PTEN Autoregulates Its Expression by Stabilization of p53 in a Phosphatase-Independent Manner

Yufang Tang1,3 and Charis Eng1,2,4

1Genomic Medicine Institute, Cleveland Clinic Lerner Research Institute; 2Department of Genetics, Case Western Reserve University School of Medicine; 3Human Cancer Genetics Program, Comprehensive Cancer Center, Department of Molecular Virology, Immunology and Medical Genetics, Ohio State University, Columbus, Ohio; and 4Cancer Research UK Human Cancer Genetics Research Group, University of Cambridge, Cambridge, United Kingdom

Abstract

PTEN (phosphatase and tensin homologue, deleted on chromosome 10) is a tumor suppressor with dual phosphatase activity and mutations of its gene, PTEN, have been associated with many sporadic cancers and heritable neoplasia syndromes, including Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. However, accumulating evidence now shows that PTEN may have novel functions other than as a phosphatase. In the present study, we show that PTEN is able to autoregulate its expression through the stabilization of another tumor suppressor p53. We further show that PTEN enhances p53 transactivation, a relationship that requires the interaction between PTEN and p53 and is PTEN phosphatase independent. We show that cell lines from Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome patients with germ line PTEN promoter mutations in the vicinity of the p53-binding motifs have altered p53 regulation. This seems to be due to reduced PTEN stability and decreased PTEN-p53 interactions. Our data provide clues to better understand the regulation of PTEN expression and the possible mechanisms of the pathogenesis of the subset of Cowden syndrome individuals with germ line promoter variation and who lack mutations in the PTEN coding region and splice sites. Importantly, this mechanism also holds for those sporadic tumors that lack intragenic mutations but have hemizygous deletion of PTEN, which includes the promoter region as manifested by loss-of-heterozygosity of 10q markers. The importance of our observations is underlined by the broad spectrum of neoplasias that harbor somatic PTEN or p53 alterations, or both. (Cancer Res 2006; 66(2): 736-42)

Introduction

Somatic alterations in PTEN (phosphatase and tensin homologue, deleted on chromosome 10) have been found in a variety of sporadic malignancies. PTEN somatic alterations occur at a frequency only second to those of TP53. Germ line PTEN mutations cause at least four inherited harramoma tumor syndromes, including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and Proteus and Proteus-like syndromes (1–3). Approximately 85% of Cowden syndrome cases have been found to carry germ line mutations in PTEN, comprising 80% of intragenic mutations and 5% of promoter mutations. PTEN encodes a 55 kDa protein composed of an NH2-terminal phosphatase catalytic domain, a C2-domain binding to phospholipid membranes, and a COOH-terminal regulatory tail involved in posttranslational control. PTEN is a dual-specific phosphatase, which dephosphorylates both lipid and protein substrates (4, 5). PTEN expression is regulated at the transcriptional level by a set of transcriptional factors, including p53, and at the posttranscriptional level by protein localization, modification, and degradation. p53 is a very labile protein under normal conditions, which is maintained by Mdm2-mediated ubiquitination and subsequent proteolysis (6). Although both PTEN and TP53 are frequently mutated in a variety of human cancers, their mutations are almost mutually exclusive (7). Recent evidence suggests that PTEN gene expression is activated by p53 (8). PTEN and p53 can physically interact and form a complex, which subsequently prevents p53 from Mdm2-mediated protein degradation (9). In this study, we show that PTEN up-regulates its own expression by stabilizing p53 protein through a phosphatase-independent interaction between itself and p53.

Materials and Methods

Expression vectors and reporter constructs. TP53 cDNA full-length (1-393) and COOH-terminal deletion mutant (1-363), PTEN-WT, C124S, and G129E mutants, and PTEN186-351, 186-403 truncation mutants were subcloned into pcDNA3 (Invitrogen, Carlsbad, CA). PTEN full promoter (−1,344 to −1) and shorter promoter (−1,344 to −1,001) were amplified from normal human or patient genomic DNA harboring mutations and subcloned into pGL3.1 basic vector (Promega, Madison, WI). All constructs were verified by DNA sequencing.

Cell lines and culture. MCF7 and PC3 cells were cultured in DMEM (Life Technologies-Invitrogen) supplemented with 10% fetal bovine serum (FBS), and 100 units/mL penicillin and streptomycin (Life Technologies-Invitrogen). Clonal lymphoblast cell lines were generated from Cowden syndrome patients and cultured in RPMI medium supplemented with 10% FBS and 100 units/mL penicillin and streptomycin. For MG132 (Calbiochem, San Diego, CA) treatment, lymphoblast cells were grown between 103 and 106/mL and treated with 4 μmol/L MG132 for 4 hours at 37°C.

Western blot analysis and immunoprecipitation. Whole cell extract was prepared with M-PER ( Pierce, Rockford, IL) supplement with protease inhibitor cocktail (Sigma, St. Louis, MO). Nuclear extracts were purified by NE-PER (Pierce). Target proteins were detected by Western blotting using anti-p53, Mdm2, actin (Santa Cruz Biotechnology, Santa Cruz, CA), PTEN (Cascade Biosciences, Winchester, MA), phospho-Mdm2, phospho-Akt, and Akt (Cell Signaling Technology, Beverly, MA) antibodies, accordingly. For immunoprecipitation experiments, 500 μg of cell lysate was incubated with 1 μL p53 antibody plus 50 μL Protein A agarose beads (Immobilized Protein A, Pierce) at 4°C overnight. Eluted proteins were detected using standard Western blotting procedure.

Transient transfection and luciferase-reporter assay of various PTEN promoter constructs. MCF7 or PC3 cells were seeded 24 hours before transfection with DMIRE reagent (Invitrogen) or FuGene (Roche Diagnostics, Indianapolis, IN), respectively. Cells were cotransfected with
Results

Naturally occurring PTEN promoter mutations from Cowden syndrome patients affect p53 transactivation of PTEN expression. When the PTEN promoter region was analyzed in previously believed PTEN mutation–negative Cowden syndrome cases, 10% were found to harbor heterozygous germline promoter mutations (10). In addition, 10% of Bannayan-Riley-Ruvalcaba syndrome patients were found to have large germline deletions, all of which involved the promoter (10). Among the patients reported with germline PTEN promoter mutations, five samples harbor mutations in the vicinity of the p53-binding sites: samples 4666, 13118, and 16639 have mutations inside of the p53-binding sites (−1,190 to −1,157; ref. 8), whereas samples 10587 and 11553 contain mutations 10 bp downstream from the sites (Fig. 1A). To investigate the biochemical effect of the mutations, we examined the promoter activities of PTEN from the five samples using the luciferase reporter assay and compared with that of the wild type. Both the full-length promoter (1,344 bp) and the −1,344 to −1,001 (344 bp) short promoter, which contains the two conserved p53-binding sites, were amplified from normal and five patient samples and subcloned into pGL3.1 basic vector (Fig. 1B). Luciferase activity was detected after transfection of the respective reporter plasmid alone or together with p53 in a breast cancer cell line, MCF7. The basal PTEN promoter activities from the five patient samples are not significantly different from those with wild-type sequence (data not shown). However, PTEN expression by p53 activation is reduced in patient samples (Fig. 1C), especially when using the short promoter region. The mutation −1,182T>C in patient 16639, which is located at the p53-binding site, has the most reduced promoter activity when responding to p53 activation. Mutation in patient 10587, −1,142C>T, which is outside of the p53-binding motifs, shows the least effect, and the mutation in patient 4666, −1,177 C>T, which is located between the two p53-binding motifs, shows an intermediate effect on the transcriptional activity of p53 on PTEN expression. These results indicate that the cis-acting effect of mutations on the transcriptional activity of p53 correlates with the distance between the mutations and the binding motifs. However, the mutations in patient 11553, −1144A>C, and in patient 13118, −1,174 C>A, have no effect on p53 transactivation (for summary, see Table 1).

To examine whether the PTEN promoter mutations affect the transcriptional complex binding to the PTEN promoter, we did an electrophoretic mobility shift analysis (EMSA) by using the genomic fragments corresponding to −1,220 to −1,130. As shown in Fig. 1D, a shifted band is detected by nuclear extract from MCF7 cells expressing p53 but not from PC3 cells that are p53 null. The specificity of p53 in the formed complex is further confirmed by competition assay because the shifted band is abolished by a wild-type p53-binding oligonucleotide and is not affected by a mutated p53 oligonucleotide (Fig. 1D). The intensities of the shifted bands are different when using mutated PTEN promoter regions from five patient samples (Fig. 1E). Probes from samples 16639, 4666, and 10587 show reduced band intensities, whereas those from samples 11553 and 13118 have similar band intensity as wild type (Fig. 1E). The specificity of p53 binding is also confirmed by competition assay. Thus, the relative ability to form an electrophoretically retarded complex in the patient samples seems to correlate with the relative promoter activity (for summary, see Table 1), which may be the reason for the aberrant PTEN expression when responding to p53 in patient cells.

Cowden syndrome patient cells with PTEN promoter mutations possess less stable PTEN protein and PTEN–p53 interaction. Because PTEN gene expression regulation by p53 is affected by promoter mutations in the above Cowden syndrome patient cells, the biochemical outcome of these mutations on PTEN/p53 expression was examined. Both PTEN and p53 protein levels in the patient samples are similar to control (Fig. 2A). Because Akt is the direct downstream target of phosphoinositide-3-kinase (PI3K) that is antagonized by PTEN, phospho-Akt was used as functional readout of PTEN. All the patient samples and control were found to have equal amounts of phospho-Akt, when Akt served as an internal protein control (Fig. 2A). Interestingly, we observed that PTEN proteins in the patient samples were more easily degraded when experiments were done on old samples that had undergone several cycles of freezing-thawing, which is not seen when we use freshly prepared samples as in Fig. 2A (data not shown). To test the protein stability in these patient samples, protein extracts were stored at room temperature for 3 days and then analyzed. All five patient samples have decreased PTEN levels and phospho-Akt levels are increased in two samples (Fig. 2B; Table 1). However, this decreased PTEN protein stability seems to be promoter mutation specific because it is not seen in patients with mutations in PTEN coding regions, control lymphoblasts that are mutation negative, or standard cell lines. To our surprise, p53 protein levels are also increased in three patient samples (Fig. 2B). However, levels of Mdm2 and phospho-Mdm2, which regulate p53 protein stability, remain constant in all samples (Fig. 2B).

Because PTEN and p53 can be associated together and p53 levels are elevated in some of the patient samples, we tried to determine if there is a connection between the p53-PTEN complex and PTEN protein stability. The physical interaction between PTEN and p53 were analyzed by coimmunoprecipitation in the patient cells as well as in control cells. Interestingly, the two patient samples (13118 and 16639), which contain less stable PTEN proteins, show much less precipitated PTEN than control, although all the samples have equal amounts of PTEN protein in the starting extracts (Fig. 2C; left; for summary, see Table 1). When the cells were treated with a proteasome inhibitor, MG132, which stabilizes p53 protein

5 K.A. Waite and C. Eng, unpublished data.
all the samples show equal strength of PTEN-p53 interaction (Fig. 2C, right). This indicates that all the cells have an equal ability to form PTEN-p53 complex; however, the complexes are less stable in patient cells than that in control cells.

PTEN facilitates p53 transactivation of PTEN expression by stabilizing p53 protein independent of the phosphatase activity of PTEN. To determine if PTEN can autoregulate its expression through by directly interacting with p53, we coexpressed

![Figure 1](image1.png)

**Table 1. Summary of patient information and findings in this study**

<table>
<thead>
<tr>
<th>Family number</th>
<th>Promoter mutation location</th>
<th>PTEN stability</th>
<th>p53-PTEN interaction</th>
<th>Response to p53 activation</th>
<th>p53 binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal human</td>
<td>Wild type</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>4666</td>
<td>$-1177C&gt;T$</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>10587</td>
<td>$-1142C&gt;T$</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>11553</td>
<td>$-1144G&gt;C$</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>13118</td>
<td>$-1174C&gt;A$</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>16639</td>
<td>$-1182T&gt;C$</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Figure 1. Altered p53 transactivation on PTEN promoter in Cowden syndrome patient samples. A, location of mutations in the five Cowden syndrome patient samples. p53-binding motifs are shown as emboldened nucleotides. B, schematic diagram of the PTEN promoter reporter plasmids. C, transactivation of p53 on PTEN promoter is affected by the mutations occurring in Cowden syndrome patients. MCF7 cells were seeded 24 hours before transfection. PTEN full-length and 344 bp fragment promoter derived from normal human and patient samples were cloned and verified. Cells were cotransfected with 0.5 μg of pcDNA3 or pcDNA3-p53, 0.5 μg of PTEN promoter-luciferase reporter construct, either full-length or 344 bp, and 20 ng of Renilla luciferase plasmid by DMIRE reagent. Luciferase activities were measured and normalized. Columns, mean of three independent experiments. D, p53 binds to PTEN promoter. The binding of p53 to the PTEN promoter region (−1,220 to −1,130) was measured using EMSA. Fragments were amplified from normal human genomic DNA by PCR and sequence was verified. The restriction-digested fragment was labeled with [α-32P]dCTP. Nuclear extracts were prepared from both MCF7 and PC3 cells. Two micrograms of nuclear extracts were incubated with probe for 20 minutes at room temperature. Wild-type p53-binding oligonucleotide (50- and 100-fold) was used as a specific competitor (Sp. Comp.) and mutant p53-binding oligonucleotide (100-fold) was used as nonspecific competitor (Non. Sp. Comp.) for competition assay. The bound complex was resolved in a 4% nondenaturing polyacrylamide gel in 0.5 Tris-borate EDTA buffer at 150 V for 3 hours. The gel was dried and subjected to phosphoimager. E, p53-binding ability to PTEN promoter is affected by mutations in Cowden syndrome patients. The binding of p53 to the PTEN promoter region (−1,220 to −1,130) was measured as in (D). Probes were made from five Cowden syndrome patient samples and nuclear extracts were prepared from normal human lymphoblast cells. N, negative control.
PTEN or p53 or both with PTEN promoter-driven reporter plasmids and determined the promoter activity using a dual-luciferase assay. Both wild-type, full-length PTEN promoter (C01,344 to C01) and the 344 bp (C01,344 to C01,001) genomic fragment were examined. To clearly test the effect of PTEN on p53, a PTEN and p53 double-null cell line, PC3, was used for promoter activity assays. As shown in Fig. 3A (top), p53 by itself induces a 2.2 ± 0.6-fold up-regulation of full-length PTEN promoter activity. Expression of PTEN alone has no effect. However, when PTEN was coexpressed with p53, there was an increase in transactivation of p53 up to 5.8 ± 3.6-fold increase on the full-length PTEN promoter.

When the 344 bp PTEN promoter was cotransfected with p53 and PTEN, the luciferase activity increased up to 10.4 ± 2.7-fold, compared with the ~3.4 ± 0.2-fold increase after p53 expression alone (Fig. 3A, top).

PTEN and p53 protein levels in the same samples evaluated for luciferase activity were detected to confirm their equal expression.
PTEN protein levels remain equal when PTEN is expressed alone or together with p53 (Fig. 3A, top). To our surprise, p53 levels are increased when coexpressed with PTEN compared with when p53 was expressed alone (Fig. 3A, top). This increased p53 protein level is not seen when the p53 construct was cotransfected with pcDNA3 vector (data not shown). This observation suggests that the elevated p53 transactivation by the presence of PTEN protein may be due to the increased p53 protein levels and PTEN may stabilize p53 protein level.

To examine if the phosphatase activity of PTEN is involved in p53-mediated transregulation of PTEN, we used two PTEN phosphatase-dead mutants, C124S (both protein and lipid phosphatase dead) and G129E (only lipid phosphatase dead). The effects of both mutants on p53 transactivation of the PTEN promoter were detected by cotransfection with p53. Assays of luciferase activity in PC3 cells show that both PTEN-C124S and -G129E mutants increase the p53 transactivation of the full-length PTEN promoter to the same extent as wild-type PTEN (Fig. 3B, top). Our data indicate that neither the lipid nor protein phosphatase activity of PTEN is necessary for its effect on p53. Correlated with luciferase activity, p53 levels are also increased in the samples coexpressing either PTEN-C124S or PTEN-G129E, similar to wild-type PTEN (Fig. 3B, bottom).

The interaction between p53 and PTEN is required for the effect of PTEN on p53 transactivation. It has been reported that PTEN can physically associate with endogenous p53 and the interacting motif is located at the C2 domain (186-351) of PTEN (12). To examine if the interaction between PTEN and p53 is necessary for the apparent autoregulation of PTEN, we proceeded to make two PTEN truncation mutants, PTEN186-351 and PTEN186-403, which have the ability to interact with p53 (Fig. 4A; ref. 12). We found that both PTEN186-351 and PTEN186-403 sufficiently increase the transactivation of p53 on the PTEN promoter to almost the same extent as the full-length PTEN (Fig. 4B, top). This result further confirms that PTEN phosphatase activity, whose domain is located within 1 to 185, is not required for the effect of PTEN on p53. We analyzed p53 protein levels in the same samples used for luciferase assays and found that p53 levels in samples cotransfected with PTEN truncation mutants were higher than that in sample expressing p53 alone, and similar to that with wild-type PTEN coexpression (Fig. 4B, bottom). Taken together, we conclude that the interaction between PTEN and p53 is necessary and sufficient to facilitate the transactivation of p53 on PTEN expression.

To further determine if the interaction between PTEN and p53 is required for PTEN autoregulation, we generated a p53 COOH-terminal deletion mutant (1-363; Fig. 4A). It has been reported that p53 (1-363) maintains the transactivation on its target genes; however, it loses 90% of the ability to interact with PTEN (12). Luciferase assay results with the short PTEN 344 bp promoter showed that, indeed, p53 (1-363) activates even higher PTEN expression than wild-type p53 (Fig. 4C, top). This indicates that the COOH terminus of p53 has an inhibitory effect on its function, similar to the previous findings (13). However, coexpression of PTEN with p53 (1-363) has less effect in facilitating its transactivation (∼1.2-fold) than that with wild-type p53 (∼2.4 fold; Fig. 4C, top). The p53 protein levels in corresponding samples were then examined. p53 (1-363) is more stable than the wild-type, as reported before (14). However, unlike p53 wild-type, p53 (1-363) levels are not increased by PTEN (Fig. 4C, bottom). These results indicate that the PTEN-p53 interaction is required for the increase of p53 protein and, in that manner, enhances p53 transactivation of PTEN expression.

A p53 half-life study was carried out in transient transfected PC3 cells to test if PTEN affects p53 protein stability. Both p53 wild type and p53 (1-363) mutant were transfected alone or together with PTEN into PC3 cells. Cells were treated with 50 μg/mL cyclohexamide for additional indicated hours after 48 hours posttransfection incubation. Indeed, we found that the wild-type p53 half-life (∼18 hours) in p53- and PTEN-coexpressing cells is much longer than that (∼11 hours) in cells expressing p53 alone (Fig. 4D). The p53 (1-363) mutant shows almost similar half-lives in both transfections (∼24 hours in coexpression with PTEN and ∼22 hours in expression alone; Fig. 4D). Our results provide direct evidence that PTEN is able to stabilize p53 protein and, consequently, regulate its own transcription.

Discussion

The biochemical outcome of PTEN genetic alteration varies and, in many instances, remains unexamined. The typical mechanism of PTEN inactivation in neoplasms is intragenic mutation accompanied by hemizygous deletion [loss of heterozygosity (LOH)]. In some cancers, PTEN protein is reduced although no mutations and no LOH were detected in intragenic regions (15, 16), suggesting that there are additional mechanisms of regulation. PTEN protein from Cowden syndrome patients with germline promoter mutations showed alternative molecular weight bands and a laddering effect on SDS-PAGE (10). Our patient studies indicate that germline mutations in the PTEN promoter can interfere with its transcriptional regulation and protein stability. We suggest that altered p53 regulation of PTEN expression, due to PTEN itself, contributes to the development of a subset of Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome. These observations suggest that it is important to understand how PTEN expression is regulated by transcriptional factors and corresponding cis-acting elements at the 5′-untranslated region: germline mutations and, more importantly, variants of unknown significance, are being described in Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome patients. Our current data indicate that such mutations, and by implication, even some variants of unknown significance, have an effect on PTEN protein levels. Importantly, whereas TP53 mutations are the most common in sporadic neoplasms, somatic PTEN alterations are the second most frequent. Thus, the interplay and autoregulatory mechanism of these two molecules will be vital in understanding both heritable and sporadic neoplasia. It is surprising and interesting that some of the mutations we study are not located within the p53-binding motif per se, yet the binding of the transcription complex to the promoter is still affected. The mutations may affect the regulatory complex and PTEN could be a co-factor because PTEN-p53 interactions enhance the binding ability of p53 to the DNA target (12). However, additional investigation will be necessary to obtain further direct evidence of PTEN binding to DNA.

Another mechanism of gene expression regulation is to regulate the stability of transcriptional factors (i.e., p53) or the expressed proteins (i.e., PTEN protein) here. We now show that PTEN increases p53 protein level directly by increasing the half-life of p53. This provides another mechanism of PTEN-dependent regulation of p53 besides indirectly through PI3K pathway (17).
We have shown that this autoregulatory role of PTEN through p53 seems to depend on the physical interaction between PTEN and p53 independent of its phosphatase activities. Human genetic studies have revealed that the majority of mutations in the PTEN coding region occur throughout the amino-terminal catalytic domain, which is responsible for the enzymatic activity of PTEN (18). However, ~20% of mutations localize to the carboxyl terminus, suggesting PTEN has a broader function other than being a phosphatase. The precise mechanisms underlying decreased PTEN stability in patient cells remain unclear although it could be a general defect in patients carrying PTEN promoter mutations. Our findings here indicate p53 may have negative effect in the process of PTEN degradation. It is unlikely that the regulation of PTEN stability in these samples is through proteasome-mediated protein degradation because when cells were treated with proteasome inhibitors, either MG132 or Velcade, no decrease in PTEN protein degradation was seen (data not shown).

Taken together, our study provides evidence that PTEN has the ability to autoregulate its own expression and that this autoregulation occurs through stabilizing p53. The interaction between PTEN and p53 is required for the transregulation of PTEN expression and the phosphatase activities of PTEN are not necessary for the effect. Cowden syndrome and

---

**Figure 4.** Interaction between PTEN and p53 is required for the facilitation of PTEN on the transactivation of p53. A, schematic illustration of PTEN/p53 truncation mutants. PTEN (186-351), PTEN (186-403), and TP53 (1-363) were subcloned into pcDNA3 vector. B, PTEN-p53 interaction is sufficient for the effect of PTEN on p53 transactivation of the PTEN promoter. PTEN wild type, or PTEN truncation mutants, PTEN (186-351), PTEN (186-403), were co-transfected with or without p53. Luciferase assay was carried out as before. Columns, means of three independent experiments (top). Total protein extracts were collected and expression levels of p53 were detected (bottom). C, PTEN-p53 interaction is required for the effect of PTEN on p53 transactivation of PTEN promoter. p53 WT or p53 (1-363) truncation mutant was co-transfected with or without PTEN. Luciferase assay was carried out as before. Columns, means of three independent experiments (top). PTEN can stabilize p53 wild type but not the COOH-terminal deletion mutant (bottom). D, PTEN increases the half-life of wild-type p53 but not the p53 (1-363) mutant. PC3 cells were transfected with p53 wild type or p53 (1-363) mutant alone (control) or together with PTEN. Cells were treated with 50 μg/mL of cyclohexamide (CHX) in serum-free medium for the additional indicated hours, 48 hours posttransfection. Cell lysates were collected and p53 protein level was measured by Western blotting. The band intensities were measured by ImageQuaNT and the percentage of remaining p53 protein at each time point was calculated (top) and shown (bottom).
Bannayan-Riley-Ruvalcaba syndrome patients carrying mutations surrounding p53-binding sites on the *PTEN* promoter affect PTEN expression most likely due to impaired PTEN protein stability and reduced PTEN-p53 interaction. Our results contribute to the better understanding of the etiopathogenic mechanisms for Cowden syndrome/Bannayan-Riley-Ruvalcaba syndrome with *PTEN* promoter mutations or deletions as well as for sporadic neoplasia with promoter deletions and of the regulation on PTEN expression. Our observations provide an important mechanism to explain the pathogenicity of germ line promoter mutations and even variants (single nucleotide polymorphism) in Cowden syndrome/Bannayan-Riley-Ruvalcaba syndrome. Furthermore, our data provide evidence of the existence of a PTEN-p53 auto-regulatory loop, which is a vital observation because of the prominence of genetic and epigenetic alterations of both *PTEN* and *TP53* in many sporadic neoplasias.

**Acknowledgments**

Received 5/5/2005; revised 11/7/2005; accepted 11/10/2005.

Grant support: American Cancer Society grant RSG-02-151-01CCE (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 Kristin A. Waite, Xiao-Ping Zhou, Marcus Pezzolesi, and Michelle Sinden for helpful discussions and critical review of drafts of the manuscript.

---

**References**

PTEN Autoregulates Its Expression by Stabilization of p53 in a Phosphatase-Independent Manner

Yufang Tang and Charis Eng


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/66/2/736

Cited articles
This article cites 18 articles, 8 of which you can access for free at:
http://cancerres.aacrjournals.org/content/66/2/736.full.html#ref-list-1

Citing articles
This article has been cited by 19 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/66/2/736.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.