz Biotechnology), anti-p53 (Imgenex, San Diego, CA), anti–acetylated CBP (Cell Signaling Technology), anti–c-Myc (Cell Signaling Technology), anti–Bcl-XL (Cell Signaling Technology), anti–caspase-3 (Cell Signaling Technology), anti-poly (ADP-ribose) polymerase (PARP; Cell Signaling Technology), and anti–β-actin antibody (Santa Cruz Biotechnology). PRIMA-1 was from Calbiochem (San Diego, CA). All other reagents were purchased from Sigma (St Louis, MO), unless otherwise specified.

Tumor Samples

Glioblastoma patients were randomly chosen and signed separate informed consent forms for postsurgical sampling. The procedures were reviewed and approved by the Review Board of the University of Virginia Health System.

Gene Silencing with Lentivirus-Based shRNA

The lentiviral vector pLKO.1-ConshRNA, pLKO.1-mp53shRNA, pLKO.1-c-McyshRNA, pLKO.1-Bcl-XLshRNA, pLKO.1-PTENshRNA, or pLKO.1-NFYAshRNA and lentivirus packaging plasmids were cotransfected into 293T cells with FuGene 6 (Roche Diagnostics, Indianapolis, IN). The viruses were harvested and filtered with low-protein binding filters (Millex-HV, 0.45-mm polyvinylidene difluoride; Millipore Corp, Billerica, MA). The cells were infected at multiplicity of infection (MOI) = 10 by incubation with respective viruses. The virus-infected cells were selected by puromycin (1 μg/ml).

Adenoviruses and Infection

Adenoviruses encoding PTEN or control green fluorescent protein (GFP) were constructed in our laboratory according to the method described by Vogelstein and colleagues. Adenoviruses encoding the mut-p53 (R273H) were a kind gift by Dr Sumitra Deb (Virginia Commonwealth University, Richmond, VA). Adenoviruses encoding the R175H mut-p53 were from Vector Biolabs (Philadelphia, PA). Cells were infected with the relevant adenoviruses (MOI = 10). PTEN and mut-p53 expressions were verified by immunoblot analysis for all experiments.

Cell Proliferation Assay

Cell proliferation was assessed with an 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium (MTS) assay and by cell counting. The CellTiter96 AQ nonradioactive cell proliferation kit (Promega Corp, Madison, WI) was used according to the manufacturer's instructions. The percentages of surviving cells from each group relative to controls were determined by reduction/increase of MTS. Cells were also counted with a hemocytometer for 5 days after respective treatments and growth curves were established.

Apoptosis Assay

Apoptosis was assessed by measuring cytoplasmic histone-associated DNA fragmentation (mononucleosomes and oligonucleosomes) using an ELISA kit (Roche Diagnostics). The photometric enzyme immunoassay was performed according to the manufacturer's instructions.

Cell Invasion Assays

Cells were placed in the upper chamber of a collagen IV–coated membrane. Ten percent FBS media were placed in the lower chamber. After incubation overnight, cells on the upper membrane surface were mechanically removed. Cells that had migrated to the lower side of the membrane were fixed, stained, and counted under a microscope in three randomly chosen fields and photographs were taken.

Colony Formation Assay

Cells were seeded in triplicates in media containing 10% FBS. After 3 weeks, the cell clones were fixed and stained with a solution containing 0.5% crystal violet and 25% methanol. Colony numbers were counted by a gel documentation system (EAGLE EYETM II; Stratagene, La Jolla, CA).

Immunoblot Analysis

Protein expression levels were determined by immunoblot analysis. Briefly, cells were lysed, and protein concentration was determined by the Coomassie Plus protein assay reagent (Pierce Chemical Co, Rockford, IL). Equal amounts of cell lysates were subjected to immunoblot analysis with specific antibodies as previously described [10]. Selected blots were subjected to densitometry on film, and signal intensities were quantified.

Immunoprecipitation

IP assays were performed as previously described [10]. Briefly, equal amounts of cell or tissue lysates were incubated with primary antibody followed by incubation with protein A agarose (Roche Diagnostics). The immunoprecipitates were boiled in sodium dodecyl sulfate (SDS) sample buffer, resolved by SDS-PAGE, transferred to nitrocellulose (Bio-Rad, Hercules, CA), and probed with respective primary antibodies. After the blots were incubated with HRP-labeled secondary antibody (Jackson ImmunoResearch, West Grove, PA), the signals were detected using the enhanced chemiluminescence reagents (Amersham Life Science, Arlington Heights, IL).

Immunodepletion

Protein samples were extracted from cells or human glioblastoma tissues. The extracts were immunodepleted with anti-p53/anti-NFYA antibodies. Thereafter, supernatants were immunoprecipitated with anti-NFYA/anti-p53 antibody. The immunoprecipitates were then subjected to immunoblot analysis with anti-CBP antibody.

Detection of Protein Complex with Native PAGE Analysis

U373 cells were infected with adenovirus encoding PTEN (Ad-PTEN) for 2 to 3 days. After collection and lysis, equal amounts of cell lysates were prepared in sample buffer without SDS and resolved by a native PAGE in Tris-glycine running buffer without SDS. After transferring the proteins from the native PAGE to nitrocellulose membranes, the membranes were probed with primary antibody followed by Western blot analysis.

Chromatin IP

Chromatin IP (ChIP) assays were performed using the modified enzymatic express ChIP kit (Active Motif, Carlsbad, CA). Briefly, DNA/protein complexes were cross-linked, lysed, and vortexed and ground to help the release and break the DNA. The DNA in the sample was then sheared at 37°C with the enzyme mix provided by the kit. The lysates were centrifuged, and soluble chromatin was

immunoprecipitated with the indicated antibody. The protein-DNA complexes were collected after incubating with protein A or G agarose beads and reverse cross-linked by heating at 65°C overnight. DNA released from the complexes was purified using the QIAQuick spin kit (Qiagen Inc, Valencia, CA). The purified DNA samples from the input, immunoprecipitates without antibody (mock), or immunoprecipitates with anti-RNA polymerase II antibody (positive control) or specific IgG (experimental) were subjected to conventional polymerase chain reaction analyses with specific primers for the promoter regions of c-Myc (forward primer: 5′-AGGCGCGCGTAGTTAATTCA-3′ and reverse primer: 5′-TCGCATTATAAAGGGCCGGT-3′) or Bcl-XL (forward primer: 5′-CGATGGAGGAGGAAGCAAGC-3′ and reverse primer: 5′-GCACCACCTACATTCAAATCC-3′). DNA extracted from soluble chromatin was used as input control for each reaction. Each experiment was repeated at least three times with multiple samples.

Immunohistochemistry and Immunofluorescence

PTEN protein levels in human glioblastoma tissues were determined by immunohistochemistry. The localization of mut-p53, CBP, or NFYA was determined by immunofluorescence. The cells were plated on coverslips, fixed, and permeabilized. The cells were then incubated with relevant antibodies followed by incubation with fluorescein isothiocyanate– or Texas Red–conjugated secondary antibodies. Similar procedures were also performed with a second primary antibody and a differently conjugated secondary antibody.

Sequencing of p53 Exons

p53 mutations were analyzed by direct DNA sequencing. DNA was extracted from cells or frozen tumor samples. All exons of p53 were amplified by polymerase chain reaction with frequently used and published specific primers for each exon as recommended by the p53 database of the International Agency for Research on Cancer (http://www-p53.iarc.fr/index.thml). Similarly processed samples from normal brain or normal astrocytes were used as controls. The resulting sequences were compared to wt-p53 sequences and mutations were identified.

Statistics

When appropriate, two group comparisons were analyzed with a t test and P values were calculated. The log rank test was used to analyze correlations between the combined PTEN/p53 mutational status and patient survival. $P < .05$ was considered significant.

Results

PTEN Exerts Oncogenic Effects that Are Mediated by Gain-of-Function Mut-p53

We previously observed that PTEN has oncogenic properties in mut-p53 glioblastoma cells. To verify and expand this observation and determine if the oncogenic effects of PTEN are mediated by mut-p53, we tested the effects of PTEN on cell growth and survival in the settings of expressed or inhibited mut-p53. We restored PTEN to PTEN-null/mut-p53 glioblastoma cells U373 and SNB19 with Ad-PTEN and inhibited gain-of-function mut-p53 (R273H) with specific shRNAs and studied the effects of these manipulations on cell proliferation using an MTS assay and cell counting and on apoptosis by measuring DNA fragmentation with an ELISA. PTEN restoration increased the growth of U373 and SNB19 cells. Knockdown

of mut-p53 inhibited cell growth, consistent with the gain-of-function characteristics of this mutant. The induction of cell growth by PTEN was reversed when mut-p53 was inhibited (Figures 1*A* and W1). PTEN restoration also inhibited apoptosis in U373 and SNB19 cells. Knockdown of mut-p53 increased apoptosis in the cells, consistent with the gain-of-function characteristics of the mutant. The inhibition of apoptosis by PTEN was reversed when mut-p53 was inhibited (Figure 1*B*). PTEN reconstitution and mut-p53 knockdown in the cells were confirmed by immunoblot analysis (Figure 1*C*).

We also investigated the effects of PTEN on clonogenicity and the involvement of mut-p53 in mediating these effects using a colony formation assay. PTEN restoration increased the clonogenicity of mut-p53 cells. Mut-p53 knockdown significantly suppressed the clonogenicity of the cells. Mut-p53 knockdown not only inhibited but also reversed the clonogenic effects of PTEN (Figure 2). One of the most detrimental hallmarks of malignant glioblastoma is invasiveness, which is considered a major cause of tumor recurrence and patient lethality [26]. We tested the effects of PTEN and mut-p53 on cell invasion

using a transwell invasion assay. PTEN restoration increased and mut-p53 knockdown inhibited the invasive ability of U373 and SNB19 cells. The effects of PTEN on invasion were reversed after knockdown of mut-p53 expression (Figure 1*D*).

Additionally, knockdown of PTEN or mut-p53 suppressed cell growth and invasion and induced apoptosis in wt-PTEN/mut-p53 GBM6 primary glioblastoma cells (Figure 3). To exclude off-target effects of shRNA, we performed similar experiments to all of the above with a second shRNA for mut-p53 and obtained consistent results (Figure W2). In addition, to determine if restored PTEN is expressed in the cytoplasm and/or nucleus, we assessed the presence of PTEN protein in cytoplasmic and nuclear fractions of U373 and SNB19 cells after infection with Ad-PTEN. PTEN was protein found mainly in the cytoplasm but was also detected in the nucleus (Figure W3).

Altogether, the above data demonstrate that PTEN exerts tumor oncogenic effects that are mediated by gain-of-function mut-p53. We next investigated the mechanisms underlying this intriguing phenomenon.
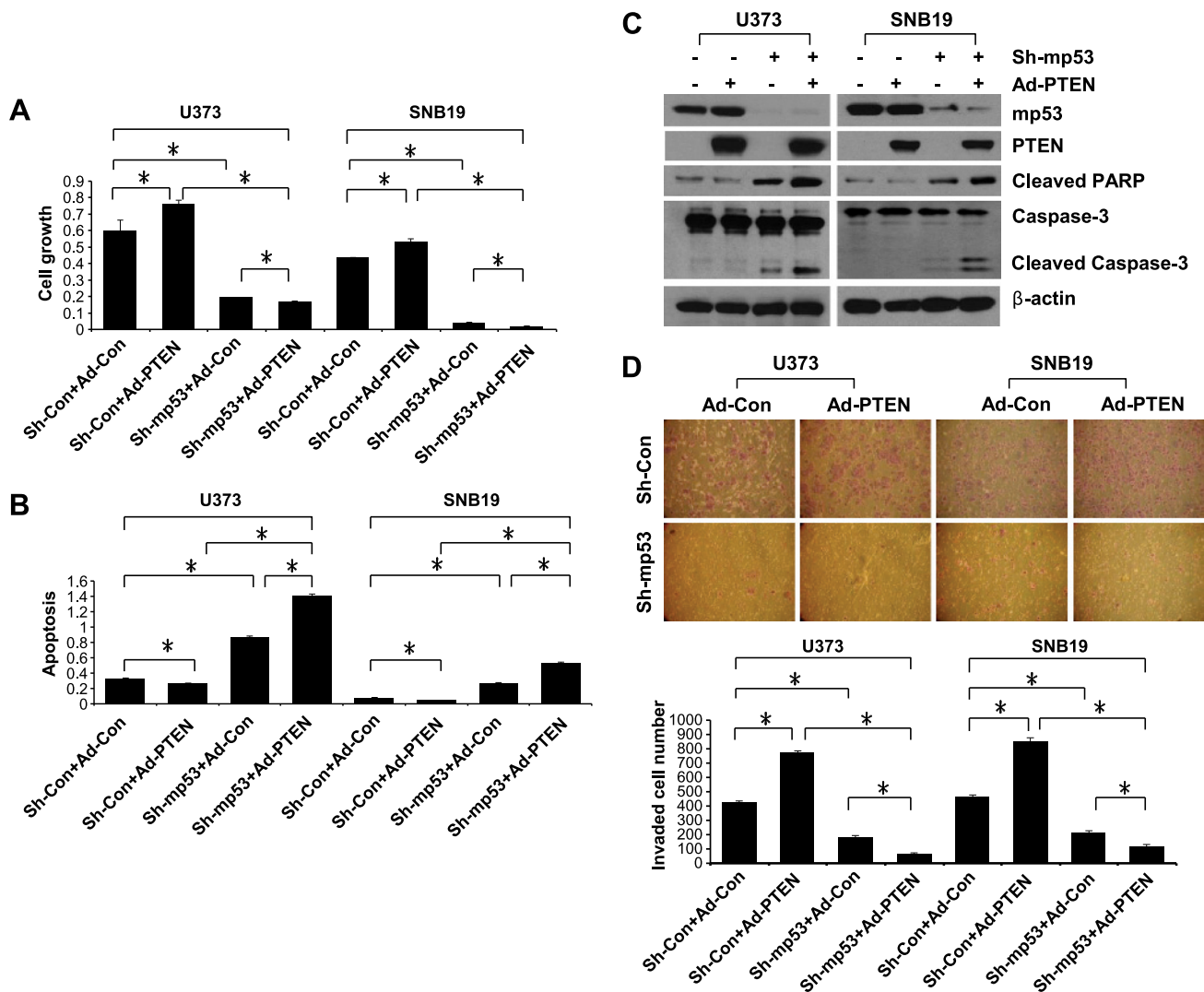
Figure 1. PTEN exerts oncogenic effects that are mediated by gain-of-function mut-p53. (A) MTS assay of PTEN-null/mut-p53 U373 and SNB19 glioblastoma cells with or without PTEN restoration with Ad-PTEN and/or with or without mut-p53 silencing with shRNA (sh-mp53). (B) Apoptosis assay of U373 and SNB19 cells with or without Ad-PTEN and/or with or without sh-mp53. (C) Immunoblot showing the levels of mut-p53 silencing and PTEN restoration and their effects on apoptotic regulators PARP and caspase-3 in glioblastoma cells. (D) Transwell invasion assay of U373 and SNB19 cells with or without Ad-PTEN and/or with or without sh-mp53. Con, control; *$P < .05$.
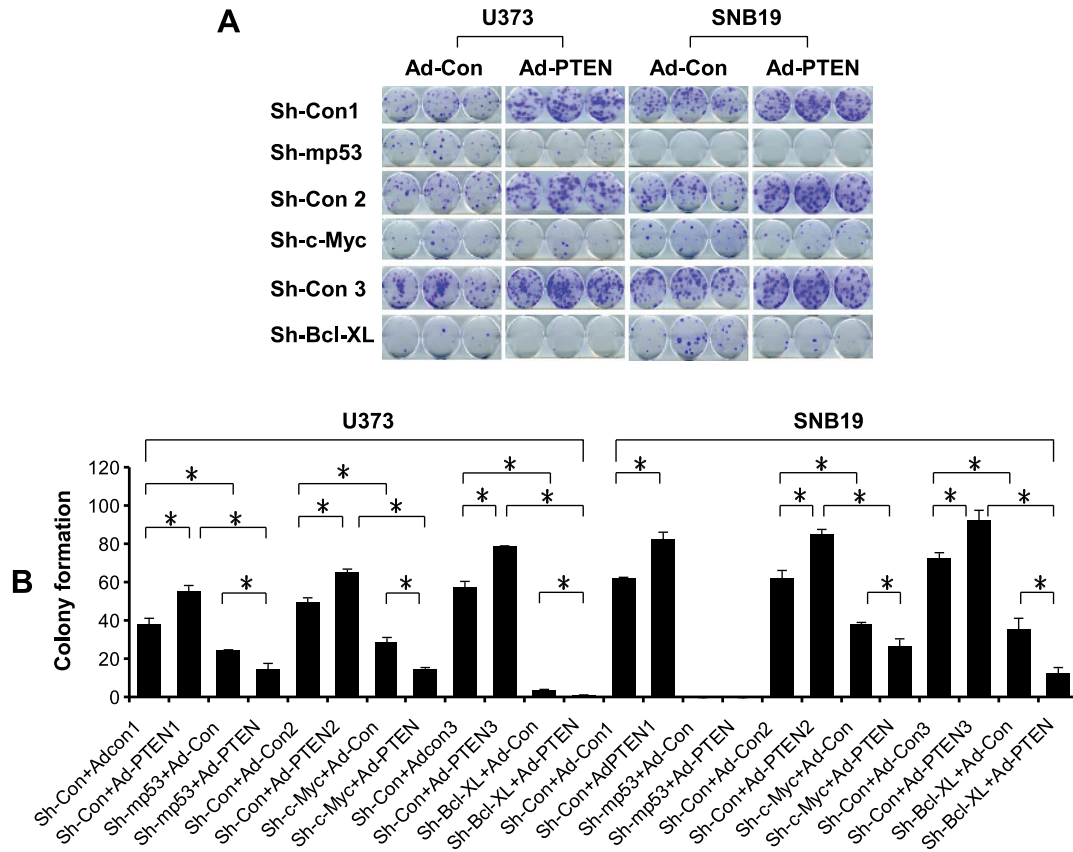
Figure 2. PTEN induces clonogenicity of glioblastoma cells through mut-p53/c-Myc/Bcl-XL. Colony formation assay of PTEN-null/mut-p53 U373 and SNB19 glioblastoma cells with or without PTEN restoration with Ad-PTEN and/or with or without mut-p53/c-Myc/Bcl-XL silencing with shRNA (sh-mp53/sh-c-Myc/sh-Bcl-XL). (A) Colony formation assay. (B) Quantification of A.

PTEN Induces the Expression of Gain-of-Function Mut-p53 Target Genes in Glioblastoma Cells

The best studied mechanism of action of gain-of-function mut-p53 is the transcriptional regulation of genes other than those regulated by wt-p53. We therefore hypothesized that PTEN might acquire tumor-promoting effects by enhancing the transcriptional activities of mut-p53. To test this hypothesis, we investigated the effects of PTEN on mut-p53 target genes *c-Myc*, *Bcl-XL*, *p27*, *E2F1*, *Id2*, *NFκB*, *cdc34*,
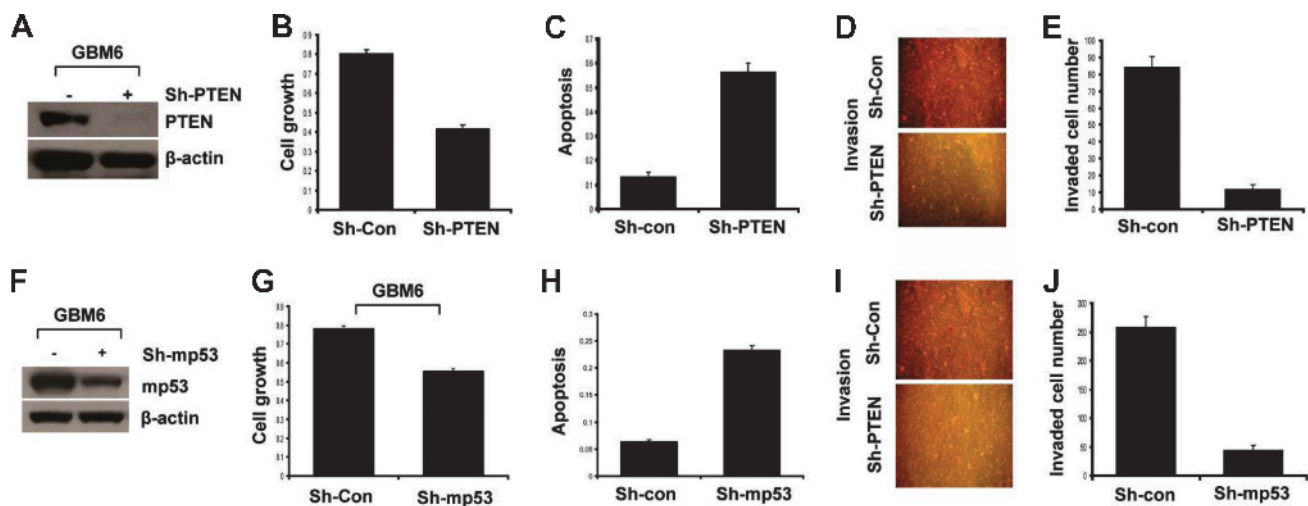
Figure 3. PTEN and mut-p53 silencing inhibits malignancy in primary glioblastoma GBM6 cells. (A) Immunoblot showing PTEN silencing with shRNA (sh-PTEN) in wt-PTEN/mut-p53 GBM6 cells. (B) Proliferation assay of GBM6 cells with or without sh-PTEN. (C) Apoptosis assay of GBM6 cells with or without sh-PTEN. (D) Phenotypic changes of GBM6 cells with or without sh-PTEN. (E) Transwell invasion assay of GBM6 cells with or without sh-PTEN. (F) Immunoblot showing mut-p53 silencing with shRNA (sh-mp53) in wt-PTEN/mut-p53 GBM6 cells. (G) Proliferation assay of GBM6 cells with or without sh-mp53. (H) Apoptosis assay of GBM6 cells with or without sh-mp53. (I) Phenotypic changes of GBM6 cells with or without sh-mp53. (J) Transwell invasion assay of GBM6 cells with or without sh-mp53.

JNK, *Rab27a*, and *TGFIIRβ*, which are also known to play a role in GBM malignancy. We restored PTEN to U373 and SNB19 by infection with Ad-PTEN and assessed the cells for target protein expression with immunoblot analysis. PTEN restoration did not affect the expression of putative mut-p53 target genes *Id2*, *NFκB*, *cdc34*, *JNK*, *Rab27a*, and *TGFIIRβ* (data not shown). However, PTEN restoration did increase the expression of c-Myc by 4.2- and 1.6-fold in SNB19 and U373, respectively. Similarly, PTEN restoration increased the expression of Bcl-XL by 3.7- and 1.8-fold in SNB19 and U373, respectively (Figure 4*A*). Conversely, PTEN knockdown with specific shRNA reduced the expressions of c-Myc by 96% and Bcl-XL by 60% in GBM6 cells (Figure 4*B*). To determine if mut-p53 mediates the induction of c-Myc and Bcl-XL by PTEN, we tested the effects of PTEN restoration on c-Myc and Bcl-XL with or without mut-p53 knockdown with shRNA. Mut-p53 knockdown decreased the expressions of Bcl-XL and c-Myc in SNB19 cells but not in U373 cells. PTEN restoration increased the expressions of both proteins in the setting of expressed mut-p53 but not when mut-p53 was silenced with shRNA (Figure 4*C*). Mut-p53 knockdown did not influence p-Akt level in U373 and SNB19 cells. PTEN reconstitution reduced p-Akt in both cells (Figure 4*C*). Mut-p53 knockdown and PTEN restoration were verified by immunoblot analysis. Altogether, the above data show that PTEN induces c-Myc and Bcl-XL in a mut-p53–dependent manner.

To further confirm these data, we used the PTEN-null/p53-null GBM cell line LNZ308. We restored PTEN and mut-p53 expression to the cells by infection with Ad-PTEN and/or Ad-mut-p53 (R273H). Immunoblot analysis confirmed the expressions of PTEN and mut-p53 in the cells. Restoration of PTEN or mut-p53 alone did not signifi-cantly alter the expression levels of Bcl-XL and c-Myc in the cells. However, combined PTEN and mut-p53 expressions significantly increased the levels of c-Myc and Bcl-XL (Figure 4*D*). To determine if the effects of PTEN on c-Myc and Bcl-XL are specifically mediated by the R273H mutant, we also conducted similar experiments to the above using the R175H mutant. Neither PTEN nor R175H mut-p53 affected c-Myc or BcL-XL expressions, suggesting that specific p53 mutations are required for PTEN to exert its tumor-promoting effects (Figure W4).

Altogether, the data presented in this section demonstrate that PTEN enhances the induction by gain-of-function mut-p53 of the levels of the oncogenes *c-Myc* and *Bcl-XL* in glioblastoma.

c-Myc Partially Mediates the Oncogenic Effects of PTEN in Mut-p53 Glioblastoma Cells

We investigated the possible involvement of mut-p53–induced c-Myc in mediating the oncogenic effects of PTEN. We studied the effects of PTEN on cell proliferation, apoptosis, and invasion of U373 and SNB19 cells in the settings of inhibited or expressed c-Myc. PTEN was restored to the cells by Ad-PTEN infection and c-Myc expression was silenced with specific shRNA, before evaluation of cell proliferation, apoptosis, and clonogenicity. PTEN restoration enhanced the growth of U373 and SNB19 cells. c-Myc knockdown inhibited growth of the cells. Particularly, c-Myc knockdown not only inhibited but also reversed PTEN-induced cell growth (Figure 5*A*). PTEN restoration suppressed apoptosis in U373 and SNB19 cells. c-Myc knockdown increased apoptosis, reduced PTEN-induced suppression of apoptosis in U373 cells, and reversed the effects of PTEN on SNB19 cells (Figure 5*B*). We also assessed the effects of PTEN restoration and
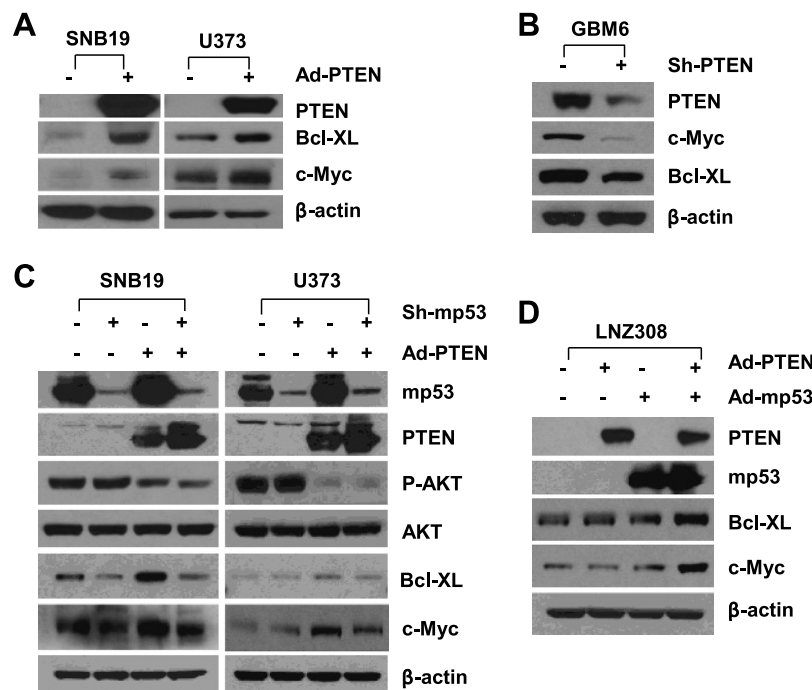
Figure 4. PTEN induces the expressions of mut-p53 target genes *c-Myc* and *Bcl-XL* in cancer cells. (A) Immunoblots showing the expressions of Bcl-XL and c-Myc in U373 and SNB19 cells with or without PTEN restoration with Ad-PTEN. (B) Immunoblots showing the expressions of c-Myc and Bcl-XL in wt-PTEN/mut-p53 GBM6 primary glioblastoma cells with or without Ad-PTEN. (C) Immunoblots showing the expressions of Bcl-XL and c-Myc in U373 and SNB19 cells with or without Ad-PTEN and/or with or without mut-p53 silencing with shRNA (sh-mp53). (D) Immunoblots showing the expressions of Bcl-XL and c-Myc in PTEN-null/p53-null LNZ308 cells with or without Ad-PTEN and/or with or without mut-p53 restoration with adenovirus-encoding mut-p53 (Ad-mp53).
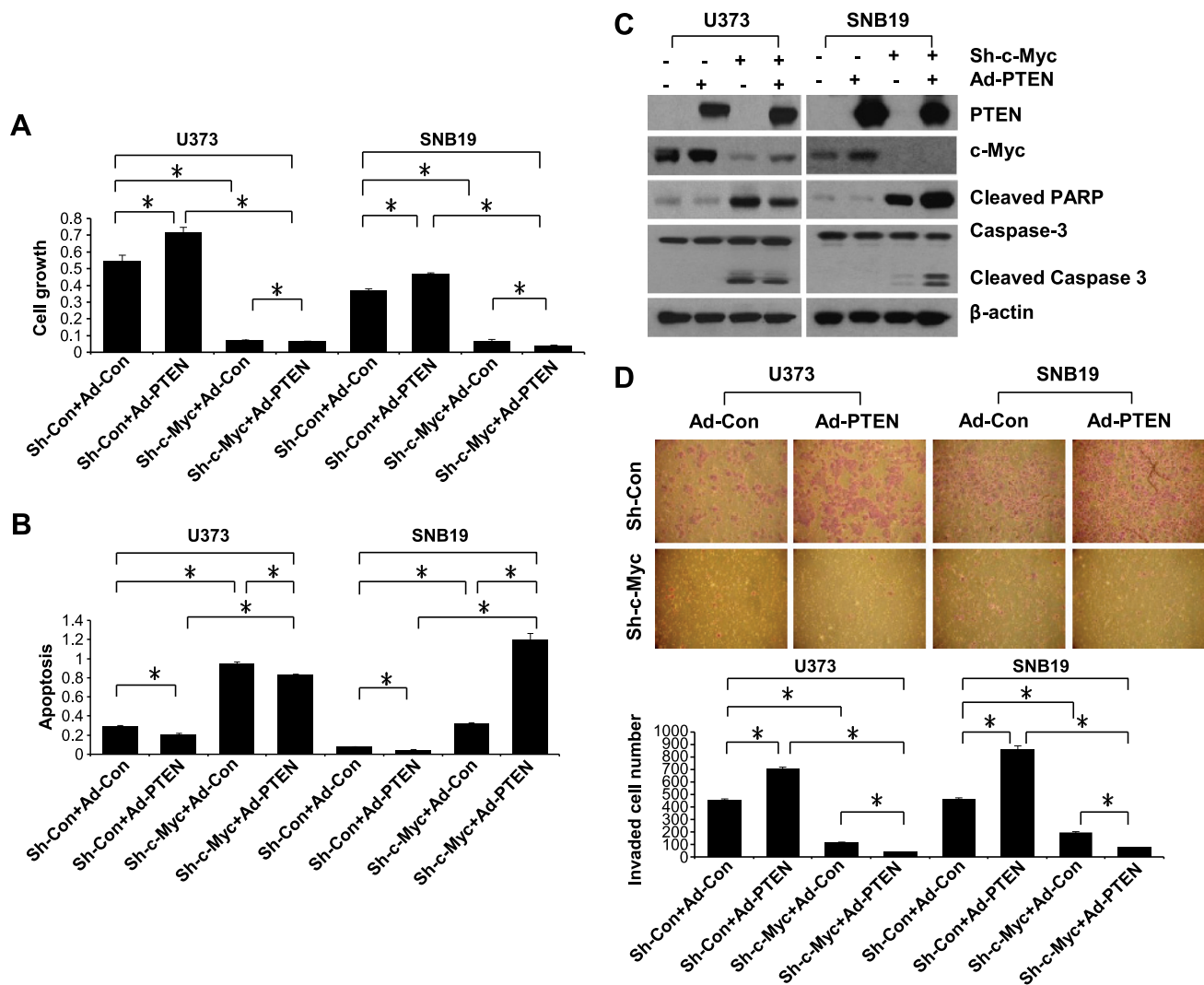
Figure 5. c-Myc partially mediates the oncogenic effects of PTEN in mut-p53 glioblastoma cells. (A) Proliferation assay of U373 and SNB19 cells with or without PTEN restoration with Ad-PTEN and/or with or without c-Myc silencing with shRNA (sh-c-Myc). (B) Apoptosis assay of U373 and SNB19 cells with or without Ad-PTEN and/or with or without sh-c-Myc. (C) Immunoblot showing the levels of c-Myc silencing and PTEN restoration and their effects on apoptotic regulators PARP and caspase-3 in glioblastoma cells. (D) Transwell invasion assay of U373 and SNB19 cells with or without Ad-PTEN and/or with or without sh-c-Myc. Con, control; *$P < .05$.

c-Myc inhibition on apoptosis by immunoblot analysis for PARP and caspase-3. PTEN restoration increased the expression of c-Myc and inhibited PARP cleavage in U373 and SNB19 cells. c-Myc knockdown induced PARP cleavage and caspase-3 activation and counteracted PTEN-induced inhibition of PARP cleavage in the cells. PTEN restoration further enhanced c-Myc knockdown-induced PARP cleavage and caspase-3 activation in SNB19 cells but not in U373 cells (Figure 5C). PTEN reconstitution increased the clonogenic ability of U373 and SNB19 cells. c-Myc knockdown suppressed the clonogenicity of the cells. c-Myc knockdown reversed the effects of PTEN on clonogenicity (Figure 2). PTEN restoration increased transwell invasion of the above mut-p53 cells. c-Myc knockdown inhibited the transwell invasion of the cells. c-Myc knockdown reversed the invasion-promoting effects of PTEN in the cells (Figure 5D). PTEN restoration and c-Myc silencing were verified by immunoblot analysis for all experiments. In addition, similar results to the above were obtained with a second shRNA for c-Myc to exclude off-target effects of the shRNAs (Figure W2).

Altogether, the above data demonstrate that c-Myc partially mediates the oncogenic effects of PTEN in mut-p53 cells.

Bcl-XL Partially Mediates the Oncogenic Function of PTEN in Mut-p53 Glioblastoma Cells

We also investigated the potential role of mut-p53–induced Bcl-XL in mediating the oncogenic effects of PTEN in glioblastoma cells. We studied the effects of PTEN on cell proliferation, apoptosis, invasion, and colony formation of U373 and SNB19 cells in the setting of inhibited or expressed Bcl-XL. PTEN was reconstituted to the cells by Ad-PTEN infection and Bcl-XL expression was silenced with specific shRNA. Bcl-XL knockdown not only inhibited but also reversed PTEN-induced cell proliferation (Figure 6A). PTEN restoration inhibited and Bcl-XL knockdown increased apoptosis in the mut-p53 cells. Bcl-XL knockdown not only inhibited but also reversed PTEN-induced inhibition of apoptosis (Figure 6B). We also evaluated the effects of PTEN reconstitution and Bcl-XL inhibition on apoptosis by immunoblot analysis for PARP and caspase-3. Restoration of

PTEN increased expression of Bcl-XL in both U373 and SNB19 cells. PTEN reconstitution inhibited PARP cleavage and Bcl-XL knockdown enhanced PARP cleavage and caspase-3 activation in the cells. PTEN restoration further increased Bcl-XL knockdown-induced PARP cleavage and caspase-3 activation in the cells (Figure 6C). PTEN restoration enhanced and Bcl-XL knockdown inhibited clonogenicity of the cells. Bcl-XL knockdown reversed the PTEN effects on clonogenicity (Figure 2). Bcl-XL knockdown did not affect the invasive ability of the cells. However, Bcl-XL knockdown did inhibit PTEN-induced invasion but to a lesser extent than that observed in c-Myc knockdown experiments (Figure 6D). PTEN restoration and Bcl-XL silencing were verified by immunoblot analysis for all experiments. In addition, similar results to above data were obtained with another shRNA for Bcl-XL to exclude off-target effects of the shRNAs (Figure W2).

Altogether, the above results show that Bcl-XL partially mediates specific oncogenic effects of PTEN in mut-p53 glioblastoma cells.

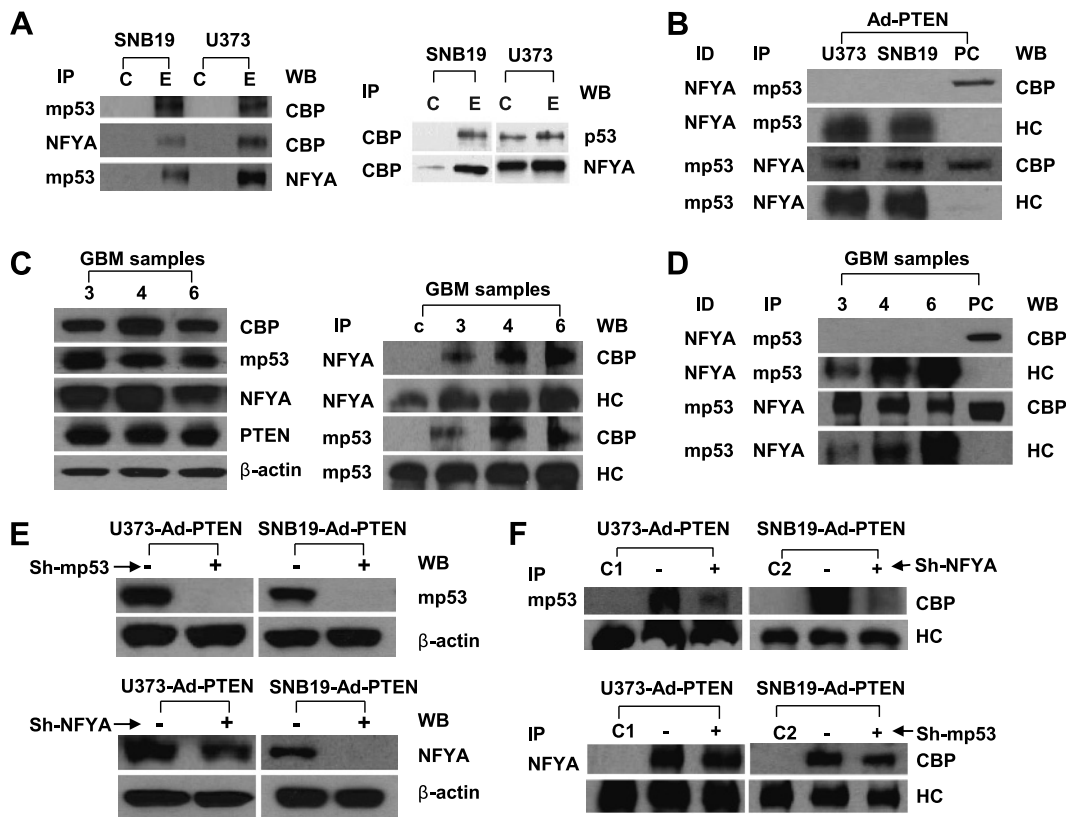## There Exists a Mut-p53/CBP/NFYA Complex in Human Glioblastoma Cells and Tumors

We next investigated the mechanism underlying the PTEN-induced increase in c-Myc and Bcl-XL in mut-p53 glioblastoma cells. NFY is a heterotrimeric transcription factor that is present in 30% of eukaryotic promoters. It consists of three subunits, NFYA, -B, and -C [27]. NFY can interact with both wt-p53 and mut-p53. CBP plays an important role in increasing histone acetylation and transcription of target genes [18,21]. To determine if a mut-p53/CBP/NFY complex exists in mut-p53 glioblastoma cells, we performed IP experiments with the protein extracts from U373 and SNB19 cells. The results identified complexes containing at least two of the three proteins (Figure 7A). Immunostaining experiments further confirmed these results (Figure W5).

These above data suggest two possibilities for associations of the three proteins: 1) all three proteins interact to form one single protein complex, and 2) three possible heterodimers consisting of mut-p53/
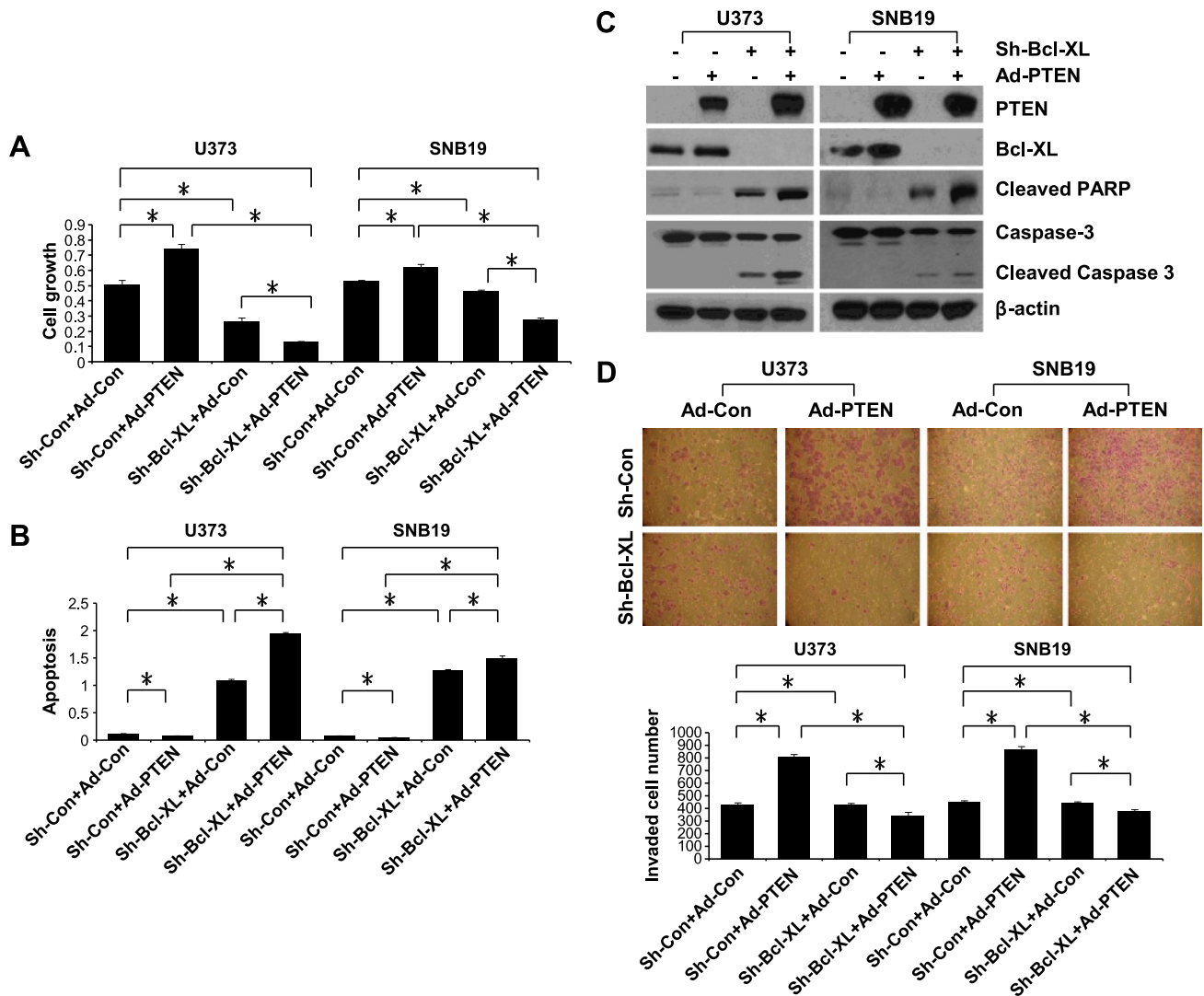
Figure 6. Bcl-XL partially mediates the oncogenic effects of PTEN in mut-p53 glioblastoma cells. (A) Proliferation assay of U373 and SNB19 cells with or without PTEN restoration with Ad-PTEN and/or with or without Bcl-XL silencing with shRNA (sh-Bcl-XL). (B) Apoptosis assay of U373 and SNB19 cells with or without Ad-PTEN and/or with or without sh-Bcl-XL. (C) Immunoblot showing the levels of Bcl-XL silencing and PTEN restoration and their effects on apoptotic regulators PARP and caspase-3 in glioblastoma cells. (D) Transwell invasion assay of U373 and SNB19 cells with or without Ad-PTEN and/or with or without sh-Bcl-XL. Con, control; $*P < .05$.

Figure 7. There exists a mut-p53/CBP/NFYA complex in glioblastoma cells. (A) IP experiments showing the interaction between two of the three proteins, mut-p53, CBP, and NFYA, in U373 and SNB19 cells. C, control samples immunoprecipitated with control IgG; E, experimental samples immunoprecipitated with relevant antibody; WB, immunoblot. (B) ID experiments to define the relationship between the three proteins mut-p53, CBP, and NFYA in U373 and SNB19 cells. HC, heavy chain; PC, positive control. (C and D) IP and ID experiments showing the relationship between the three proteins mut-p53, CBP, and NFYA in human glioblastoma (GBM) tissue samples. C, control samples immunoprecipitated with control IgG; 3, 4, 6 are distinct GBM samples. (E) Immunoblots (WB) showing the expressions of mut-p53 or NFYA in glioblastoma cells with or without mut-p53 and NFYA silencing with specific shRNA, sh-mp53, or sh-NFYA. (F) IP experiments showing the changes in the interaction between mut-p53/NFYA and CBP with or without NFYA/mut-p53 silencing with sh-NFYA or sh-mp53. C1, C2, control samples immunoprecipitated with control IgG.

CBP, NFYA/CBP, and mut-p53/NFYA separately coexist in mut-p53 glioblastoma cells. To distinguish between these possibilities, total cell lysates (supernatants) from U373 and SNB19 cells were immuno-depleted with an anti-p53 antibody and immunoprecipitated with an anti-NFYA antibody followed by immunoblot analysis with an anti-CBP antibody. The interaction between NFYA and CBP still existed, suggesting that the existence of mut-p53/CBP/NFYA complex could not be confirmed. However, when the protein extracts were immuno-depleted with anti-NFYA antibody followed by IP with an anti-p53 antibody and immunoblotted for CBP, the interaction between mut-p53 and CBP completely disappeared, suggesting that the mut-p53/CBP/NFYA complex exists in mut-p53 glioblastoma cells. Together, the above data indicate the existence of a mut-p53/CBP/NFYA complex but do not exclude the existence of free NFYA and/or CBP/NFYA heterodimers outside the protein complex (Figure 7B).

To determine if the mut-p53/CBP/NFYA complex also exists in human tumor tissues, we extracted protein from three human glioblastoma tumor specimens. Immunoblot analysis showed the expression of CBP, mut-p53, and NFYA in all three samples. All three samples were determined to harbor mut-p53 based on the high expression of p53 confirmed by immunoblot analysis (Figure 6C) and immunohistochemistry (not shown), as mut-p53 is overexpressed in

tumor cells [15], due to mut-p53 inability to effectively activate MDM2 [28]. IP experiments confirmed the co-existence of mut-p53, NFYA, and CBP in human glioblastoma tissues (Figure 7C). ID experiments in mut-p53 glioblastoma tissues showed similar results to those obtained from glioblastoma cells, demonstrating that the mut-p53/CBP/NFYA complex also exists in glioblastoma tissues (Figure 7D).

To further explore the relationship between the different proteins in the mut-p53/CBP/NFYA complex, we disrupted the complex by knockdown of either mut-p53 or NFYA in U373 and SNB19 cells before PTEN restoration. The results revealed that mut-p53 knock-down slightly reduced the interaction between NFYA and CBP, whereas NFYA knockdown strongly reduced the interaction between mut-p53 and CBP (Figure 7, E and F). These results further support the existence of the complex in mut-p53 cells.

Altogether, the above data show that mut-p53/CBP/NFYA exists as a multiprotein complex in glioblastoma cells and tissues.
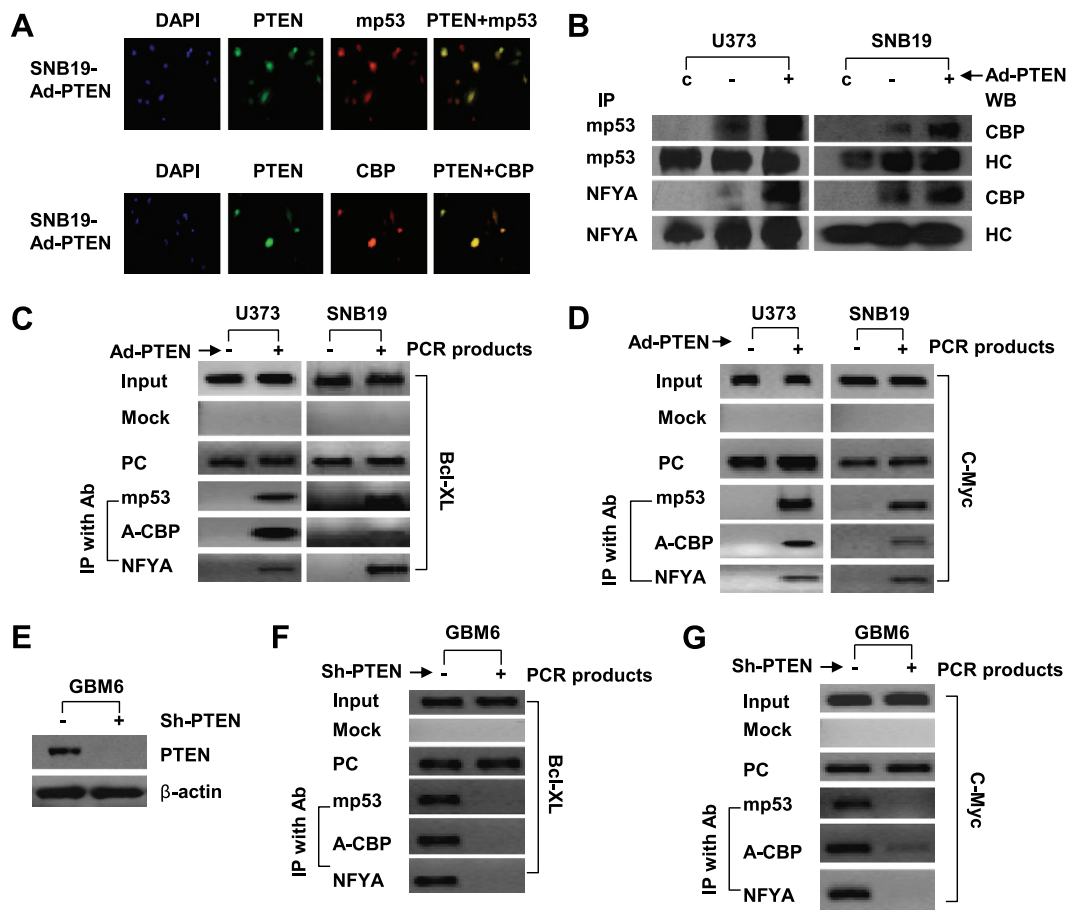
PTEN Regulates Mut-p53/CBP/NFYA Binding to the Promoters of the Mut-p53 Target Genes c-Myc and Bcl-XL

Having demonstrated the existence of a mut-p53/CBP/NFYA complex in human glioblastoma cells and tissues, we next investigated the

effects of PTEN on this complex as well as the effects of the complex on the promoters of the mut-p53 target genes *c-Myc* and *Bcl-XL*. Immunofluorescence assays confirmed the interaction between PTEN and mut-p53, which is consistent with our previous report [10]. Interestingly, we found a previously unknown association of PTEN and CBP in SNB19 cells (Figure 8*A*). We then performed IP experiments to determine if PTEN restoration affects the interaction between mut-p53/NFYA and CBP. IP experiments showed that PTEN increased the associations between mut-p53/NFYA and CBP in mut-p53 glioblastoma cells (Figure 8*B*). To further ascertain that PTEN affects the association of the mut-p53/CBP/NFYA protein complex, PTEN was reconstituted to U373 cells by Ad-PTEN infection. We then performed native PAGE that maintains the multiprotein complex intact. Western blot analyses with mut-p53, CBP, and NFYA antibody following native PAGE revealed a major band >300 kDa that was clearly enhanced in response to PTEN restoration (Figure W6). The molecular weight of the protein complex containing mut-p53, CBP, and NFYA is 393 kDa [53 kDa (mut-p53) + 300 kDa (CBP) + 40 kDa (NFYA)]. Consistent with the above, we also demonstrate that free mut-p53, CBP, and NFYA decrease following PTEN restoration (Figure W6). Altogether, these above results suggest that PTEN restoration enhances

the association of the protein complex in mut-p53 glioblastoma cells. Alternatively, increased complex formation could be a resultant of increased mut-p53 expression induced by PTEN.

CBP and p300 are highly conserved and functionally related transcriptional co-activators that associate with transcriptional regulators and signaling molecules, integrating multiple signal transduction pathways with transcriptional machinery [29]. CBP also has histone acetyltransferase activity, allowing it to acetylate histones and other proteins [30]. Acetylation of CBP has been demonstrated to enhance its histone acetyltransferase activity and affect a wide variety of signaling events [31]. Therefore, we chose acetylated CBP instead of CBP itself as target to perform ChIP experiments. ChIP experiments showed that PTEN restoration increased the binding of mut-p53, acetyl-CBP, and NFYA to the promoter region of *Bcl-XL* and *c-Myc* (Figure 8, *C* and *D*). PTEN knockdown suppressed the binding of mut-p53, acetyl-CBP, and NFYA to the promoter region of both genes (Figure 8, *E–G*). Additionally, knockdown of NFYA and mut-p53 to disrupt the protein complex decreased the binding of acetyl-CBP and mut-p53/NFYA to the promoter region of both *c-Myc* and *Bcl-XL*. Both mut-p53 and NFYA knockdown reversed the PTEN tumor-promoting function in mut-p53 glioblastoma cells (Figures 1 and W6). These

Figure 8. PTEN regulates mut-p53/CBP/NFYA binding to mut-p53 target genes *c-Myc* and *Bcl-XL* promoters. (A) Immunofluorescence assays showing the interaction between PTEN and mut-p53/CBP in mut-p53 glioblastoma cells. (B) IP experiments showing the interaction between mut-p53/NFYA and CBP in mut-p53 glioblastoma cells in response to PTEN restoration with Ad-PTEN. C, control samples immunoprecipitated with control IgG. (C and D) ChIP experiments showing the binding of mut-p53, acetyl-CBP, and NFYA to the promoter regions of *Bcl-XL* and *c-Myc* with or without Ad-PTEN. PC, positive control; Ab, antibody. (E) Immunoblot showing PTEN expression in wt-PTEN/mut-p53 GBM6 cells with or without silencing with specific shRNA (sh-PTEN). (F and G) ChIP experiments showing the binding status of mut-p53, acetyl-CBP, and NFYA to the promoter regions of *Bcl-XL* and *c-Myc* in GBM6 cells with or without sh-PTEN.
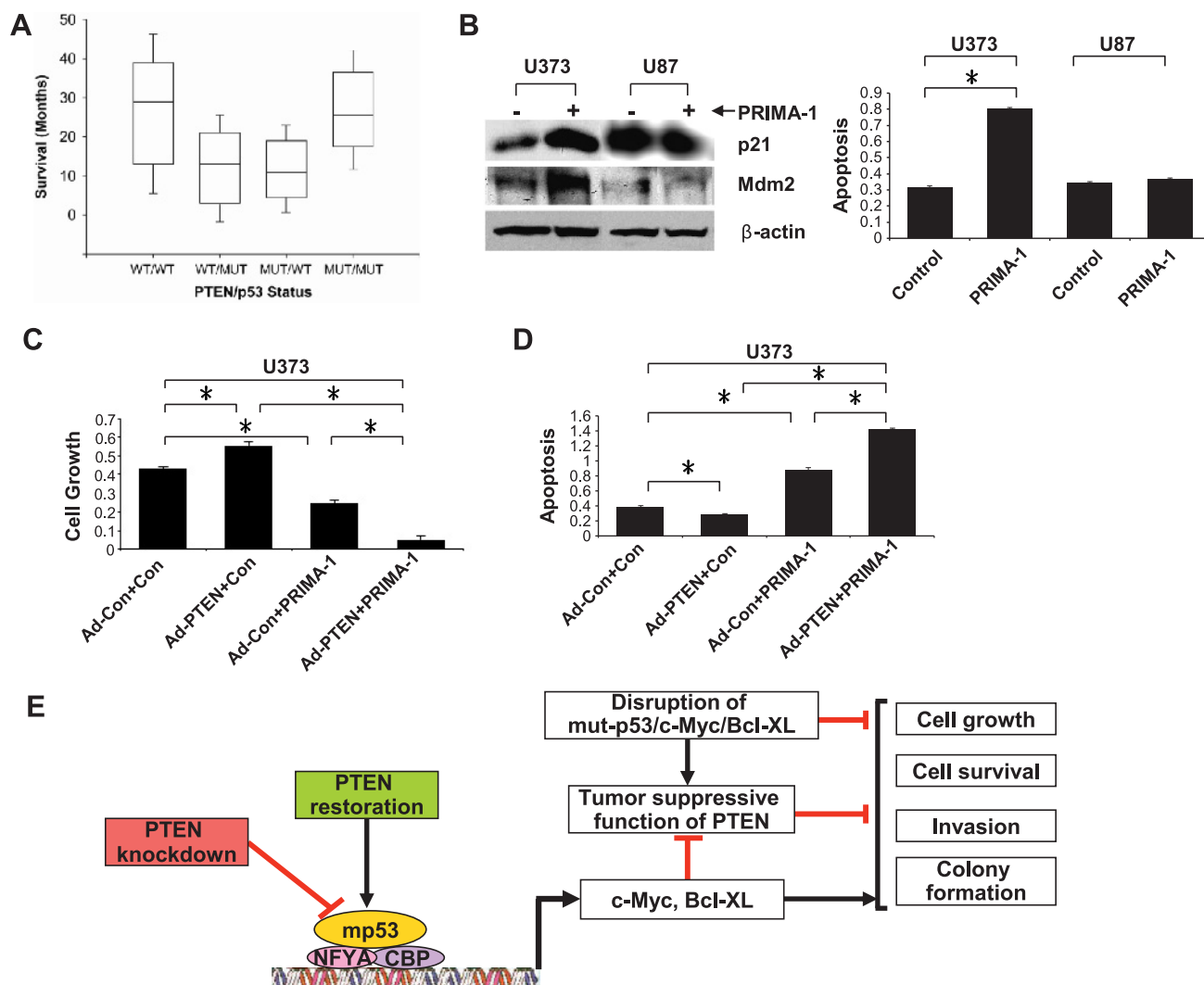
Figure 9. Prognostic and experimental therapeutic implications of the PTEN/mut-p53 oncogenic effects. (A) All p53 exons were sequenced in 38 human glioblastoma samples and PTEN protein expression was determined by immunohistochemistry in the same tumor specimens. The combined PTEN/p53 mutational status was plotted against patient survival. (B) Immunoblot showing the expressions of wt-p53 target genes *p21* and *Mdm2* in mut-p53 U373 and wt-p53 U87 cells in response to PRIMA-1 treatment (left panel). Apoptosis assay of U373 and U87 cells in response to PRIMA-1 treatment (right panel). (C) Proliferation assay of U373 cells with or without PTEN restoration with Ad-PTEN and/or with or without PRIMA-1 treatment. (D) Apoptosis assay of U373 cells with or without PTEN restoration with Ad-PTEN and/or with or without PRIMA-1 treatment. (E) Schematic representation of the mechanisms underlying the dual oncogenic/tumor-suppressive effects of PTEN in mut-p53 cancer cells. PTEN enhances a transcriptional complex containing mut-p53, NFY, and CBP. The mut-p53/CBP/NFY complex binds to the promoter of the oncogenes c-Myc and Bcl-XL and induces their transcription, leading to increased cell proliferation, survival, invasion, and clonogenicity. Disruption of the mut-p53/c-Myc/Bcl-XL axis unmasks the hidden tumor-suppressive effects of PTEN in mut-p53 cancers.

results suggest that PTEN regulates mut-p53/CBP/NFYA complex binding and activation of mut-p53 target gene promoters.

Analysis of the Correlations between PTEN/p53 Mutational Status and Glioblastoma Patient Survival

The above findings show that PTEN exerts oncogenic effects through a novel PTEN/mut-p53/c-Myc/Bcl-XL axis in glioblastoma. An important potential implication from these findings would be that PTEN expression in mut-p53 tumors would negatively affect prognosis and that consequently patients with PTEN-positive/mut-p53 tumors would have a worse clinical outcome than the patients with PTEN-negative/mut-p53 tumors. To verify if this is true, we sequenced

all *p53* exons in 38 human glioblastoma samples and determined PTEN protein expression by immunohistochemistry (because PTEN mutations usually lead to protein truncation and loss of protein expression) in the same tumor specimens. We then analyzed the relationship between the combined *PTEN/p53* mutational status and patient survival. We found that patients with PTEN-positive/mut-p53 tumors exhibited a trend toward lesser survival than the patients with PTEN-negative/mut-p53 tumors (12.2 *vs* 26.5 months; $P = .058$). Interestingly, patients with PTEN-negative/mut-p53 exhibited a comparable survival magnitude to the patients with PTEN-positive/wt-p53 tumors. Furthermore, only four tumors were PTEN-negative/mut-p53 (Figure 9A). These clinical data are consistent with our

findings in cells and further validate the oncogenic effects of PTEN in mut-p53 glioblastoma.

Reactivation of wt-p53 Function with a Small Molecule Modulator Reverses the Oncogenic Effects of PTEN

To determine if our findings might be exploited for future cancer therapies, we used the small molecule modulator of p53, PRIMA-1, to reactivate wt-p53 in mut-p53 cells before testing the effects of PTEN restoration on apoptosis. Previous research identified PRIMA-1 as a small molecule modulator that restores wt-p53 conformation and DNA binding to mut-p53 (R273H and R175H) cells and induces apoptosis preferentially in mut-p53–expressing tumor cells [32]. Mut-p53 U373 and wt-p53 U87 cells were treated with PRIMA-1, and the expressions of the wt-p53 transcriptional targets p21 and Mdm2 were assessed with immunoblot analysis. PRIMA-1 preferentially induced p21 and Mdm2 expressions in U373 cells, indicating that it could reactivate wt-p53 transcriptional activity in mut-p53 cells (Figure 9*B*). We also tested the effects of PRIMA-1 on apoptosis in U373 and U87 cells and found that PRIMA-1 induced greater apoptosis in mut-p53 U373 cells (Figure 9*B*). We then tested the effects of PRIMA-1 on U373 cells with or without PTEN restoration. PTEN restoration enhanced and PRIMA-1 suppressed the growth of U373 cells. PRIMA-1 reversed the effects of PTEN on the growth of U373 cells (Figure 9*C*). PTEN restoration inhibited and PRIMA-1 induced apoptosis in U373 cells. PRIMA-1 reversed the effects of PTEN on apoptosis in U373 cells (Figure 9*D*). These results suggest that PRIMA-1 and other p53 modulators exert greater antitumor effects in PTEN-expressing cancer cells and that combining PTEN restoration with PRIMA-1 in mut-p53 tumors might have therapeutic value.

Discussion

In this study, we show that PTEN has mut-p53–dependent oncogenic effects. We demonstrate, for the first time, that PTEN exerts these effects by increasing the levels of a transcriptional complex containing mut-p53, CBP, and NFYA, which activates the oncogenes *c-Myc* and *Bcl-XL*. We also describe an association between the combined *PTEN/p53* mutational status and patient survival and determine the effects of PTEN expression on the experimental therapeutic restoration of p53 function.

There is accumulating evidence that several hotspot p53 mutants not only lose the tumor-suppressive functions of wt-p53 but also acquire new pro-oncogenic properties leading to the concept of "gain-of-function" mut-p53 [15,17]. Many gain-of-function phenotypes of mut-p53 have been reported, including increased invasion, metastasis, and genomic instability, resistance to chemotherapy, and regulation of pro-inflammatory and anti-apoptotic pathways [16,20], all of which give mut-p53–expressing cells a selective growth and survival advantage. Recent research has uncovered a multilevel and complex cooperation between wt-PTEN and wt-p53 [23–25,33]. However, almost nothing is known about the interactions between wt-PTEN and mut-p53. Our study provides novel and detailed insights into these interactions and their implications for cancer.

We show that PTEN increases the levels of gain-of-function mut-p53 and the proliferation, survival, clonogenicity, and invasion of glioblastoma cells in a mut-p53–dependent manner. Mut-p53 knockdown not only inhibited but also reversed the oncogenic effects

of PTEN, suggesting that knockdown of mut-p53 unmasks the hidden tumor-suppressive forces of PTEN, supporting our novel idea of dual context-dependent effects of PTEN in cancer. These above findings led us to explore the underlying mechanisms of the unconventional PTEN oncogenic effects in mut-p53 glioblastoma. We found that PTEN induces c-Myc and Bcl-XL gene expression and malignancy through enhancement of a mut-p53–containing protein complex.

c-Myc is a key regulator of cellular outcomes in normal cells, and its dysregulation is associated with cancer development and progression [34–36]. A key role for c-Myc is to integrate various signals so as to orchestrate an extensive transcription program that regulates cell proliferation, differentiation, and death [37]. We previously showed that PTEN increases the stability and expression of mut-p53 in glioblastoma cells [10]. A previous study found that gain-of-function mut-p53 increased the expression of c-Myc in cancer cells [20]. Although PTEN was reported to inhibit expression of c-Myc in tumor cells [38], we hypothesized that PTEN might increase the expression of c-Myc in mut-p53 cells. Our data confirmed this hypothesis and showed that PTEN can induce c-Myc in a mut-p53–dependent manner. Functionally, c-Myc can elicit epithelial-mesenchymal transition (EMT) and metastasis in carcinomas [39]. We found that c-Myc knockdown reversed the effects of PTEN on the growth, invasion, and clonogenicity of the cells, suggesting that c-Myc knockdown unveils the tumor-suppressive effects of PTEN, supporting our hypothesis for the dual context-dependent effects of PTEN in cancer.

Bcl-XL is a mitochondrial membrane protein that promotes cell survival by regulating the electrical and osmotic homeostasis of mitochondria in response to a variety of stimuli [40]. Inhibition of Bcl-XL induces apoptosis of glioblastoma cells [41]. Gain-of-function mut-p53 was shown to upregulate the expression of Bcl-XL in cancer cells [20]. We therefore hypothesized that PTEN might induce the expression of Bcl-XL in mut-p53 cancer cells. Indeed, we showed for the first time that PTEN enhances the expression of Bcl-XL in a mut-p53–dependent manner. Furthermore, Bcl-XL knockdown suppressed the PTEN effects on cell growth, apoptosis, and colony formation, suggesting that Bcl-XL mediates specific oncogenic effects of PTEN in mut-p53 glioblastoma cells. Interestingly, Bcl-XL knockdown reversed the effects of PTEN on the growth, clonogenicity, and survival in the cells, suggesting that Bcl-XL knockdown unmasks the tumor-suppressive effects of PTEN.

We further investigated the molecular mechanisms of induction of c-Myc and Bcl-XL by PTEN and mut-p53. To the best of our knowledge, we are the first to report the existence of a protein complex mut-p53/CBP/NFYA in glioblastoma cells and human tissues. PTEN interacts with both mut-p53 and CBP and increases the association of the proteins in the complex and the binding of the proteins to the promoter region of the target genes, resulting in enhanced expressions of *c-Myc* and *Bcl-XL*, leading to functional changes including induction of cell growth, survival, colony formation, and invasion. Knockdown of any component of the novel mut-p53/c-Myc/Bcl-XL axis and complex reversed the oncogenic effects of PTEN and the PTEN-induced activation of the c-Myc and Bcl-XL promoters (Figures 8 and 9*E*). We therefore describe a new mechanism through which PTEN promotes oncogenic parameters through novel PTEN/mut-p53/c-Myc/Bcl-XL axis. However, considering the complex roles of p53 mutants, it cannot be excluded that additional mechanisms are involved in mediating the oncogenic effects of PTEN [20].

To determine if the PTEN oncogenic effects in the setting of mut-p53 have potential clinical implications, we assessed the

correlations between the *PTEN/p53* mutational status and survival of glioblastoma patients. On the basis of our findings, one would predict that PTEN expression in tumors harboring gain-of-function p53 mutations would negatively affect clinical outcome. In fact, we found that patients with PTEN-positive/mut-p53 tumors had worse clinical outcome than patients with PTEN-negative/mut-p53 tumors. The differences in survival were barely below statistical significance, likely due to the moderate sample size of 38 tumors. Moreover, we found only four cases of PTEN-negative/mut-p53 tumors, suggesting that the tumors lose selective advantage by losing PTEN protein expression in the setting of gain-of-function mut-p53. These findings are consistent with our *in vitro* data and in line with previous reports that mutations in *TP53* and *PTEN* are mutually exclusive in other cancers [42]. These findings confirm the context-dependent oncogenic potential of PTEN. In the setting of mut-p53, PTEN effects are likely a balance between the well-known lipid phosphatase-mediated tumor-suppressive effects and the oncogenic effects mediated by mut-p53 and its gene targets. While PTEN mostly acts as a tumor suppressor, in specific contexts its oncogenic effects might prevail as shown in our study. Even in the latter case, the tumor-suppressive effects of PTEN still exist because PTEN restoration in U373 and SNB19 cells do reduce the level of p-Akt (Ser-473), resulting in decreased PI3K-AKT signaling. We therefore cannot exclude the possibility that, in specific circumstances, the tumor-suppressive effects might dominate the role of PTEN, also in the setting of gain-of-function mut-p53. The oncogenic effects of PTEN in the setting of gain-of-function mut-p53 possibly also apply to human malignancies other than glioblastoma based on preliminary data from our laboratory (not shown). If so, our findings will be very valuable because p53 is mutated in about half of all human tumors [14]. Our findings also have potential prognostic and therapeutic implications. They show that the combined *PTEN/p53* mutational status is a more accurate predictor of clinical outcome than the status of either single gene. They imply that restoration of PTEN expression or function should take into consideration the p53 mutational status to avoid unwanted effects. Our data also suggest that small molecule modulators of p53 are likely to have greater therapeutic effects in PTEN-expressing cells and/or that combining PTEN restoration with p53 modulators or c-Myc/Bcl-XL inhibitors might represent new strategies for cancer therapy.

Altogether, our study describes a novel, unconventional, and context-dependent function of PTEN and unravels its mechanistic basis. The findings shed new light on the complex molecular interactions between PTEN, mut-p53, and its gene targets *c-Myc* and *Bcl-XL* and have potentially important implications for prognosis and future therapies of cancer.

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