p53 Down-Regulates Phosphatase and Tensin Homologue Deleted on Chromosome 10 Protein Stability Partially through Caspase-Mediated Degradation in Cells with Proteasome Dysfunction

Yufang Tang[1,2] **and Charis Eng**[1,2,3,4,5,6]

[1]Genomic Medicine Institute, [2]Lerner Research Institute, and [3]Taussig Cancer Center, Cleveland Clinic Foundation; [4]Department of Genetics and [5]Case Comprehensive Cancer Center, Case Western Reserve University School of Medicine, Cleveland, Ohio and [6]Cancer Research UK Human Cancer Genetics Research Group, University of Cambridge, Cambridge, United Kingdom

Abstract

There has been intense investigation regarding the interaction between the phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and p53 tumor suppressors. p53 has been shown to up-regulate PTEN expression as a transcriptional activator. However, clinical observations by immunohistochemistry studies indicate that significant increases in p53 protein levels coexist with reduced or absent expression of PTEN protein in a variety of neoplasias. In this study, we propose a mechanism that begins to explain how p53 can both up-regulate and down-regulate PTEN. We have found that PTEN protein is down-regulated under proteasome dysfunction induced by proteasome inhibitor MG132 in both human lymphoblast cells and MCF7 cells. The reduction of PTEN is coincident with elevated p53 protein levels and the association between PTEN and p53 but independent of its phosphatase activities. Quantitative reverse transcription-PCR indicates that proteasome inhibition does not reduce PTEN message levels but affects PTEN protein stability. The p53 inhibitor, pifithrin-α, is able to attenuate the effect of proteasome inhibition. Using ectopic expression studies in p53-null mouse embryonic fibroblasts and p53/PTEN-null PC3 cells, we show that PTEN is more stable in p53-null cells compared with p53-expressing cells. Inhibition of caspases, the downstream targets of p53, particularly caspase-3, can partially restore the stability of PTEN. This study provides the first evidence that p53 is able to down-regulate PTEN protein stability in stressed cells. Our study sheds some light on the mechanisms that regulate PTEN protein stability, which is important to fully elucidate to comprehend the broad neoplastic manifestations of Cowden syndrome/Bannayan-Riley-Ruvalcaba and sporadic cancers. (Cancer Res 2006; 66(12): 6139-48)

Introduction

Phosphatase and tensin homologue deleted on chromosome 10 (PTEN), located on chromosome 10q23, is one of the most commonly altered tumor suppressors in human cancers, perhaps second only to p53. Interestingly, whereas somatic intragenic *PTEN* mutations are uncommon in the great majority of primary noncultured sporadic solid tumors, with the exception of glioblastoma multiforme and endometrial cancer, reduced PTEN protein expression is common across solid tumors even in the absence of structural alterations (i.e., mutation; for review, see ref. 1). Germline mutations in *PTEN* have been reported in ~85% of cases of Cowden syndrome, an autosomal dominant disorder characterized by multiple harmatomas with an increased risk of breast, thyroid, and endometrial neoplasias (1, 2). Interestingly, germline *PTEN* mutations have been found in 65% of cases of Bannayan-Riley-Ruvalcaba syndrome, a congenital developmental disorder characterized by mental retardation, lipomas, haemangiomas, and pigmentation of the penile shaft (3). *PTEN* mutations span from the coding region to the intronic and untranslated regions (4). However, in the presence of a germline *PTEN* mutation, the mechanism of complete PTEN protein shutoff in Cowden syndrome–related cancers is not known because 89% still possess the remaining wild-type allele (5).

PTEN plays a pivotal role in cell migration, proliferation, and apoptosis; therefore, cellular PTEN levels have to be regulated tightly. Interestingly, PTEN deficiency observed by immunohistochemical staining in patient cells was found to be associated with elevated p53 protein levels in the early phase of carcinogenesis (6–8). We have recently reported that PTEN protein is easily degraded in lymphoblast cells from Cowden syndrome patients possessing *PTEN* promoter mutations in the context of elevated p53 levels (9). However, it remains unclear what is the relationship between p53 function and PTEN protein level.

Recent evidence suggest that the functions of PTEN and p53 are connected as p53 transcriptionally activates PTEN (10, 11) and PTEN stabilizes p53 (12). Yet, it is difficult to understand why significant increases of p53 protein level correlate with no or reduced expression of PTEN protein in variety of cancers, particularly considering that p53 is known to up-regulate *PTEN* expression (6, 7). By using both normal human lymphoblast cells and MCF7 breast cancer cells, we now show that p53 is able to down-regulate PTEN partially by activating caspases under stress induced by proteasome inhibition.

Materials and Methods

PTEN and p53 expression constructs and reagents. *TP53* and *PTEN* cDNA were subcloned into pcDNA3 vector as described previously (9). All constructs were verified by DNA sequencing. MG132, doxorubicin, caspase inhibitors (Z-DEVD-FMK, Z-LEHD-FMK, and Z-VAD-FMK), and negative control (Z-FA-FMK) were purchased from Calbiochem (San Diego, CA). Cyclohexamide and mithramycin A were purchased from Sigma (St. Louis, MO). Pifithrin-α was purchased from A.G. Scientific (San Diego, CA).

Cell lines and culture and transient transfection. MCF7 and PC3 cells were cultured in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (Invitrogen) at 37°C with 5% CO_2. MCF7 Tet-Off breast cancer cells expressing either PTEN wild-type, protein and lipid phosphatase inactive mutant PTEN (C124S), or

Requests for reprints: Charis Eng, Genomic Medicine Institute, Cleveland Clinic Foundation, 9500 Euclid Avenue, Mailstop NE-50, Cleveland, OH 44195. Phone: 216-444-3440; Fax: 216-636-0655; E-mail: engc@ccf.org.

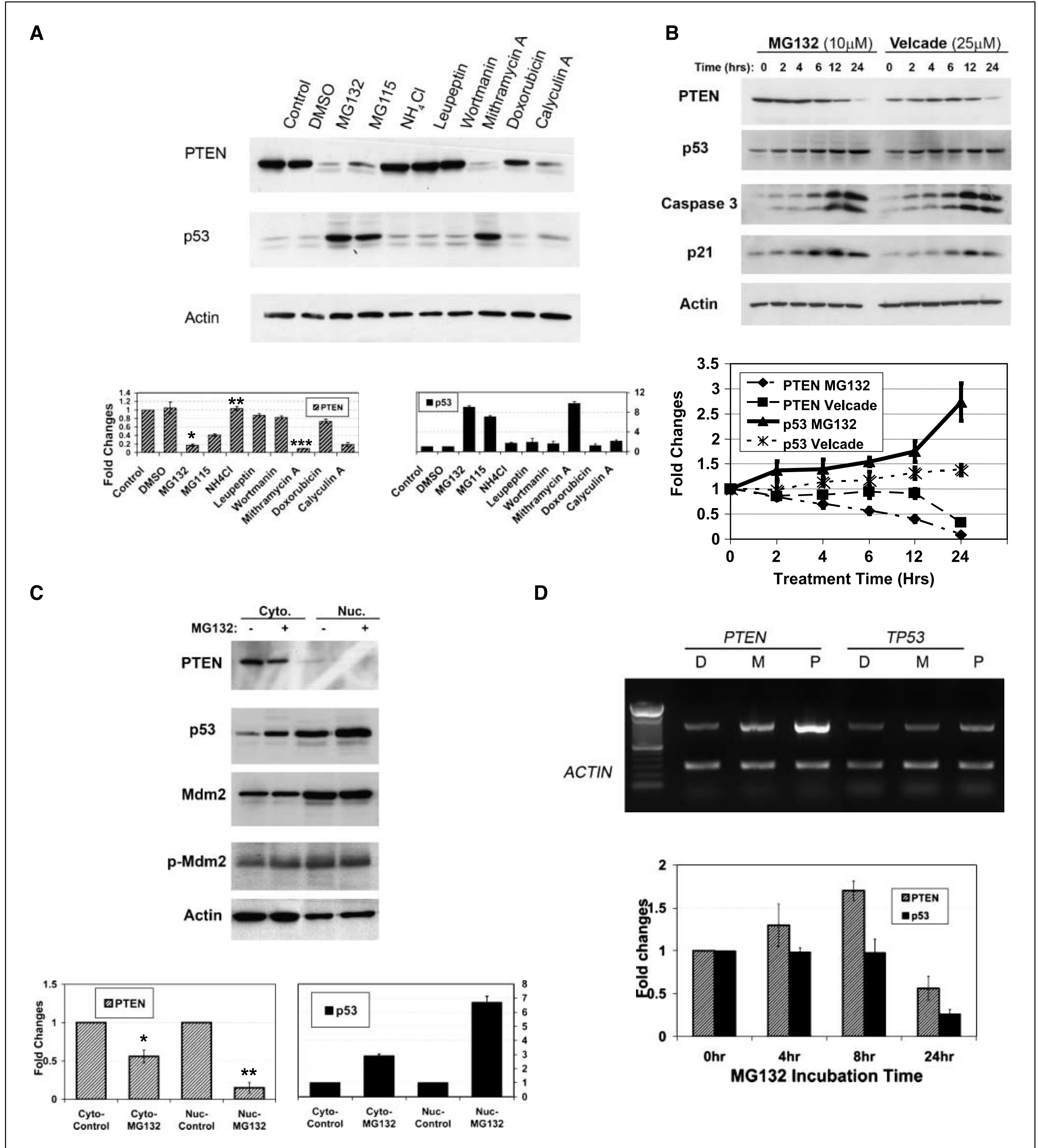
©2006 American Association for Cancer Research.

doi:10.1158/0008-5472.CAN-06-0772

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

lipid phosphatase inactive protein phosphatase active mutant PTEN (G129E) were generated as described previously (13). The MCF7 Tet-Off cell lines (BD Clontech, Palo Alto, CA), which contain a tetracycline-controlled cassette, were grown in DMEM high glucose supplemented with 10% FBS, 1% penicillin-streptomycin, 100 μg/mL G418 (Invitrogen), and 2 μg/mL tetracycline. Mouse embryonic fibroblast (MEF) wild-type and *TP53*−/− cells (kindly provided by Dr. Lawrence Kirschner, Ohio State University, Columbus, OH) were cultured in DMEM supplemented with 14% FBS (Hyclone, Logan, UT) and 1% penicillin-streptomycin.

PC3 cells or MEFs were cultured in six-well plate 24 hours before transient transfection. FuGene 6 (Roche Diagnostics, Indianapolis, IN) was used for transfection and cells were cotransfected with 0.5 μg PTEN or p53 or both. After transient transfection for 48 hours, cells were treated as indicated.



Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

Western blot analysis and immunoprecipitation. Cells were harvested and washed with PBS before protein extraction. Whole-cell extract was prepared by lysing the cells with M-PER Mammalian Protein Extraction Reagent (Pierce, Rockford, IL) supplemented with protease inhibitor cocktail (Sigma). Nuclear and cytoplasmic protein extracts were purified by NE-PER nuclear and cytoplasmic extraction reagents (Pierce) according to the manufacturer's protocols. Protein (10 μg) from each sample was subjected to SDS-PAGE and transferred onto nitrocellulose. Target proteins were detected by Western blot analysis using anti-p53, Mdm2 (Santa Cruz Biotechnology, Santa Cruz, CA), PTEN (Cascade Biosciences, Winchester, MA), phosphorylated Mdm2, and p21, caspase-3, caspase-9, and cleaved poly(ADP-ribose) polymerase (PARP; Cell Signaling Technology, Beverly, MA) antibodies, respectively. Protein bands were quantified by densitometry using ImageQuant (GE, Piscataway, NJ).

For immunoprecipitation experiments, 500 μg whole-cell lysates (cells treated with indicated concentration of either MG132 or DMSO for 4 hours at 37°C) were incubated with 1 μL p53 antibody or IgG only (Santa Cruz Biotechnology) plus 50 μL protein A agarose beads (Pierce) at 4°C overnight. Beads were washed thrice with PBS supplemented with protease inhibitor cocktail and 0.1% Triton X-100 and centrifuged for 5 minutes at 5,000 × *g* between each wash. Protein was eluted from the beads with 40 μL of 2× Laemmli sample buffer. Samples were resolved on a 10% SDS-PAGE gel and transferred onto nitrocellulose membrane. PTEN protein was detected using standard Western blotting procedure.

Semiquantitative reverse transcription-PCR and real-time PCR. MCF7 cells were released by trypsin treatment and subsequently washed thrice with PBS. RNA was extracted from the cells following the RNeasy Mini Protocol (Qiagen, Valencia, CA). A quantity of 1 μg total RNA was reverse transcribed in a 50 μL reaction mixture containing 0.5 μg oligo$(dT)_{15}$ as primer by SuperScript II reverse transcriptase (Invitrogen). The resultant cDNA was subjected to multiplex PCR using primers specific to *PTEN* (5′-TCAAGAGGATGGATTCGACTT-3′ and 5′-TGAAGTACAGCTTCACCT-TAAA-3′), *TP53* (5′-GGGATCCATGGAGGAGCCGCAGTCAGATCC-3′ and 5′-GGAATTCTCAGTCTGAGTCAGGCCC-3′), and β-actin (Quantum RNA β-actin; Ambion, Austin, TX). Primers were allowed to anneal at 58°C for 30 seconds. The products from PCR were resolved on a 1% agarose gel.

Quantitative (real-time) PCR was carried out with the iCycler instrument (Bio-Rad, Hercules, CA) using the SYBR Green PCR Reagent kit (Bio-Rad). Gene-specific PCR primers are *PTEN* (5′-TGGATTCAAAGCATAAAAACCA-3′ and 5′-AAAAGGATATTGTGCAACTCTGC-3′) and *TP53* (5′-GGGATC-CATGGAGGAGCCGCAGTCAGATCC-3′ and 5′-CGGCAAGGGGGACA-GAACG-3′). Expression levels of the *PTEN*/*TP53* gene were normalized using glyceraldehyde-3-phosphate dehydrogenase (5′-CCATCTTCCAG-GAGCGAGA-3′ and 5′-AAATGAGCCCCAGCCTTCT-3′) to correct minor variations in mRNA extraction and reverse transcription.

Statistics. Statistical analysis was done using Student's *t* test. Data are mean ± SD of three independent experiments. Each group was normalized to β-actin and compared with a control. $P < 0.05$ is considered significant.

Results

Proteasome inhibition reduces PTEN protein stability. We have reported previously that PTEN is less stable in lymphoblast cell lines derived from a subset of Cowden syndrome patients compared with normal (non–Cowden syndrome) human cells (9). To investigate the mechanisms of reduced protein stability, we first examined the ubiquitin-mediated protein degradation pathway. Two proteasome inhibitors, MG115 and MG132, were incubated with lymphoblast cells for 12 hours. Unexpectedly, PTEN protein stability was not protected by proteasome inhibition; instead, PTEN protein levels decreased with exposure to either of the inhibitors (5.8 ± 0.7–fold decrease after MG132 treatment compared with untreated cells, $P = 0.0004$, versus 2.5 ± 0.2–fold decrease after MG115 treatment, $P = 0.0013$; Fig. 1*A*). To evaluate if PTEN destabilization is proteasome dysfunction specific, we treated the normal lymphoblast cells with inhibitors of different protein degradation pathways. PTEN protein showed no change after treatment with a lysosome inhibitor, NH_4Cl, or an inhibitor for trypsin-like proteases and cysteine proteases, leupeptin (Fig. 1*A, lane 5*, NH_4Cl, $P = 0.21$, and *lane 6*, leupeptin, $P = 0.17$). The phosphatidylinositol 3-kinase (PI3K) inhibitor wortmannin also had no effect on PTEN stability, suggesting that this phenomenon is at least PTEN lipid phosphatase independent and PI3K independent (Fig. 1*A*, *lane 7*, $P = 0.08$). Surprisingly, cells incubated with mithramycin A, a transcriptional inhibitor, showed a dramatic PTEN protein reduction (Fig. 1*A*, *lane 8*, −11 ± 1.5–fold, $P = 0.0002$). Furthermore, two apoptosis inducers, doxorubicin and calyculin A, cause a 1.3 ± 0.1– and 5.7 ± 1.1–fold PTEN protein reduction, respectively (Fig. 1*A*, $P = 0.024$ and 0.0004, respectively). These results indicate that PTEN destabilization is not specific to proteasome dysfunction, as inhibitors of other cellular pathways can also induce a decrease in PTEN protein levels. Calyculin A, doxorubicin, and MG132 have been reported to induce cellular apoptosis (14, 15), and mithramycin A is a strong activator of p53 (16), suggesting that PTEN protein reduction might be related to either an apoptotic event and/or the increase of p53. Our previous study showed that p53 protein levels were elevated in some Cowden syndrome patients with increased PTEN degradation (9). Based on these results, we next set out to examine the level of p53 protein in samples treated with the above inhibitors. We observed that MG132-, MG115-, or mithramycin A–treated samples with reduced PTEN always contained increased (∼8-fold; $P < 0.0001$) levels of p53, whereas samples with intact PTEN showed no changes of p53 protein levels (Fig. 1*A*).

To better understand the relationship between PTEN and p53 under cellular stress, we examined the time course of proteasome inhibitors on both PTEN and p53 protein levels. Consistent with the results in Fig. 1*A*, PTEN protein levels decreased in a time-dependent manner when cells were exposed to either MG132 or Velcade when compared with untreated cells (Fig. 1*B*). p53, known

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

Figure 1. Proteasome inhibitors induce reduction of PTEN while increasing p53 protein. *A,* detection of PTEN protein after the treatment of cells with different inhibitors. Normal human lymphoblast cells were treated for 12 hours with each of the following inhibitors: proteasome inhibitors MG132 or MG115 (10 μmol/L), lysosome inhibitor NH_4Cl (50 mmol/L), trypsin-like and cysteine protease inhibitor leupeptin (10 μmol/L), PI3K inhibitor wortmannin (2 μmol/L), transcription inhibitor mithramycin A (1 μmol/L), apoptosis inducer doxorubicin (0.5 μg/mL), and calyculin A (10 μmol/L). β-Actin served as a loading control. Relative levels of PTEN and p53 were quantitated by densitometry and normalized to β-actin. Results are fold change compared with nonstimulated cells. *Columns,* mean of three independent experiments; *bars,* SD. *, $P = 0.0004$, MG132 treated compared with untreated control; **, $P = 0.21$, no difference in PTEN levels after NH_4Cl treatment compared with untreated controls; ***, $P = 0.0002$, mithramycin A–treated compared with untreated controls (Student's *t* test). *B,* either MG132 or Velcade exposure resulted in accumulation of p53 and reduction of PTEN protein. Normal human lymphoblast cells were treated with 25 μmol/L Velcade or 10 μmol/L MG132 for the indicated times. Protein levels of PTEN, p53, p21, and caspase-3 were detected by Western blotting. β-Actin served as loading control. *C,* both nuclear and cytoplasmic PTEN/p53 proteins were affected by MG132. Normal human lymphoblast cells were treated with 10 μmol/L MG132 for 12 hours. Cytoplasmic (*Cyto*) and nuclear (*Nuc*) fractions were purified. Protein levels of PTEN, p53, Mdm2, and phosphorylated Mdm2 were detected in both fractions. β-Actin is served as loading control. *, $P = 0.0059$, cytoplasmic PTEN level after MG132 treatment compared with untreated cytoplasmic control; **, $P = 0.0004$, nuclear PTEN levels after MG132 treatment compared with untreated control nuclei. *A* to *C,* representative blots of three individual experiments. *D,* MG132 increases the transcription of *PTEN* while not affecting *TP53* transcription. MCF7 cells were treated with 10 μmol/L MG132 for 4 hours. Semiquantitative PCR was done. PTEN-overexpressing MCF7 cells were used as a positive control of *PTEN* (*top*). Quantitative RT-PCR was carried out to test the time course of MG132 effect on *PTEN/TP53* transcription in MCF7 cells (*bottom*). *Columns,* mean of three independent experiments; *bars,* SE. *D,* DMSO; *M,* MG132; *P,* sample from PTEN-expressing cells.

as a labile protein whose stability is regulated closely by the ubiquitin-mediated degradation pathway, gradually accumulated with extended incubation with both proteasome inhibitors (Fig. 1*B*), consistent with previous reports (17). MG132 was reported to increase p53 activity (17); therefore, we examined the downstream targets of p53, p21, and caspase-3 under MG132 treatment. Both proteins showed increased levels after proteasome inhibitor treatment in lymphoblast cells (Fig. 1*B*) as expected.

Protein redistribution between cytoplasmic and nuclear fractions is one regulatory mechanism for protein stability in a variety of proteins, including p53. We know that PTEN protein is localized to both fractions; furthermore, the subcellular localization of PTEN correlates with its distinct lipid and protein phosphatase functions (18). Based on this, we next examined if the reduction of PTEN protein due to proteasome inhibitor exposure was related to changes in PTEN nuclear-cytoplasmic shuttling. We found that PTEN levels were reduced in both nuclear (-7.4 ± 2.1–fold; $P = 0.0004$) and cytoplasmic (-1.8 ± 0.1–fold; $P = 0.0059$) fractions of normal lymphoblast cells under induced proteasome dysfunction (Fig. 1*C*). We next checked if the stabilization of p53 occurred in the cytoplasmic fraction, where p53 is degraded through the ubiquitination pathway. To our surprise, p53 protein levels were increased in both cytoplasmic (3-fold; $P < 0.0001$) and nuclear (6.5-fold; $P < 0.0001$) fractions after MG132 treatment (Fig. 1*C*). The protein levels of Mdm2 or phosphorylated Mdm2, the major regulators of p53 protein stability, did not change in the presence of proteasome inhibitor (Fig. 1*C*), suggesting the existence of an alternative pathway to monitor intracellular p53 levels in human lymphoblast cells.

Based on the above results, we next sought to determine if proteasome inhibitor treatment affects either the transcription or the protein stability of PTEN and/or p53 using a breast cancer cell line, MCF7, in which MG132 treatment is associated with decreased protein levels of PTEN in the presence of increased protein levels of p53 (data not shown). MCF7 cells were treated with 10 μmol/L MG132 (Fig. 1*D, M*) and transcripts of both *TP53* and *PTEN* were examined by both semiquantitative reverse transcription-PCR (RT-PCR; 4 hours of treatment, Fig. 1*D, top*) and quantitative RT-PCR (with a time course of 0, 4, 8 and 24 hours of treatment, Fig. 1*D, bottom*). MCF7 cells, overexpressing PTEN, were used as a positive control for PTEN transcription (Fig. 1*D, P*) and DMSO (Fig. 1*D, D*) was used as a vehicle control. We found that there was no increase in *TP53* transcript levels after MG132 incubation (Fig. 1*D*), suggesting that MG132 regulates p53 protein stability but does not do so by increasing *TP53* transcription. Interestingly, in the presence of MG132, we found that *PTEN* transcripts were increased, not decreased, as seen with PTEN protein (Fig. 1*D*). p53 is a transcriptional activator of *PTEN* expression; therefore, one would expect that increased p53 levels would result in the increased *PTEN* message. The seemingly paradoxical observations indicate that proteasome inhibitor treatment reduces protein stability, not transcription, of PTEN.

Increased p53 protein levels are responsible for PTEN protein degradation and facilitate the interaction between PTEN and p53. Our above data suggested that there is an inverse correlation between PTEN and p53 protein levels. In other words, the higher the p53 protein level, the lower the level of PTEN protein. Therefore, we next determined if p53 plays a direct role in regulating the stability of PTEN protein. MCF7 cells with inducible PTEN overexpression (PTEN overexpression in the absence of tetracycline) were treated with pifithrin-α, a p53 inhibitor, in combination with MG132. Exposure to pifithrin-α alone can reduce intracellular p53 protein levels (-2.0 ± 0.2, $P = 0.0036$; Fig. 2*A*). Similarly, as in lymphoblast cells, MG132 induced a 9.0 ± 0.3–fold increase of p53 in MCF7 cells ($P < 0.0001$). In contrast, cells incubated with the combined MG132 and pifithrin-α induced only a 2.3 ± 0.4–fold increase of p53 (versus 9.0 ± 0.3–fold of MG132 treatment alone; $P = 0.0022$; Fig. 2*A*). We found that pifithrin-α exposure attenuated the effect of MG132 on PTEN protein stability. In cells treated with MG132, there was a 3.1 ± 0.3–fold ($P = 0.0015$) reduction in PTEN protein compared with untreated cells. When pifithrin-α was added to MG132, there was a 1.4 ± 0.2–fold (versus 3.1 ± 0.3–fold decrease with MG132 alone; $P = 0.0076$) reduction of PTEN protein compared with untreated cells (Fig. 2*A*). Taken together, these data show that PTEN protein levels are directly regulated by intracellular p53 (i.e., p53 down-regulates PTEN protein stability).

As reported earlier, PTEN can physically interact with p53 (19), and exposure to proteasome inhibitor stabilizes p53 (17). In addition, we have recently reported that MG132 stabilizes the interaction between PTEN and p53 in a subset of Cowden syndrome patient cells yet possesses less stable PTEN protein (9). This suggests that the formation of a PTEN-p53 complex may interfere with PTEN protein stability. Here, we try to elucidate if the status of their association correlates with the observed reduction of PTEN protein in human lymphoblast cells. Cells were incubated with increasing concentrations of MG132, as indicated, for 4 hours at 37°C and subjected to coimmunoprecipitation. The protein levels of PTEN and p53 were detected by Western blotting and quantitated (Fig. 2*B*). We found that MG132 exposure gradually increased the association between PTEN and p53 in a dose-dependent manner, with maximal association occurring at 10 μmol/L (6.4 ± 0.5–fold increase compared with control, $P < 0.0001$; Fig. 2*B*). However, when cells were exposed to a higher MG132 concentration (20 μmol/L), the PTEN-p53 association began to be attenuated (only 1.4 ± 0.2–fold increase compared with control, $P = 0.031$, versus to 6.4 ± 0.5–fold at 10 μmol/L; Fig. 2*B*). The response of PTEN protein degradation to MG132 exposure is also dose dependent (Fig. 2). Interestingly, the extent of the disappearance of PTEN correlated with the strength of the association between PTEN and p53 when MG132 ranged from 1 to 10 μmol/L. However, although p53 levels peaked at 20 μmol/L, the association between PTEN and p53 already started to decrease at 20 μmol/L (Fig. 2*B*). These findings suggest that the association between PTEN and p53 is a regulated process and not simply due to the level of involved proteins.

p53-dependent PTEN degradation does not require PTEN protein and lipid phosphatase activities. The dual-phosphatase activities, especially lipid phosphatase activity, are critical for PTEN tumor suppressor function (20). Based on this, we examine if PTEN phosphatase activities are involved in its p53-regulated protein stability. We used two cell lines with inducible expression of PTEN phosphatase-inactivated mutants, C124S (both protein and lipid phosphatase inactive) and G129E (lipid phosphatase inactive, protein phosphatase active; ref. 13), together with wild-type PTEN and control cells (vector only). All the cells were induced to express exogenous genes by tetracycline removal for 48 hours before proteasome inhibitor treatment. In all cell lines, including both control cells and PTEN-overexpressing cells (wild-type and mutant), PTEN levels were reduced after treatment with either proteasome inhibitor (10 μmol/L MG132 and 25 μmol/L Velcade) to a similar extent (1.9- to 2.7-fold for all four cells and for either of

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

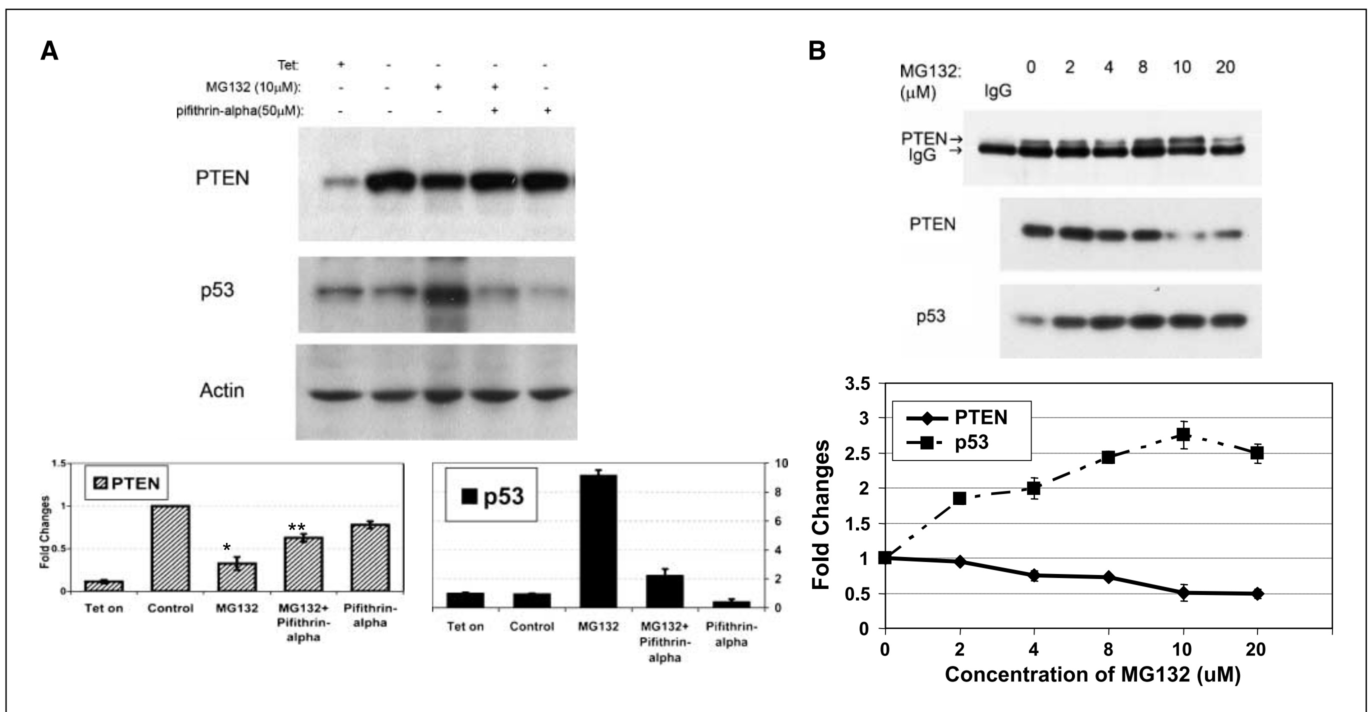


Figure 2. Increased p53 is responsible for PTEN protein degradation and facilitates the association between PTEN and p53. *A,* chemical inhibitor of p53, pifithrin-α, can attenuate the effect of MG132 on PTEN stability. MCF7 cells expressing wild-type PTEN were growing in medium with or without tetracycline (*Tet*) for 48 hours. Cells were treated with the indicated chemicals for an additional 24 hours at 37°C. Protein levels of PTEN, p53, and β-actin were detected in 10 μg total extracts. *B,* proteasome inhibitor MG132 facilitates the association between PTEN and p53. Normal human lymphoblast cells were treated with MG132 at the indicated concentration for 4 hours. Total extracts were prepared and coimmunoprecipitation was done. Protein levels of PTEN were detected in 10 μg total extracts or the precipitated elutes. p53 was detected in total extracts. *A* and *B,* representative blots of three individual experiments. Relative levels of PTEN and p53 were quantitated and normalized. Results are fold change compared with control. *Columns,* mean of three independent experiments; *bars,* SD. *, $P = 0.0015$, difference in PTEN levels after MG132 treatment alone compared with untreated controls; **, $P = 0.0076$, difference in PTEN levels after combined MG132 and pifithrin-α treatment compared with untreated controls (Student's *t* test).

the proteasome inhibitors; Fig. 3). Our data indicate that both phosphatase activities of PTEN are not required for p53-dependent PTEN protein degradation. For all cells, the changes of p53 protein levels were almost identical (Fig. 3). Considering the important role of Mdm2 in regulating p53 protein levels inside the cell, we examined Mdm2 (total and phosphorylated) protein levels. We found that there was no change of total Mdm2 under either condition; however, the level of p-Mdm2 was increased with proteasome inhibitor treatment, mirroring the increased levels of p53 (Fig. 3). This change is different from what we saw in control human lymphoblast cells, where proteasome inhibitors had no effect on the level of p-Mdm2. The discrepancy may indicate that proteasome inhibitors regulate the protein stability of p53 through cell type–specific pathways.

PTEN is more stable in cells lacking p53. To consolidate our evidence for the involvement of p53 in the regulation of PTEN protein stability, we used p53−/− MEFs, and wild-type MEFs as control. Both cells were treated with MG132 (10 μmol/L for 24 hours) and PTEN and p53 protein levels were detected by Western blotting. p53 protein levels were increased 3.6 ± 0.6–fold ($P = 0.0001$) after MG132 treatment in MEFs when compared with untreated cells (Fig. 4*A*), similar to that observed in MCF7 cells and human lymphoblast cells. Although PTEN protein levels were reduced in both p53 wild-type and p53-null MEFs, PTEN was reduced to a greater extent in cells expressing p53 (i.e., in wild-type MEF; −2.0 ± 0.3, $P = 0.0029$; Fig. 4*A, lane 2*) than that in p53-null cells (−1.1 ± 0.2, $P = 0.018$; Fig. 4*A, lane 4*). This supports our previous finding that p53 destabilizes PTEN. PTEN protein stability was further examined by treatment with cyclohexamide. Both p53 wild-type and p53-null MEFs were incubated with 50 μmol/L cyclohexamide for 24 hours before addition of MG132 with the indicated concentrations for another 4 hours. We found that after cyclohexamide treatment the level of PTEN protein in wild-type MEFs (Fig. 4*B, lane 4*) was reduced (−15 ± 5.0–fold decreased compared with untreated cells; $P < 0.0001$) 6-fold more than that in p53-null MEFs (−2.5 ± 0.3, $P = 0.0005$; Fig. 4*B, lane 10*). This further confirms that PTEN protein is more stable in cells that do not express p53. We also noticed that MG132 lost its p53 elevating effects when cyclohexamide was present and at the same time lost the ability to further reduce PTEN (Fig. 4*B, lanes 4-6*). These results show that increased p53 protein is the key molecule modulating PTEN protein when cells are treated with proteasome inhibitor.

To determine whether the p53 effect on PTEN protein stability is caused by the presence of p53 or the increase of p53 levels induced by cellular stress, we introduced p53 back into the p53-null MEFs. We did not see PTEN protein change after we exogenously expressed p53 in p53-null cells (Fig. 5*A, lanes 1* and *4*). However, when cells were treated with combined MG132 and cyclohexamide, PTEN protein in p53-transfected cells (labeled as p53−/− + p53 MEFs) was found to decrease by −12.7 ± 3.1–fold ($P < 0.0001$; Fig. 5*A, lane 6*), which is a much greater reduction than the 2.2-fold noted in parental p53-null MEFs (Fig. 5*A, lane 3*). Our data indicate that it is the increase in p53 protein after stress

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

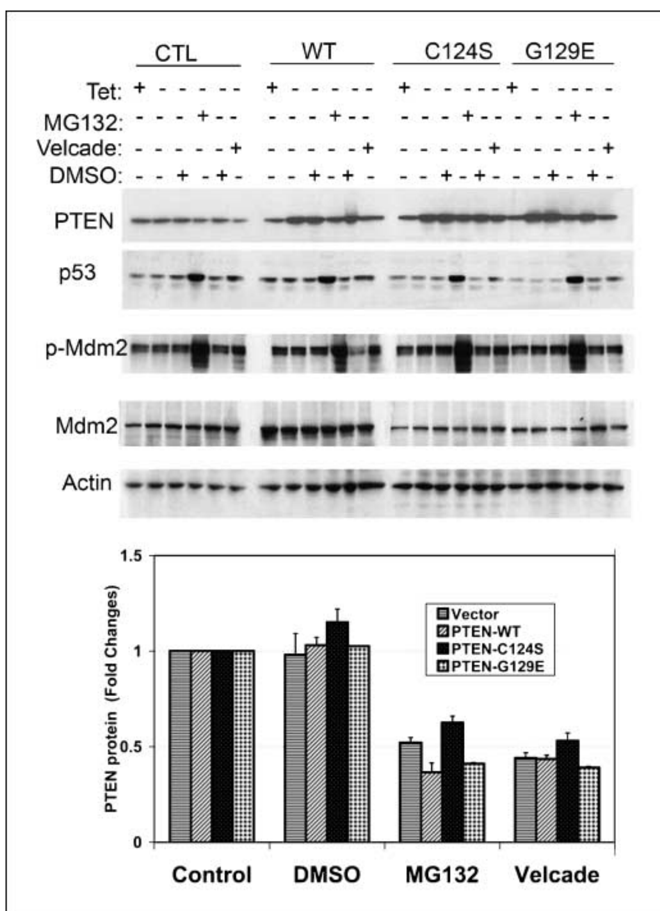


Figure 3. PTEN protein reduction induced by proteasome dysfunction does not require its phosphatase activities. MCF7 cells expressing wild-type PTEN, PTEN-C124S (both lipid and protein phosphatase inactivated) or PTEN-G129E (protein phosphatase inactivated) mutants, or vector only (as control) were incubated in medium with or without tetracycline for 48 hours. All the cells were treated equally with indicated chemicals for an additional 24 hours. Protein levels of PTEN, p53, phosphorylated Mdm2, Mdm2, and β-actin were detected. Representative blots of three separate experiments. Results are fold change compared with control.

induction, such as proteasome inhibition, which directly results in PTEN degradation. Similar to endogenous p53, the exogenously expressed p53 was also able to accumulate (manifested as a 3-fold increase) after MG132 incubation (Fig. 5*A*).

We then examined if exogenous p53 had a similar ability as endogenous p53 to destabilize PTEN protein. Toward this end, p53 was introduced into MEFs 2 days before the MG132 (10 μmol/L) treatment with indicated time. The untransfected MEF p53-null and wild-type MEFs were used as controls for these experiments. Similar to the results shown in Figs. 4*B* and 5*A*, p53-null MEFs showed the most PTEN protein remaining after MG132 incubation for 24 hours (−1.5 ± 0.1–fold; *P* = 0.006; Fig. 5*B, lane 3*). To our surprise, although we introduced higher levels of p53 back into p53-null MEFs (labeled as p53−/− MEF + p53 cells) and the exogenously expressed p53 showed dramatic increases (14-fold) after 24-hour MG132 treatment, PTEN protein in these cells only decreased by 2.6 ± 0.2–fold compared with controls (*P* = 0.0005; Fig. 5*B, lane 9*). In contrast, PTEN in wild-type MEFs decreased by 6.45 ± 1.5–fold (*P* = 0.0002; Fig. 5*B, lane 6*) when p53 levels increased 8-fold. These results suggest that although exogenous p53 can affect PTEN stability, the degradation of PTEN under induced stress is triggered by a complicated pathway, including but not limited to p53.

To further elucidate the effect of p53 on PTEN protein stability, we used an unambiguous exogenous expressing system and protein half-time study. PTEN, or p53, or both were introduced into PC3 cells, which is a *PTEN* and *TP53* double-null cell line. Consistent with previous findings, the presence of p53 alone did not affect PTEN (9). However, in the presence of cyclohexamide, PTEN protein was found to be more stable (half-life, ∼20 hours) when expressed alone than coexpressed with p53 (half-life, ∼12 hours;

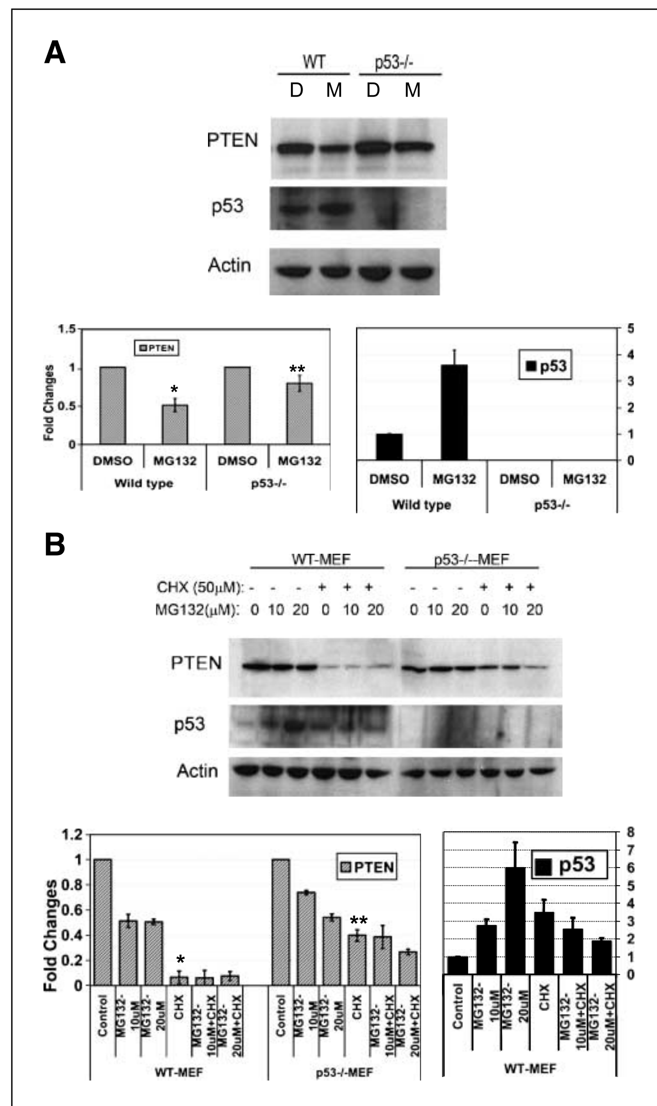
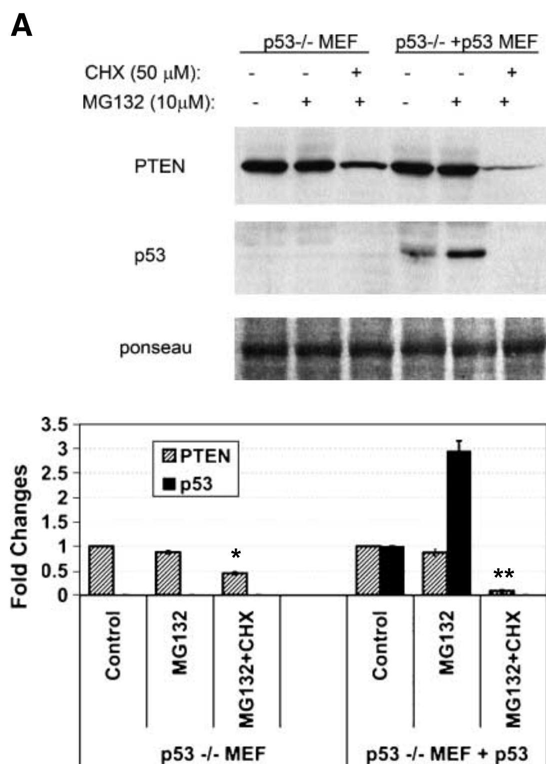
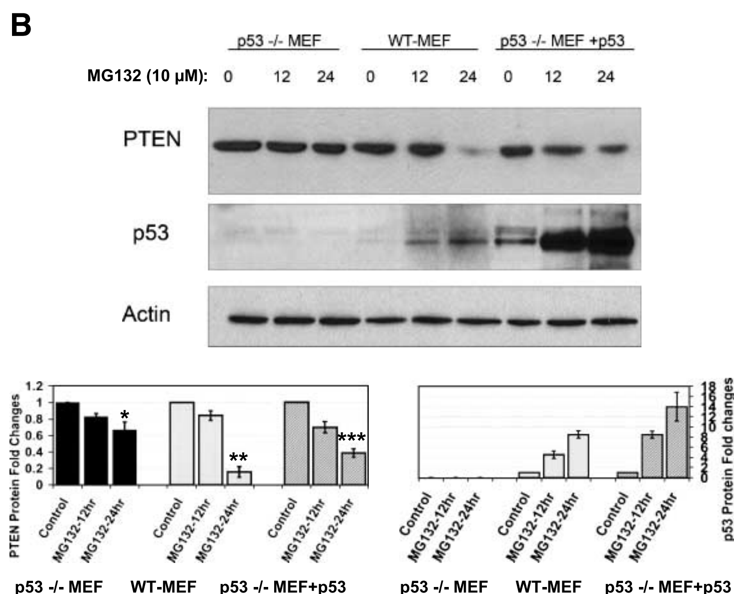


Figure 4. PTEN protein is more stable in cells lacking p53. Wild-type and p53−/− MEFs were treated with 10 μmol/L MG132 for 24 hours (*A*) or with the indicated dosage of MG132 for 4 hours after 24-hour incubation with 50 μmol/L cyclohexamide (*CHX; B*) at 37°C. PTEN, p53, and β-actin levels were detected by Western blotting. *A* and *B,* representative blots of three individual experiments. Relative levels of PTEN and p53 were quantitated and normalized. Results are fold change compared with control. *Columns,* mean of three independent experiments; *bars,* SD. *A,* *, *P* = 0.0029, difference in PTEN protein levels after MG132 treatment compared with no treatment in wild-type MEFs; **, *P* = 0.0018, difference in PTEN protein levels after MG132 treatment compared with no treatment in p53−/− MEFs (Student's *t* test). *B,* *, *P* < 0.0001, difference in PTEN protein levels after cyclohexamide treatment compared with no treatment in wild-type MEFs; **, *P* = 0.0005, difference in PTEN protein levels after cyclohexamide treatment compared with no treatment in p53−/− MEFs (Student's *t* test).

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024





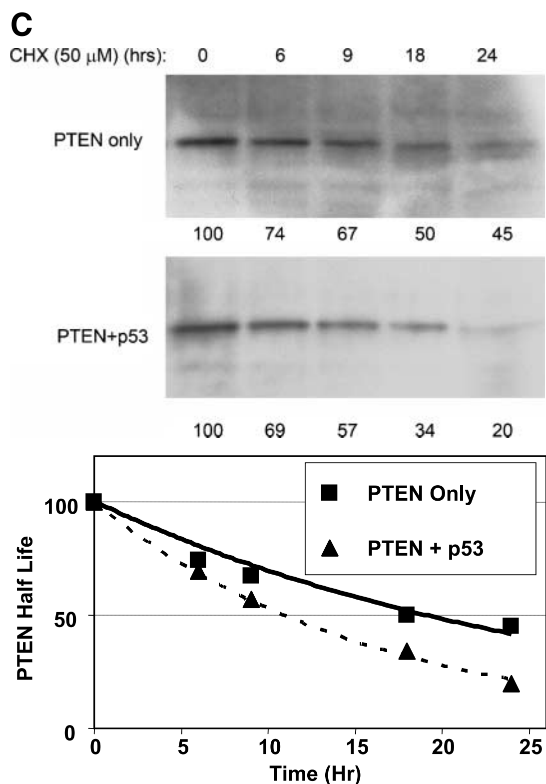


Figure 5. Exogenously introduced p53 destabilizes PTEN protein but at a lesser extent compared with the endogenous counterpart. p53 expression plasmid or empty vector was transfected into p53−/− MEFs for 2 days as described in Materials and Methods. Transfected cells together with control cells (MEF *p53*−/− and wild-type) were treated with 50 μmol/L cyclohexamide for an additional 8 hours after incubation with MG132 for 24 hours (*A*) or treatment only with MG132 at the indicated times (*B*). PTEN and p53 levels were detected by Western blotting. *A* and *B,* representative blots of three individual experiments. Relative levels of PTEN and p53 were quantitated and normalized. Results are fold change compared with control. *Columns,* mean of three independent experiments; *bars,* SD. *A,* *, $P = 0.0007$, PTEN protein levels after MG132 combined with cyclohexamide treatment compared with no treatment in p53−/− MEFs; **, $P < 0.0001$, PTEN protein levels after MG132 combined with cyclohexamide treatment compared with no treatment in p53−/− + p53 MEFs (Student's *t* test). *B,* *, $P = 0.0065$, PTEN protein levels after MG132 treatment alone compared with no treatment in p53−/− MEFs; **, $P = 0.0002$, PTEN protein levels after MG132 treatment alone compared with no treatment in wild-type MEFs; ***, $P = 0.0005$, PTEN protein level after MG132 treatment alone compared with no treatment in p53−/− + p53 MEFs (Student's *t* test). *C,* p53 reduces PTEN protein stability in ectopic expression system. PC3 cells were transfected with *PTEN* with or without *TP53* for 48 hours. Transfected cells were treated with 50 μmol/L cyclohexamide for the indicated hours at 37°C. PTEN protein levels were examined by Western blotting. *Numbers,* percentage of remaining PTEN. *Points,* PTEN half-lives.

$P = 0.001$; Fig. 5*C*). These results clearly indicate that p53 has a negative effect on PTEN protein stability.

PTEN protein is partially degraded by caspases. Although the ubiquitin-mediated proteasome pathway is the major degradation pathway for most proteins, degradation of PTEN must occur through alternative pathways under proteasome inhibition. In examining the degradation pattern of PTEN in a group of Cowden syndrome patients, we noticed that the pattern was similar to that

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

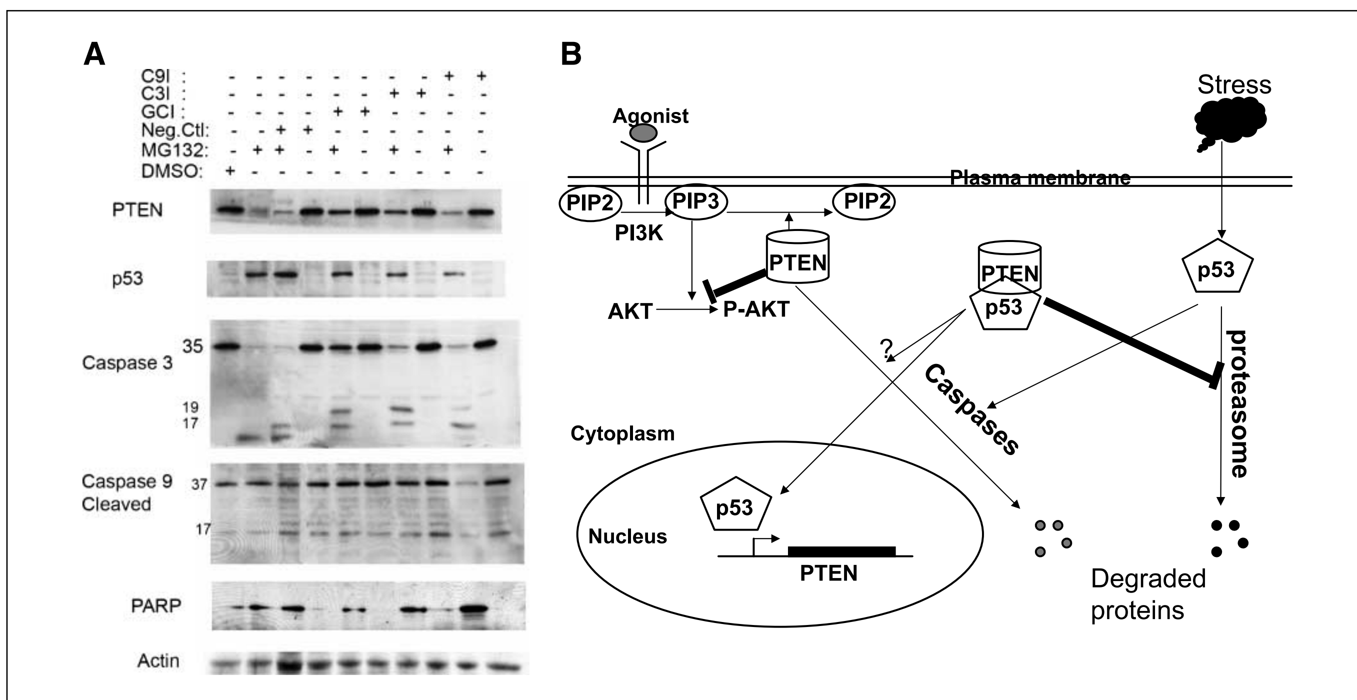


Figure 6. p53 regulates PTEN protein levels partially through caspases. *A,* caspases partially degrade PTEN under proteasome inhibition. Normal human lymphoblast cells were treated with MG132 (10 μmol/L) alone or together with caspase inhibitors (caspase-3 inhibitor Z-DEVD-FMK, caspase-9 inhibitor Z-LEHD-FMK, general caspase inhibitor Z-VAD-FMK, and negative control Z-FA-FMK; 100 μmol/L each) or with each of the inhibitors alone for 12 hours. Protein levels of PTEN, p53, caspase-3, caspase-9, cleaved PARP, and β-actin were detected by Western blotting. *B,* a model for the effect of p53 on PTEN protein degradation under cellular stress. PTEN antagonize PI3K kinase activities. PTEN gene expression is up-regulated by p53. PTEN also stabilizes p53 protein by protecting p53 from Mdm2-mediated protein degradation, which in turn auto-up-regulates its expression. However, p53 promotes PTEN protein degradation in part by increasing intracellular active caspases independent of proteasomal pathways of PTEN degradation. *Arrow,* activation; *blunt line,* inhibition.

reported for caspase-3-mediated protein digestion (21).[7] Therefore, we sought to determine if caspase-3 is involved in the degradation of PTEN in our system. We combined the MG132 treatment with the presence/absence of inhibitors for caspases (general caspase inhibitor, caspase-3- or caspase-9-specific inhibitors). PTEN protein showed no change when incubated with caspase inhibitors alone (Fig. 6*A*). However, caspase inhibitors only partially recovered PTEN protein stability after MG132 incubation (Fig. 6*A*). The general inhibition of caspases showed the maximal recovery of PTEN protein stability, whereas the caspase-9 inhibitor showed the minimal effect. Although the levels of active forms of caspase-3 (17 and 19 kDa) were correlated with p53 protein levels, both were inversely correlated with PTEN protein level (Fig. 6*A*). The activity of caspase-3 was further confirmed by the readout target protein, cleaved PARP (Fig. 6*A*). However, the activation of caspase-9 showed no direct correlation with PTEN protein stability (Fig. 6*A*). These data suggest that caspases, including but not limited to caspase-3, are involved in PTEN degradation under proteasome dysfunction.

Discussion

There has been intense investigation on the ability of PTEN to stabilize p53 protein through a variety of pathways, including AKT and Mdm2 (9, 12, 21). p53 activates *PTEN* gene expression, which explains a decreased PTEN expression in p53-null MEFs as seen by Wang et al. (22) and in 293 cells by Chappell et al. (23) under stress induced by serum deprivation. However, it is difficult to understand why *in vivo* when p53 is significantly increased we do not see the corresponding PTEN protein increase, which would be expected based on the transcriptional regulation of PTEN of p53 (6, 24, 25). One possible explanation is that the increased p53 protein noted on immunohistochemistry reflects increased p53 stability (hence increased immunostaining) in the context of *TP53* mutations. Yet, it is possible that this stable, mutant p53 can still complex with PTEN protein leading to the degradation of the latter. This may help explain why in most these primary human cancers PTEN protein levels actually go down in the early stage of carcinogenesis. All in all, the many seemingly paradoxical observations involving PTEN and p53 suggest that the interplay between p53 and PTEN is complicated and that p53 can negatively regulate PTEN at the protein level in a manner and complexity that is beyond the positive role of p53 in *PTEN* transcriptional activation. In this study, we found that lower PTEN protein levels are paradoxically correlated with higher levels of p53 under different induced cell stresses. By using *p53−/−* MEF and double *PTEN−/−*, *TP53−/−* PC3 cells as well as an ectopic expression system, we are able to conclude that the stabilization and activation of p53 by proteasome inhibition leads to down-regulation of PTEN protein. We have shown that p53 functions as a negative regulator of PTEN protein stability in both Mdm2-dependent and Mdm2-independent pathways for both cancer cell lines and normal human cells. We also provide evidence that the effect is directly connected with p53, and specifically, it is the increase of p53 protein that results in PTEN protein degradation.

[7] Tang and Eng, unpublished observations.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

Therefore, intracellular PTEN protein levels are a result of the fine balance between p53-activated PTEN transcription and p53-promoted PTEN degradation.

It is not surprising to find that the phosphatase activities are not involved in stress-induced PTEN degradation (Figs. 1*A* and 3) because PTEN protein stability is mainly regulated at COOH-terminal domains (two PEST domains and several caspase recognition sites; refs. 26, 27). However, we do have evidence that PTEN enzymatic activities may regulate its degradation in certain instances.[8] Further elucidation is required to fully understand the role of the association between PTEN and p53 in the PTEN degradation process as well. Binding of p53 to PTEN may alter the spatial structure of PTEN for degradation (28). Alternatively, the PTEN-p53 complex may bring in enzymes, such as caspases, to facilitate the digestion of PTEN, which is very different from its role to protect p53 from Mdm2-mediated degradation (12). Combined effect of PTEN and p53 implicates the checkpoint of tumorigenesis in which cells survive with only one tumor suppressor inactivation; however, combined lose of both PTEN and p53 elicits evasive cancer phenotype. This may explain why lost of PTEN and elevated p53 were observed in early-stage cancer cases in which cells still struggle to survive.

We have shown the correlation of decreased PTEN levels with increased p53 levels has been confirmed in three different cell types (human lymphoblast cells, cancer cell lines MCF7 and PC3, and MEFs) and in both the cytoplasm and the nucleus, indicating that regulation of PTEN stability by p53 is a general pathway. Elevated p53 functions to trigger downstream targets, such as caspases (reviewed in ref. 29). The onset of degradation of PTEN has a relatively long delay (>8 hours) under stress induced by proteasome dysfunction (Fig. 1*B*), a phenomenon favoring caspase involvement (30, 31). However, PTEN protein is continually degraded despite MG132 treatment in *TP53*-null MEFs (Figs. 4 and 5). In addition, the reintroduced p53 destabilizes PTEN to a lesser extent compared with endogenous p53 (Fig. 5*B*). These findings together indicate that p53, despite being a key negative regulator of PTEN stability, may not be the exclusive effector in the increasingly complex degradation process of PTEN.

The precise underlying mechanisms of regulation of PTEN protein stability remain unclear. It is unlikely that the reduction of PTEN stability is through ubiquitin-mediated protein degradation because we cannot detect an increase of overall ubiquitinated PTEN after induced stress (data not shown). Furthermore, when cells were treated with proteasome inhibitors, including MG132, MG115, or Velcade, PTEN protein levels actually decreased by ∼6-fold (Fig. 1*A, lane 3*). Similar phenomena have been reported with other genes, such as E2F1 (32, 33). Previously, Torres et al. linked PTEN stability to proteasome-mediated degradation by using the MG132 inhibitor (27). In our hands, we see the opposite results. The discrepancy could be due to the high concentration (50 μmol/L) of MG132 used in their studies. Concentrations this high may promote the transcriptional activation of heat shock genes and then prevent apoptotic cell death by binding to and inactivating caspase-3 and caspase-9 (34). Here, we provide evidence that caspases, especially caspase-3, may be one of the executors of PTEN digestion and hence PTEN destabilization, which would stand to reason that the inactivation of caspase-3 would result in an increase in PTEN. However, this does not exclude the involvement of other enzymes given our observations that the inhibition of caspases can only partially rescue PTEN degradation induced by proteasome dysfunction (Fig. 6*A*). Although our studies suggest that apoptosis may play a role in the degradation of PTEN, we cannot definitively link PTEN degradation to the apoptosis pathway *per se* because mithramycin A, which possesses antiapoptotic effects, causes a decrease in PTEN levels.

Taken together, we propose a model (Fig. 6*B*) that, under cellular stress, increased p53 down-regulates PTEN protein stability by activating caspases, although p53 is a transcriptional activator for *PTEN* expression. Although the PTEN-p53 complex protects p53 from degradation, the association may also facilitate PTEN degradation, thus setting up a finely balanced feedback loop. Our studies provide clues to better understand the regulatory mechanisms of PTEN protein stability and the mutual effect between two important tumor suppressors, PTEN and p53, involved in multiple neoplasias.

[8] Waite and Eng, unpublished data.

Acknowledgments

Received 2/28/2006; revised 4/11/2006; accepted 4/14/2006.

Grant support: American Cancer Society grant RSG-02-151-01CCE and Doris Duke Distinguished Clinical Scientist Award (C. Eng).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank Dr. Lawrence Kirschner for the MEFs (both wild-type and *TP53*−/−), Michelle Sinden for helpful discussions, Dr. Kristin Waite for critical review of several drafts of this article, and Dr. Michael C. Ostrowski for continued support.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024

References

1. Eng C. PTEN: one gene, many syndromes. Hum Mutat 2003;22:183–98.
2. Marsh DJ, Coulon V, Lunetta KL, et al. Mutation spectrum and genotype-phenotype analyses in Cowden disease and Bannayan-Zonana syndrome, two hamartoma syndromes with germline PTEN mutation. Hum Mol Genet 1998;7:507–15.
3. Marsh DJ, Kum JB, Lunetta KL, et al. PTEN mutation spectrum and genotype-phenotype correlations in Bannayan-Riley-Ruvalcaba syndrome suggest a single entity with Cowden syndrome. Hum Mol Genet 1999;8:1461–72.
4. Zhou XP, Waite KA, Pilarski R, et al. Germline PTEN promoter mutations and deletions in Cowden/Bannayan-Riley-Ruvalcaba syndrome result in aberrant PTEN protein and dysregulation of the phosphoinositol-3-kinase/Akt pathway. Am J Hum Genet 2003;73:404–11.
5. Marsh DJ, Dahia PL, Coulon V, et al. Allelic imbalance, including deletion of PTEN/MMACI, at the Cowden disease locus on 10q22-23, in hamartomas from patients with Cowden syndrome and germline PTEN mutation. Genes Chromosomes Cancer 1998;21:61–9.
6. Inaba F, Kawamata H, Teramoto T, Fukasawa I, Inaba N, Fujimori T. PTEN and p53 abnormalities are indicative and predictive factors for endometrial carcinoma. Oncol Rep 2005;13:17–24.
7. Liao XB, Hu DX, Zhou XM, Yu FL, Yuan YC, Chen MJ. Expression of PTEN, p16, p21, and p53 proteins in lung cancer using tissue microarray and their clinical significance. Ai Zheng 2004;23:334–8.
8. Macwhinnie N, Monaghan H. The use of P53, PTEN, and C-erbB-2 to differentiate uterine serous papillary carcinoma from endometrioid endometrial carcinoma. Int J Gynecol Cancer 2004;14:938–46.
9. Tang Y, Eng C. PTEN autoregulates its expression by stabilization of p53 in a phosphatase-independent manner. Cancer Res 2006;66:736–42.
10. Sheng X, Koul D, Liu JL, Liu TJ, Yung WK. Promoter analysis of tumor suppressor gene PTEN: identification of minimum promoter region. Biochem Biophys Res Commun 2002;292:422–6.

11. Stambolic V, MacPherson D, Sas D, et al. Regulation of PTEN transcription by p53. Mol Cell 2001;8: 317–25.
12. Mayo LD, Dixon JE, Durden DL, Tonks NK, Donner DB. PTEN protects p53 from Mdm2 and sensitizes cancer cells to chemotherapy. J Biol Chem 2002;277: 5484–9.
13. Weng LP, Brown JL, Eng C. PTEN coordinates G(1) arrest by down-regulating cyclin D1 via its protein phosphatase activity and up-regulating p27 via its lipid phosphatase activity in a breast cancer model. Hum Mol Genet 2001;10:599–604.
14. MacLaren AP, Chapman RS, Wyllie AH, Watson CJ. p53-dependent apoptosis induced by proteasome inhibition in mammary epithelial cells. Cell Death Differ 2001;8:210–8.
15. Okamura H, Yoshida K, Morimoto H, Haneji T. PTEN expression elicited by EGR-1 transcription factor in calyculin A-induced apoptotic cells. J Cell Biochem 2005; 94:117–25.
16. Koutsodontis G, Kardassis D. Inhibition of p53-mediated transcriptional responses by mithramycin A. Oncogene 2004;23:9190–200.
17. Latonen L, Kurki S, Pitkanen K, Laiho M. p53 and MDM2 are regulated by PI-3-kinases on multiple levels under stress induced by UV radiation and proteasome dysfunction. Cell Signal 2003;15:95–102.
18. Chung JH, Eng C. Nuclear-cytoplasmic partitioning of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) differentially regulates the cell cycle and apoptosis. Cancer Res 2005;65:8096–100.
19. Freeman DJ, Li AG, Wei G, et al. PTEN tumor suppressor regulates p53 protein levels and activity through phosphatase-dependent and -independent mechanisms. Cancer Cell 2003;3:117–30.
20. Myers MP, Pass I, Batty IH, et al. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. Proc Natl Acad Sci U S A 1998;95: 13513–8.
21. Trotman LC, Pandolfi PP. PTEN and p53: who will get the upper hand? Cancer Cell 2003;3:97–9.
22. Wang J, Ouyang W, Li J, et al. Loss of tumor suppressor p53 decreases PTEN expression and enhances signaling pathways leading to activation of activator protein 1 and nuclear factor κB induced by UV radiation. Cancer Res 2005;65:6601–11.
23. Chappell WH, Green TD, Spengeman JD, McCubrey JA, Akula SM, Bertrand FE. Increased protein expression of the PTEN tumor suppressor in the presence of constitutively active Notch-1. Cell Cycle 2005;4:1389–95.
24. Cheng LM, Wang SY, Lin JS. [Expression of phosphatase and tensin homology deleted on chromosome ten(PTEN) and p53 protein and their significance in human hepatocellular carcinomas]. Ai Zheng 2003; 22:42–5.
25. Komazawa N, Suzuki A, Sano S, et al. Tumorigenesis facilitated by Pten deficiency in the skin: evidence of p53-Pten complex formation on the initiation phase. Cancer Sci 2004;95:639–43.
26. Vazquez F, Ramaswamy S, Nakamura N, Sellers WR. Phosphorylation of the PTEN tail regulates protein stability and function. Mol Cell Biol 2000;20:5010–8.
27. Torres J, Rodriguez J, Myers MP, et al. Phosphorylation-regulated cleavage of the tumor suppressor PTEN by caspase-3: implications for the control of protein stability and PTEN-protein interactions. J Biol Chem 2003;278:30652–60.
28. Georgescu MM, Kirsch KH, Kaloudis P, Yang H, Pavletich NP, Hanafusa H. Stabilization and productive positioning roles of the C2 domain of PTEN tumor suppressor. Cancer Res 2000;60:7033–8.
29. Shen Y, White E. p53-dependent apoptosis pathways. Adv Cancer Res 2001;82:55–84.
30. Ding HF, McGill G, Rowan S, Schmaltz C, Shimamura A, Fisher DE. Oncogene-dependent regulation of caspase activation by p53 protein in a cell-free system. J Biol Chem 1998;273:28378–83.
31. Soengas MS, Alarcon RM, Yoshida H, et al. Apaf-1 and caspase-9 in p53-dependent apoptosis and tumor inhibition. Science 1999;284:156–9.
32. Lim JH, Chang YC, Park YB, Park JW, Kwon TK. Transcriptional repression of E2F gene by proteasome inhibitors in human osteosarcoma cells. Biochem Biophys Res Commun 2004;318:868–72.
33. Magae J, Illenye S, Tejima T, et al. Transcriptional squelching by ectopic expression of E2F-1 and p53 is alleviated by proteasome inhibitors MG-132 and lactacystin. Oncogene 1997;15:759–69.
34. Cowan JL, Morley SJ. The proteasome inhibitor, MG132, promotes the reprogramming of translation in C2C12 myoblasts and facilitates the association of hsp25 with the eIF4F complex. Eur J Biochem 2004;271: 3596–611.

Downloaded from http://aacrjournals.org/cancerres/article-pdf/66/12/6139/2864003/6139.pdf by guest on 09 November 2024