PTEN Suppresses Breast Cancer Cell Growth by Phosphatase Activity-dependent G1 Arrest followed by Cell Death

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ABSTRACT

PTEN/MMAC1/TEP1, a tumor suppressor gene, is frequently mutated in a variety of human cancers. Germ-line mutations of phosphatase and tensin homolog, deleted on chromosome ten (PTEN) are found in two inherited hamartoma tumor syndromes: Cowden syndrome, which has a high risk of breast, thyroid, and other cancers; and Bannayan-Zonana syndrome, a related disorder. PTEN encodes a phosphatase that recognizes both protein substrates and phosphatidylinositol-3,4,5-triphosphate. The lipid phosphatase activity of PTEN seems to be important for growth suppression through inhibition of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway. We established clones with stable PTEN expression controlled by a tetracycline-inducible system to examine the consequences of increased levels of wild-type and mutant PTEN expression in a well-characterized breast cancer line, MCF-7. When we overexpressed PTEN in MCF-7, growth suppression was observed, but only if PTEN phosphatase activity is preserved. The initial growth suppression was attributable to G1 cell cycle arrest, whereas subsequent growth suppression was attributable to a combination of G1 arrest and cell death. Of note, the decrease in Akt phosphorylation preceded the onset of suppression of cell growth. Treatment of MCF-7 cells with wortmannin, a PI3K inhibitor, caused cell growth inhibition in a way similar to the effects of overexpression of PTEN in this cell line. In general, the inverse correlation between PTEN protein level and Akt phosphorylation was found in a panel of breast cancer cell lines. Therefore, PTEN appears to suppress breast cancer growth through down-regulating PI3K signaling, which leads to the blockage of cell cycle progression and the induction of cell death, in a sequential manner.

INTRODUCTION

The tumor suppressor gene PTEN/MMAC1/TEP1 has been mapped to chromosome 10q23.3 and cloned based on homozygous deletions in breast, brain, and prostate cancers and through its homology with tyrosine phosphatase and tensin homolog, deleted on chromosome ten (PTEN) (1,2). Germ-line PTEN mutations are found in the autosomal dominant CS, which is characterized by multiple hamartomas as well as an increased risk of developing breast and thyroid cancers (3–6). A related developmental disorder, Bannayan-Zonana syndrome, has been shown to be allelic with CS (7). In the murine model, homozygous deletion of PTEN is embryonic lethal (8–10). Nonetheless, in pten-deficient mice, an increase in cell proliferation throughout the premorbid embryo, particularly in the ectoderm region, has been observed. Furthermore, embryonic fibroblasts from pten-deficient mice exhibited decreased sensitivity to apoptosis. Heterozygous knockout mice, on the other hand, develop tumors in multiple organs (9, 11). Therefore, both genetic and biological evidence suggests that PTEN has an important function in both normal embryonic development and tumorigenesis.

PTEN is a dual specificity phosphatase, i.e., it possesses phosphatase activity on phosphotyrosyl and phosphoseryl/threonyl residues. It appears to be a major phosphatase in the PI3K pathway, acting as the phosphatidylinositol 3-phosphate (8, 12). PTEN has been shown to be implicated in cell migration and spreading through interaction with focal adhesion kinase (13) and in cell growth suppression through inhibition of PI3K/Akt-mediated signal transduction (8, 14–16). Several studies (14, 16, 17) have shown that transient introduction of PTEN into PTEN-null glioma cell lines causes cell cycle arrest at the G1 phase but not apoptosis (16, 18). In contrast, one group reported that transient expression of PTEN by adenoviral DNA delivery into breast cancer cell lines induces apoptosis but not cell cycle arrest (15). Hence, it is still unclear whether PTEN plays distinct tissue-specific roles, i.e., only apoptosis in breast cancer cells versus G1 arrest in glioma cells. A number of “gatekeeper” tumor suppressors, such as p53 (19), RB (20), and APC (21, 22), are involved in the regulation of both cell cycle progression and cell death. Whether the tumor suppressor PTEN could function through regulating both the cell cycle and cell death in the same cell type is unclear. Therefore, we sought to determine whether PTEN could indeed mediate both the cell cycle and cell death and whether one is dependent on the other in the context of breast cancer, the major component cancer of CS, by using a Tet-inducible stable transfection system. Furthermore, we chose MCF-7, a breast cancer cell with endogenous wild-type functional PTEN, so that when mutant constructs are transfected, it would mimic the in vivo human situation; CS mutations are heterozygous, and the great majority of CS and primary sporadic breast tumors do not have biallelic PTEN mutations (23).

MATERIALS AND METHODS

Cell Culture. Breast cancer cell lines BT-549, BT-20, MDA-MB436, and MDA-MB-468 were obtained from the American Type Culture collection; HS-5787, MDA-MB-231, and T-47D were generous gifts from Dr. Jim Xiao (Boston Medical Center, Boston, MA), and ZR-75-1 was from Dr. Kornelia Polyak (Dana-Farber Cancer Institute, Boston, MA). The breast cancer cells were grown in DMEM/10% fetal bovine serum (Life Technologies, Inc., Grand Island, NY) with 100 units/ml penicillin G (Sigma Chemical Co., St. Louis, MO) and 100 μg/ml streptomycin sulfate (Sigma). The MCF-7/Tet cell line (Clontech, Palo Alto, CA) and PTEN stable expressing clones were maintained in similar media plus 100 μg/ml Geneticin and 1 μg/ml Tet (Sigma).

Plasmid Construction and Transfection. The PTEN cDNA was obtained by PCR, using the normal human thyroid cDNA as template and the following primers: 5′-CATCTTCTCTCCATTTTCTTCA and 5′-TTTTCTATGTTTATCCCTTCT and which span the entire coding region of PTEN (27). The
resulting PCR product was cloned into pCR2.1 vector. The inducible mammanian cell expression vector pUHD10–3 (24), 25), which contains a Tet-suppressible (Tet-off) promoter, was used to generate two sets of PTEN expression constructs. The wild-type PTEN cDNA, including full-length PTEN coding sequence, was cloned into pUHD10–3 to generate pUHD10–3/PTEN.WT. The conserved cysteine residue at codon 124 was mutated to serine by PCR-based site-directed mutagenesis to generate phosphatase dead mutant pUHD10–3/PTEN.CS. The MCF-7/Toff cell line, which was stably transfected with the Tet-controlled transactivator expression plasmid pUHD15–1, was used to establish two sets of stable expression cell lines that can be induced to express wild-type PTEN, MCF-7/PTEN-wt, and phosphatase-dead PTEN, MCF-7/PTEN-cs. Transfections were done using Lipofectamine (Life Technologies). Exponentially growing cells (5 × 10^6) were transfected with 1 μg of plasmid DNA on a 35-mm dish. The cells were transferred to 100-mm dishes 24 h after transfection and selected by puromycin (Sigma) at 1 μg/ml for 6 days. Tet (1 μg/ml) was included in the culture medium to silence the ectopic expression of PTEN during the process of cloning.

**Induction of PTEN Expression.** Subconfluent stock cells were washed three times with medium, once with phosphate-buffered NaCl solution (150 mM NaCl, 1.35 mM KH₂PO₄, and 2.7 mM Na₂HPO₄, pH 7.2), and then trypsinized. Equal numbers of cells were plated into Tet-free culture medium to induce PTEN expression and into medium containing 1 μg/ml Tet as a control and cultured for various lengths of time as indicated in "Results."

**Cell Growth Assay.** Cell growth was measured by methylene blue. Equal numbers of cells were plated into Tet + and Tet − media in 12-well plates and cultured for various times. After incubation, medium was removed, and cells were washed with phosphate-buffered NaCl solution and fixed with 12.5% glutaraldehyde (Fisher, Fair Lawn, NJ) for 20 min at room temperature. Cells were rinsed with distilled water and incubated with 0.05% methylene blue (Sigma) for 30 min, again rinsed with water, and then incubated with 800 μl of 0.33 M HCl for 30 min to extract the methylene blue. Absorption was measured at 595 nm. The ratio of the absorption in Tet + cultures to the absorption in Tet − cultures at each time point was calculated and presented as percentage of cell growth.

**FACS Analysis.** Assays were performed in p100-mm dishes. At the end of incubation, cells were trypsinized and washed into ice-cold phosphate-buffered NaCl solution. Cells were then fixed by adding them dropwise into ice-cold 80% ethanol while vortexing, followed by incubation on ice for 60 min. The fixed cells were washed with cold phosphate-buffered NaCl solution and incubated at 37°C for 30 min in 0.5 ml phosphate-buffered NaCl solution containing 10 μg/ml of propidium iodine (Sigma) and 5 μg/ml RNase A (New England Biolab, Beverly, MA). DNA content was determined by FACS scan analysis (Becton Dickinson).

**Cell Death Assay.** Dead cells were determined by trypan blue staining. Both floating cells and trypsinized attached cells were collected and incubated with 0.2% trypan blue for 5 min at room temperature. Blue cells and total cells were counted. Cell death was presented as percentage of blue cells from cell lysates by centrifugation at 4°C. Protein concentration was calculated by Bradford reagent. The Bradford reagent and other chemicals were purchased from Sigma.

**Protein Extraction and Immunoblotting.** After PTEN induction, cells were washed twice with ice-cold phosphate-buffered NaCl solution and lysed in cold lysis buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton X-100, 0.5% NP40, 1 mM EDTA, 1 mM EGTA, 5 μM phenylmethylsulfonyl fluoride, 5 μg/ml of leupeptin, pepstatin A, and aprotinin, 1 mM Na₃VO₄, 2 mM NaF, and 2 mM Na₃PO₄] for 10 min on ice. Insoluble material was removed from cell lysates by centrifugation at 4°C. Protein concentration was calculated by Bradford reagent and other chemicals were purchased from Sigma.

**Cell lysates were mixed with equal volumes of 2× Laemmli sample buffer, boiled for 10 min, resolved by 10% SDS-PAGE, and transferred onto nitrocellulose membrane. The membranes were blocked with 5% nonfat dry milk in TBST (10 mM Tris-HCl, pH 8.0, 100 mM NaCl, and 0.05% Tween 20) for 1 h at room temperature and then incubated with appropriate primary antibody for 2 h at room temperature or overnight. The membranes were washed and incubated with horseradish peroxidase-conjugated secondary antibody (Promega Corp., Madison, WI) at 1:5000 dilution for 1 h at room temperature. Protein signals were detected by enhanced chemiluminescence (Amersham, Piscataway, NJ).

For growth factor stimulation, cells were starved by exposure to serum free medium for 24 h before adding growth factors. Both insulin and EGF were purchased from Life Technologies, Inc. The anti-PTEN monoclonal antibody (26) or polyclonal TB-166 (27) was used at 1:250 or 1:1000 dilution, respectively. The polyclonal anti-phospho-Akt, anti-Akt, anti-phospho-MAPK, and anti-MAPK (New England Biolab, Boston, MA) were used at 1:1000 dilution, and monoclonal anti-a-tubulin (Sigma) was used at 1:5000 dilution.

**RESULTS**

**Tightly Controlled Expression of PTEN in MCF-7 Cells.** To investigate the function of PTEN in breast cancer and the mechanism of tumorigenesis, we attempted to alter PTEN activity by stable overexpression of PTEN in cultured breast cancer cells. For our studies, we wished to ensure generation of stable clones with controlled expression. Among several available mammalian inducible expression systems, the Tet-off system has been successfully used in some cell lines (25, 28, 29). Our initial efforts were to use this system to generate stable PTEN-expressing clones in both PTEN-positive and PTEN-null breast cell lines. Among several breast cancer cell lines, we chose the MCF-7 line for two major reasons: (a) the Tet-off system seemed to work well in this line; and (b) more importantly, we wanted to eventually examine mutant PTEN in a heterozygous state that mimics the in vivo situation in CS breast cancer and sporadic primary breast carcinomas.

Constructs expressing either wild-type PTEN or a phosphatase-dead mutant, C124S, under the control of the Tet-off promoter were transfected into MCF-7/Toff cells. Stable clones, which could be induced to express either wild-type PTEN or mutant PTEN protein, were generated and screened for PTEN expression by Western blot using anti-PTEN antibody (Fig. 1). In general, comparable amounts of PTEN protein were induced in the two sets of transfectants (Fig. 1A). Depending on the individual clone, anywhere from <1-fold to ~4-fold increased expression of PTEN, compared with control levels, could be induced. PTEN protein levels of all clones growing in the presence of Tet was indistinguishable from the endogenous level of MCF-7/Toff cells, indicating that there is no leaky activity of the Tet-off promoter in this cell line. The increase in PTEN protein expression appeared at 8 h and reached its maximum level at 36 h after withdrawing Tet (Fig. 1B). Furthermore, the level of PTEN induction could be finely controlled by the concentration of Tet (Fig. 1C). A concentration of Tet as low as 10 ng/ml is sufficient to suppress PTEN expression.

Clone MCF-7/PTEN-wt1 derived from pUHD10–3/PTEN.WT transfectants and clone MCF-7/PTEN-cs3 from pUHD10–3/PTEN.CS transfectants express equivalent amounts of PTEN protein and were chosen for additional experiments.

**PTEN Suppresses Cell Growth.** To investigate whether increased levels of either wild-type PTEN or phosphatase-dead mutant PTEN in MCF-7 had effects on growth properties, we induced both wild-type and mutant PTEN expression over a time course. Parental MCF-7/Toff, MCF-7/PTEN-wt, and MCF-7/PTEN-cs cells were seeded at equal density and cultured in the presence or absence of Tet. The number of cells after culture at each time point was determined by methylene blue staining, and the ratio of the number of cells in Tet− cultures to the number in the Tet+ cultures was calculated. After 48 h induction, the cell number of MCF-7/PTEN-wt grown in the absence of Tet started to decrease, and at 120 h, reached 50% of the cell number compared with that of MCF-7/PTEN-wt grown in the presence of Tet (Fig. 2). The phosphatase-dead mutant carries the C124S mutation described in the germ-line of CS patients (30). It has been shown that this substitution completely abrogates PTEN phosphatase activity (18). In contrast to overexpression of wild-type PTEN, over-
expressing the C124S mutant in MCF-7 cells did not alter cell growth over time, indicating that growth suppression by PTEN is mediated through its phosphatase activity.

**PTEN Blocks G1 Progression.** To determine whether the PTEN-mediated growth inhibition in MCF-7 cells was attributable to cell cycle arrest, cell cycle distribution was analyzed by FACS analysis. The MCF-7/Toff, MCF-7/PTEN-wt, and MCF-7/PTEN-cs cells were grown in Tet-free media to induce PTEN expression and in parallel, in media containing Tet as a control, for 72 h. As shown in Fig. 3A, the induction of PTEN expression resulted in a significant increase in the numbers of cells at G1 and decrease in the number of cells at S phase. The number of cells at G1 increased from 52.1% (MCF-7/PTEN-wt, Tet−) to 68.6% (MCF-7/PTEN-wt, Tet+), and the number of cells at S decreased from 31.0 to 19.5%. No significant changes in cell cycle distribution were observed in MCF-7/PTEN-cs cells, suggesting that the phosphatase activity was required for PTEN-mediated G1 arrest. Tet alone had no effect on cell cycle distribution in MCF-7 cells.

To examine the precise time point at which PTEN exerted its effect on cell cycle distribution, a time course of cell cycle phase distribution was performed. Fig. 3B demonstrated that a significant increase in the number of cells in G1 phase began at 36 h of PTEN induction, 12 h before the reduction in cell number (Fig. 2). Interestingly, no cell death could be detected at the 72-h induction time (Fig. 4), suggesting that the mechanism of growth suppression mediated through PTEN in MCF-7 cells was G1 arrest, at least, until this time point.

**PTEN Induces Cell Death.** Having demonstrated that PTEN overexpression in MCF-7 cells resulted in reduction in cell number attributable to G1 arrest, we asked whether overexpressing PTEN could also cause cell death in MCF-7 cells. Trypan blue staining was used to detect dead cells. No significant change in the number of dead cells was detected at 72 h after withdrawing Tet from control MCF-7/Toff, MCF-7/PTEN-wt, and MCF-7/PTEN-cs cells (Fig. 4). In contrast, >3-fold increased dead cells (from 5.5 to 17.5%) were noted in the MCF-7/PTEN-wt-transfected line but not in the MCF-7/PTEN-cs-transfected line after 120 h induction (Fig. 4). Thus, it would appear that PTEN exerts cell growth suppression through both cell cycle blockade and cell death in MCF-7 cells in a sequential manner; G1 arrest appeared first, followed by cell death. The cell death induced by overexpressing PTEN was apoptotic cell death, as confirmed by TUNEL assay (Fig. 4B). The difference of the percentage of cell death detected by TUNEL assay was similar to that detected by trypan blue staining.

**Negative Regulation of PI-3K/AKT Pathway by PTEN.** PI3K has been implicated in transducing both proliferation and survival signals (31, 32). It has been shown both in vivo and in vitro that the products of PI3K
by Western blot using antibody recognizing both phosphorylated and unphosphorylated forms; therefore, the decreased signal in phosphorylated Akt was attributable to phosphorylation. Overexpression of PTEN caused a partial block of Akt phosphorylation in response to the stimulation of insulin and epithelial growth factor (Fig. 5C), two potent stimulators of the PI3K pathway. In this instance, the partial block was not attributable to the insufficient induction of PTEN expression under these experimental conditions, because the consistently increased levels of PTEN were noted by Western blot. The effect of PTEN on Akt phosphorylation appeared to be specific because MAPK phosphorylation was not affected by overexpression of PTEN under the same conditions (Fig. 5C).

If blocking PI-3K signal transduction was the cause of PTEN-mediated growth inhibition, then we would expect that the reduction of Akt phosphorylation should occur before the onset of growth suppression. To test this, we examined the time course of Akt phosphorylation during induction of PTEN expression (Fig. 5C). A clear decrease in the amount of phosphorylated Akt appeared at 24 h of PTEN induction and remained low thereafter. Note that the accumulation of cells at G1 in PTEN-wt cells occurred at 36 h after withdrawing Tet. The appearance of Akt hypophosphorylation before G1 arrest would suggest that Akt underphosphorylation could be the direct cause of G1 arrest.

To determine the correlation of Akt phosphorylation with the endogenous level of PTEN in breast cancer, we examined the PTEN protein and Akt phosphorylation in a panel of 10 breast cancer cell lines with known PTEN gene status. Three lines, BT-549, MDA-MB-468, and ZR-75-1, have one allele deleted; they also have mutations in their respective second alleles, a 44-bp deletion within exon 6, a 2-bp insertion within exon 4, and missense mutation L108R.4 No mutation or deletion of the coding regions in the other seven lines has been noted. In general, lines with structurally abnormal PTEN have no PTEN protein. However, it should be noted that MDA-MB-436 and MDA-MB-435S, without genomic PTEN abnormalities, had no PTEN or low levels of PTEN protein. An inverse correlation of PTEN with Akt phosphorylation appeared in 9 of the 10 lines (Fig. 5D). Three lines (BT-549, MAD-MB-436, and MAD-MB-468) with no detectable PTEN and one line (ZR-75-1) with very low levels of PTEN had high levels of phosphorylated Akt. Five lines with relatively high levels of PTEN (MDA-MB-435, BT-20, T47-D, MDA-MB-231, and MCF-7) had low levels of phosphorylated Akt. The exception to the inverse correlation rule was seen in HS-578T; it had high levels of phosphorylated Akt and high levels of PTEN.

**Inhibition of PI-3K Leads to G1 Arrest and Cell Death.** To further investigate whether the effects of PTEN on both cell growth and cell death in the MCF-7 breast cancer cell line were mediated by blocking PI3K signaling, we analyzed the cell cycle and cell death in MCF-7 cells treated with wortmannin, a specific inhibitor of PI3K. Treatment of MCF-7 cells with wortmannin at 200 nM for 72 h led to a 40% reduction in cell number (Fig. 6A). If the cells were treated with wortmannin for only 24 h, the number of G1 phase cells increased (Fig. 6B), and the number of dead cells remained unchanged (Fig. 6C). A significantly increased number of dead cells was detected after 72 h of wortmannin treatment (Fig. 6C). These results are consistent with the growth suppressive action of PTEN on this cell line. At a concentration of 200 nM, wortmannin sufficiently inhibited PI3K activity, and the effects were sustained for 24 h, as judged by levels of Akt phosphorylation (Fig. 5C and Fig. 6D). The similarity of growth inhibition and mechanism of action between overexpression of PTEN and treatment with a specific inhibitor of PI3K provided
additional evidence that PTEN inhibits cell growth in a breast cancer cells by blocking the PI3K signal transduction pathway, which in turn leads to G1 cell cycle arrest and sequential cell death.

DISCUSSION

Our data demonstrate that overexpression of PTEN in a breast cancer cell line causes growth suppression mediated initially by G1 arrest, followed by cell death. Thus, we show that both G1 arrest and cell death can occur in a single tissue type, in this case, breast cancer. This is in contradistinction to several previous studies. In the glioma model, growth suppression was felt to be secondary to G1 arrest (14, 16, 17, 33) or anoikis (34). In contrast, it appeared that transient ectopic but high level expression of PTEN in breast cancer cell lines led to apoptosis, but no clear evidence of G1 arrest was found (15). Because the known cell survival factor Akt/PKB lies downstream of...
PTEN via the PI3K pathway (32, 35–39), the apoptotic response secondary to transient PTEN expression in breast cancer lines is understandable. However, why ectopic expression of PTEN in glioma cells led to G1 arrest, but not apoptosis (16, 18), is thought provoking. Given those two lines of evidence, a plausible hypothesis is that response to PTEN expression was tissue specific: G1 arrest in glioma cells and apoptosis in breast cancer cells. This hypothesis is clearly not universal, because our present data demonstrated that when PTEN is expressed in breast cancer cells, they experience phosphatase-dependent growth suppression attributable initially to G1 arrest, followed subsequently by cell death. Thus, downstream response to PTEN with respect to cell cycle control and cell death is not necessarily tissue specific, because breast cancer cells appear to be capable of undergoing both PTEN-mediated G1 arrest and cell death. Of note, these events occurred in a serial manner; G1 arrest occurred first, followed by cell death.

Our data clearly showed that exogenous wild-type PTEN inhibits cell cycle progression in MCF-7 breast cancer cell line, and others have reported that reintroduction of wild-type PTEN blocks cell cycle progression in glioma (14, 16, 33). An increase in cell proliferation throughout the premorbid embryo, particularly in the ectoderm region, has been shown in pten-deficient mice. Those data suggest that PTEN may play an universal role in regulation of cell cycle. The role of PTEN in the regulation of apoptosis is intriguing. It is clear from our results and those of Li et al. (15) that exogenous wild-type PTEN induces apoptosis in breast cancer cell lines, but exogenous expression of wild-type PTEN in glioma results in G1 cell cycle arrest without cell death (16, 18). Another contrasting point is the previous observation that exogenous PTEN transiently transplanted into cells with both wild-type copies of PTEN did not alter growth characteristics (33, 40, 41). Our data obtained from a stable expressing system clearly show that ectopic expression of PTEN in PTEN wild-type MCF-7 cells clearly can produce an effect on cell growth characteristics, and Li et al. (15) has shown that exogenous PTEN inhibits cell growth regardless of endogenous PTEN status. These discrepancies may be attributable to the differences of PTEN signaling between breast epithelial and other cell types.

Although it is clear from our data and previous data that growth suppression of PTEN in cancer cell line models and in vivo neoplasia is mediated by blocking the PI3K signaling pathway, it is becoming obvious that the precise response of a cell and the panoply of pathways downstream of Akt is not straightforward. PI3K has been implicated in transducing both proliferation and survival signals. On the one hand, it is required for G1 to S phase progression stimulated by a variety of growth factors (42); it is involved in up-regulation of cyclin D expression in NIH 3T3 fibroblasts and the MCF-7 breast cancer line (43), and it is involved in down-regulation of p27KIP1 in NIH 3T3 fibroblasts and smooth muscle cells (44) and activation of p70s6k, which regulates a subset of mRNA species thought to be important for cell cycle progression (45, 46). On the other hand, recent reports reveal that activation of PI3K or Akt protects various cell types from apoptosis induced by withdrawing survival factors (32, 35–39), induces the expression of antiapoptotic Bcl-2 (47), and phosphorylates and inactivates the proapoptotic Bcl-2 family member BAD (35), as well as phosphorylates and inhibits the Forkhead family of transcription factors, which can induce the expression of genes critical for cell death, such as the FAS ligand (48).

It has been shown that PTEN interacts and dephosphorylates focal adhesion kinase, which is involved in the regulation of cell growth and apoptosis (49) through transducing cell adhesion-mediated signaling. Although the data from our laboratory and others support that the growth inhibition of PTEN is mediated through its lipid phosphatase activity, it is still not clear yet whether the protein phosphatase activity also participates in the regulation of cell growth in synergistic way with its lipid phosphatase activity. Some naturally occurring mutations, such as G129E, which retain protein phosphatase activity but lose lipid phosphatase activity, could be useful tools to help answer this question.

Cell growth requires both proliferation signals and survival signals. Tumor cells gain a growth advantage by abnormal proliferation and a defect in the regulation of cell death. A handful of tumor suppressors including p53 (19), RB (20), BRCA1 (50, 51), and APC (21, 22) exert direct effects both on cell cycle progression and cell viability. Our results and those of others do indeed suggest that PTEN participates in the regulation of cell proliferation and cell survival like p53 and RB, the well known “gatekeeper” tumor suppressor.

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