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Advances in Brief

The *PTEN/MMAC1* Tumor Suppressor Induces Cell Death That Is Rescued by the AKT/Protein Kinase B Oncogene[1]

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Abstract

***PTEN/MMAC1* is a tumor suppressor gene that is mutated in a variety of cancers. *PTEN* encodes a phosphatase that recognizes phosphoprotein substrates and the phospholipid, phosphatidylinositol-3,4,5-triphosphate. *PTEN* inhibited cell growth and/or colony formation in all of the epithelial lines tested with one exception. The decrease in cellular proliferation was associated with an induction of apoptosis and an inhibition of signaling through the phosphatidylinositol 3′-kinase pathway. *Akt/protein kinase B*, a gene whose antiapoptotic function is regulated by phosphatidylinositol-3,4,5-triphosphate, was able to rescue cells from PTEN-dependent death. PTEN, therefore, appears to suppress tumor growth by regulating phosphatidylinositol 3′-kinase signaling.**

Introduction

PTEN/MMAC1 is a tumor suppressor gene located on human chromosome 10q23 (1, 2). Somatic *PTEN* mutations have been identified in many types of cancer, including glioblastoma multiforme, endometrial carcinoma, prostate carcinoma, breast carcinoma, malignant melanoma, bladder carcinoma, small cell lung cancer, and endometrioid ovarian cancer (3, 4). Germ-line mutations of *PTEN* have been commonly found in Cowden disease and Bannayan-Zonana syndrome (5, 6). Evidence in glioma cell lines suggests that PTEN exerts its tumor suppressive effect through the inhibition of cellular growth (7, 8). In addition, PTEN disrupts the architecture of focal adhesions and the cytoskeleton when it is introduced into fibroblasts and glial tumor cells (9).

The predicted amino acid sequence of *PTEN* is homologous with tensin and auxilin, and a conserved tyrosine phosphatase domain is present in this region (1, 2). PTEN can remove phosphates from phosphotyrosine, phosphoserine, phosphothreonine, and phosphatidylinositol phosphate residues *in vitro* (10–12). Furthermore, missense mutations in *PTEN* identified in patient material cluster around the phosphatase domain and often diminish its phosphatase activity (11). These data support the hypothesis that the phosphatase activity of PTEN plays an essential role in tumor suppression. Endogenous cellular substrates of PTEN include FAK[3] (9) and PtdIns(3, 4, 5)P_3 (12). In spite of this wealth of information, the mechanism through which PTEN suppresses tumor formation remains unclear.

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[3] The abbreviations used are: FAK, focal adhesion kinase; PtdIns(3,4,5)P_3, phosphatidylinositol-3,4,5-triphosphate; MOI, multiplicity of infection; PI, phosphatidylinositol; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; Ad, adenovirus; GST, glutathione *S*-transferase; β-Gal, β-galactosidase; GFP, green fluorescence protein; CMV, cytomegalovirus; PH, pleckstrin homology.

Materials and Methods

Cell Lines. Breast cancer cell lines UACC-812, UACC-893, MDA-MB-453, MDA-MB-175-VII, MDA-MB-468, MBD-MB-361, MDA-MB-231, MDA-MB-436, MDA-MB-415, MDA-MB-157, MDA-MB-435-S, ZR75-30, ZR75-1, BT-549, BT-483, BT-474, T-47D, MCF-7, BT-20, SK-BR-3, HBL-100, human embryonic kidney 293 cells, and the mink lung epithelial line (MvLu-1) were obtained from American Type Culture Collection. Two colorectal cancer cell lines HCT-116 and SW480 were provided by Dr. Bert Vogelstein. All media were supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin sulfate.

Antibodies and Protein Detection. Polyclonal anti-PTEN antibody CS486 was obtained by injecting a rabbit with a COOH-terminal peptide. For the detection of PTEN expressed by adenovirus, a MOI of 10 was used for the infection. Protein lysates (50–100 μg) were resolved by denaturing PAGE and transferred onto polyvinylidene difluoride membranes. Total cell lysates were collected each day for 4 days, and exogenous protein was detected by Western blot with a 1:500 dilution of CS486. Blots were developed with horseradish peroxidase-conjugated secondary antibody using the enhanced chemiluminescence system (Amersham). Antibodies to Akt were purchased from New England Biolabs and used as described by the manufacturer.

PTEN Delivery Systems. A *PTEN* cDNA clone, which included the full-length PTEN coding sequence, was cloned into a eukaryotic episomal expression vector pCEP4 (Invitrogen) to generate pCEP4.PTEN. The conserved cysteine residue at codon 124 in the phosphatase catalytic domain was mutated to serine using a site-directed mutagenesis kit from Bio-Rad to generate pCEP4.PTEN.C124S. Three μg of pCEP4, pCEP4.PTEN, or pCEP4.PTEN.C124S were used to transfect cell lines with Fugene 6 (Boehringer Mannheim), and colonies were selected by incubating the transfected cells with the optimal dose of hygromycin over a period of 2–4 weeks. The colonies were then stained with crystal violet and counted. All experiments were done in triplicate and repeated at least once.

A full-length *PTEN* clone was amplified from human cDNAs by PCR. The PCR product was gel purified and cloned into pGEX-2TK (Pharmacia) to create pGEX-PTEN. The key cysteine residue at codon 124 in the phosphatase catalytic domain was mutated to alanine using a site-directed mutagenesis kit from Bio-Rad (Hercules, CA). The PCR product was gel purified, subcloned into an adenovirus vector, and sequenced. Generation of recombinant virus and amplification in 293 cells was as described (13). The viruses were concentrated on a cesium chloride gradient and subjected to dialysis. The titers were determined by using anti-adenovirus antibody (provide by Dr. Hamish Young, Columbia University, New York, NY), and positive plaques were visualized using a fluorescence microscope. Adenoviral infections were performed by inoculating cells with a small volume of growth medium supplemented with 2% serum and the appropriate viral dilution at 37°C with occasional rocking. After 1 h, additional growth medium with 10% serum was added. For growth inhibition experiments, cells were grown in medium with serum for 96 h. Cells were then trypsinized and counted. Experiments were repeated for all cell lines, and similar results were obtained.

Apoptosis Assays. TUNEL assays were performed using the *in situ* cell death detection kit (Boehringer Mannheim) with cells grown on chamber slides or T25 cm^2 flasks. The signal was initially observed with a Zeiss LSM 410 confocal laser scanning system attached to Zeiss Axiovert 100TV inverted microscope. To quantify the percentage of apoptotic cells, both floating and

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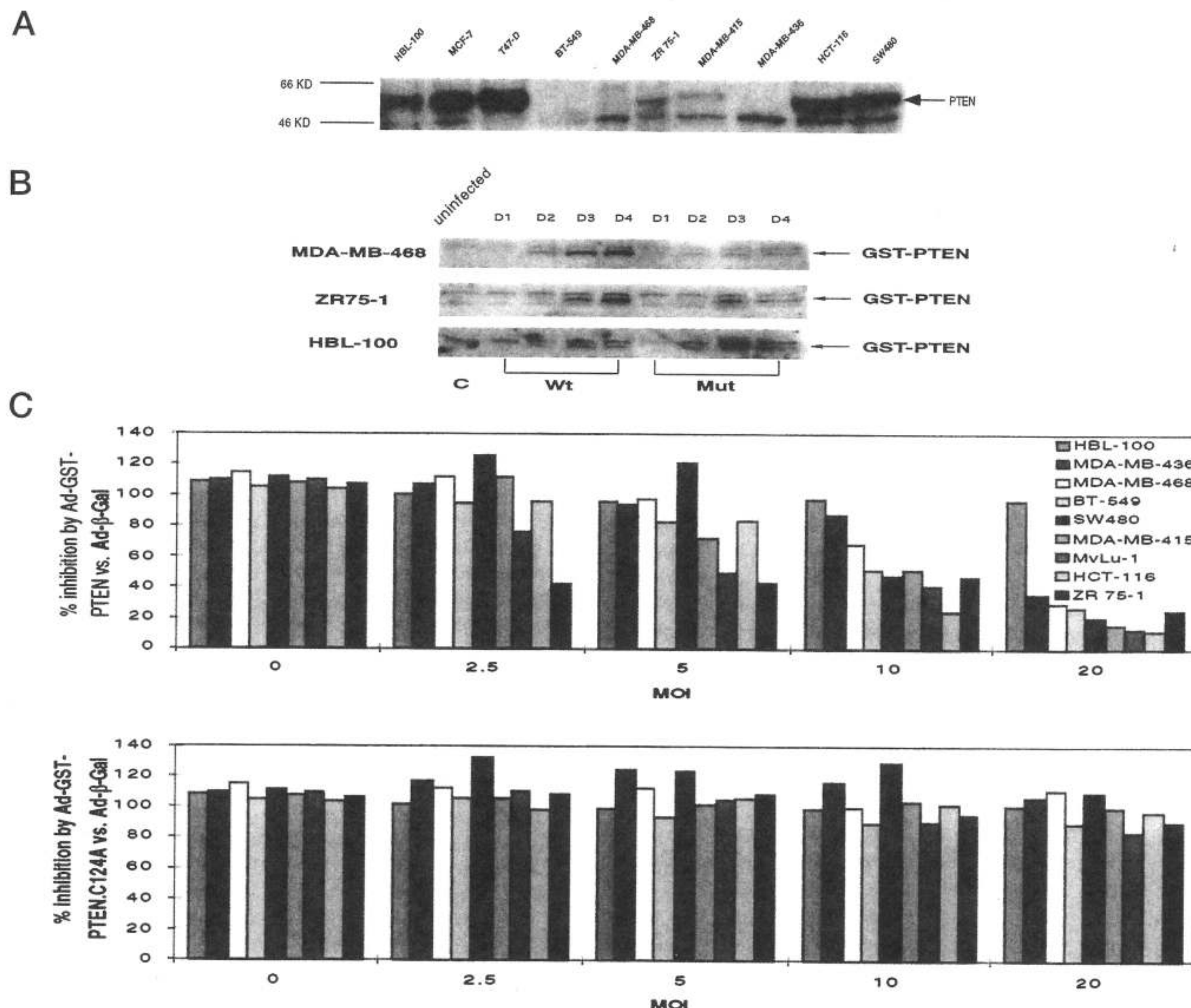

Fig. 1. Growth suppression of cell lines containing endogenous mutant and wild-type PTEN. *A,* detection of endogenous PTEN. Western analysis using an anti-PTEN antibody CS486 on total cell lysates of HBL-100, MCF-7, T47-D, BT-549, MDA-MB-468, ZR75-1, MDA-MB-415, MDA-MB-436, HCT-116, and SW480. *Arrow,* endogenous PTEN band; the lower band is nonspecific. *B,* detection of exogenous GST-PTEN delivered by Ad. The Western blot using an anti-PTEN antibody CS486 shows the expression of exogenous GST-PTEN in MDA-MB-468, ZR75-1, and HBL-100. Total cell lysates were collected over a 4-day period after Ad infection at an MOI of 10 with either Ad-GST-PTEN or Ad-GST-PTEN.C124A. The uninfected lane is the cell lysate before infection. *Lanes D1–D4* are the cell lysates at 1–4 days after infection, respectively. *Arrows* and *lines,* expression of either exogenous wild-type GST-PTEN (*Wt*) or mutant GST-PTEN.C124A (*Mut*). *C,* percentage of growth inhibition by Ad-GST-PTEN *versus* Ad-β-Gal (*upper panel*) and growth inhibition by Ad-GST-PTEN.C124A *versus* Ad-β-Gal (*lower panel* from *left* to *right*) in HBL-100, MDA-MB-436, MDA-MB-468, BT-549, SW 480, MDA-MB-415, MvLu-1, HCT116, and ZR75-1. All of the cells were seeded at 1×10^5/T25 cm^2 culture flask and infected with Ad-β-Gal, Ad-GST-PTEN, or Ad-GST-PTEN.C124A. Infections were done at four different MOI. The cells were counted at day 4 after infection.

attached cells were collected, and the TUNEL assay was performed using the same kit. The resulting samples were analyzed by flow cytometry. To test whether general caspase inhibitor would affect the TUNEL results, 50 μM of ZVAD (Val-Ala-Asp fluoro methyl ketone; Enzyme System Product, Livermore, CA) was supplemented into the medium after 24 h of infection, and TUNEL assays were performed after 96 h of infection. For apoptosis rescue assays, plasmids were transfected with Fugene 6 into MDA-MB-468 cells on six-well dishes. pGFP-PTEN was provided by K. Yamada, and pGreenlantern-1 was purchased from Life Technologies, Inc. Rescue plasmids were as follows: pcDNA3HA-FAK (K. Yamada, NIH, Bethesda, MD), p110* (A. Klippel, Chiron Corp., Emeryville, CA), pCMV-myr-AKT-HA, pCMVHA-Akt, pCMV-HA-k179m-Akt (P. N. Tsichlis, Fox Chase Cancer Center, Philadelphia, PA), pBcl-2 (J. Pietenpol, Vanderbilt University, Nashville, TN), and pCMV-β-Gal. All experiments were examined after 24 h with an inverted Nikon Eclipse TE300 microscope. Inhibition and rescue was reproduced in multiple experiments.

PI 3′-kinase reporter assay. NIH 3T3 cells were transfected with Fugene-6 overnight, switched to serum-free medium for 48 h, and luciferase activity was measured from a c-fos promoter linked to luciferase.

Results

Growth Suppression by PTEN. Previously, we identified two breast cancer cell lines that lacked wild-type *PTEN* (1). To identify additional epithelial cell lines harboring mutations of *PTEN* and to identify lines expressing the wild-type gene, we analyzed the cDNA of PTEN amplified from 19 breast cancer lines, 1 kidney cell line, and 2 colorectal cancer cell lines. Two additional mutations were found (MDA-MB-415, C136Y; ZR 75–1, L108R), which are in the phosphatase domain and may affect phosphatase function. For both of these lines, the wild-type allele was not expressed. A third line expressed no PTEN message (MDA-MB-436). A rabbit polyclonal antibody raised against a COOH-terminal peptide of PTEN recognized a M_r 56,000 protein in all of the 18 lines containing wild-type PTEN message (Fig. 1A). Very low levels of PTEN could be detected in the two lines with PTEN missense mutations, MDA-MB-415 and ZR 75–1. No protein was detected for BT 549, MDA-MB-468, or

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Table 1 *Colony inhibition by PTEN*

Cell lines	*PTEN* gene[b]	Colonies[a] pCEP4	pCEP4.PTEN	pCEP4.PTEN.C124S
MDA-MB 468	Mut	59.5 ± 9.5	3.3 ± 1.9	53 ± 12.4
BT 549	Mut	234 ± 11.7	1 ± 0.8	245.7 ± 9.1
MCF-7	Wt	194 ± 19.3	21 ± 5.3	149 ± 13.7
T47-D	Wt	267 ± 19.6	2.7 ± 0.47	317 ± 37.9
HBL-100	Wt	436 ± 36.5	430 ± 23.2	446.7 ± 19.2
293	Wt	200.5 ± 16.3	4 ± 1.4	329 ± 21.2

[a] Colonies were counted after staining and averaged from three independent experiments ± SD.

[b] Mut, mutant endogenous *PTEN* gene in these cell lines; Wt, wild-type *PTEN* gene in these cell lines.

MDA-MB 436. Thus, we documented that 19 lines expressed wild-type PTEN protein, whereas 5 lines did not.

Vectors expressing wild-type PTEN and the phosphatase dead C124S mutant were used to select for colonies in five breast cancer cell lines and one kidney cell line. Transient expression of wild-type and mutant PTEN was comparable in each cell line. Cells were grown in the presence of hygromycin for 2–4 weeks until colonies appeared. For five of the lines, MCF-7, T47-D, MDA-MB-468, BT 549, and 293, colony formation was inhibited from 10- to 200-fold by the PTEN expression vector (Table 1). This inhibition was observed in cell lines containing or lacking wild-type endogenous PTEN. Expression of mutant PTEN (C124S), which carried a point mutation at the catalytic cysteine residue in the phosphatase domain, had no effect on colony formation when compared with the empty vector. Only one cell line, HBL-100, was resistant to wild-type PTEN. Although the colony size in the PTEN-transfected HBL-100 samples was slightly smaller when compared with the colonies of the control, there was no difference in the colony number.

Adenoviruses expressing β-galactosidase (Ad-β-Gal), wild-type GST-PTEN fusion protein (Ad-GST-PTEN), or a codon 124 cysteine to alanine mutant (Ad-GST-PTEN.C124A) were used to infect a panel of cancer cell lines. Only cell lines capable of expressing β-Gal and GST-PTEN were studied for further analysis. The expression of exogenous GST-PTEN and GST-PTEN.C124A peaked at 3–4 days after infection (Fig. 1*B*). The levels of exogenous GST-PTEN and GST-PTEN-C124A were comparable, although the mutant protein was expressed at lower levels in the cell line MDA-MB-468. Expression of GST-PTEN in lines containing endogenous wild-type PTEN ranged from 0.5 to 2 times the endogenous level (data not shown).

To determine whether PTEN might affect cellular proliferation, nine lines capable of expressing GST-PTEN were infected with escalating multiplicities of infection. Four days after infection, cells were trypsinized and counted (Fig. 1*C*). Ad-GST-PTEN strongly suppressed the growth of eight of the cell lines, regardless of the mutational status of endogenous PTEN. Increasing doses of virus

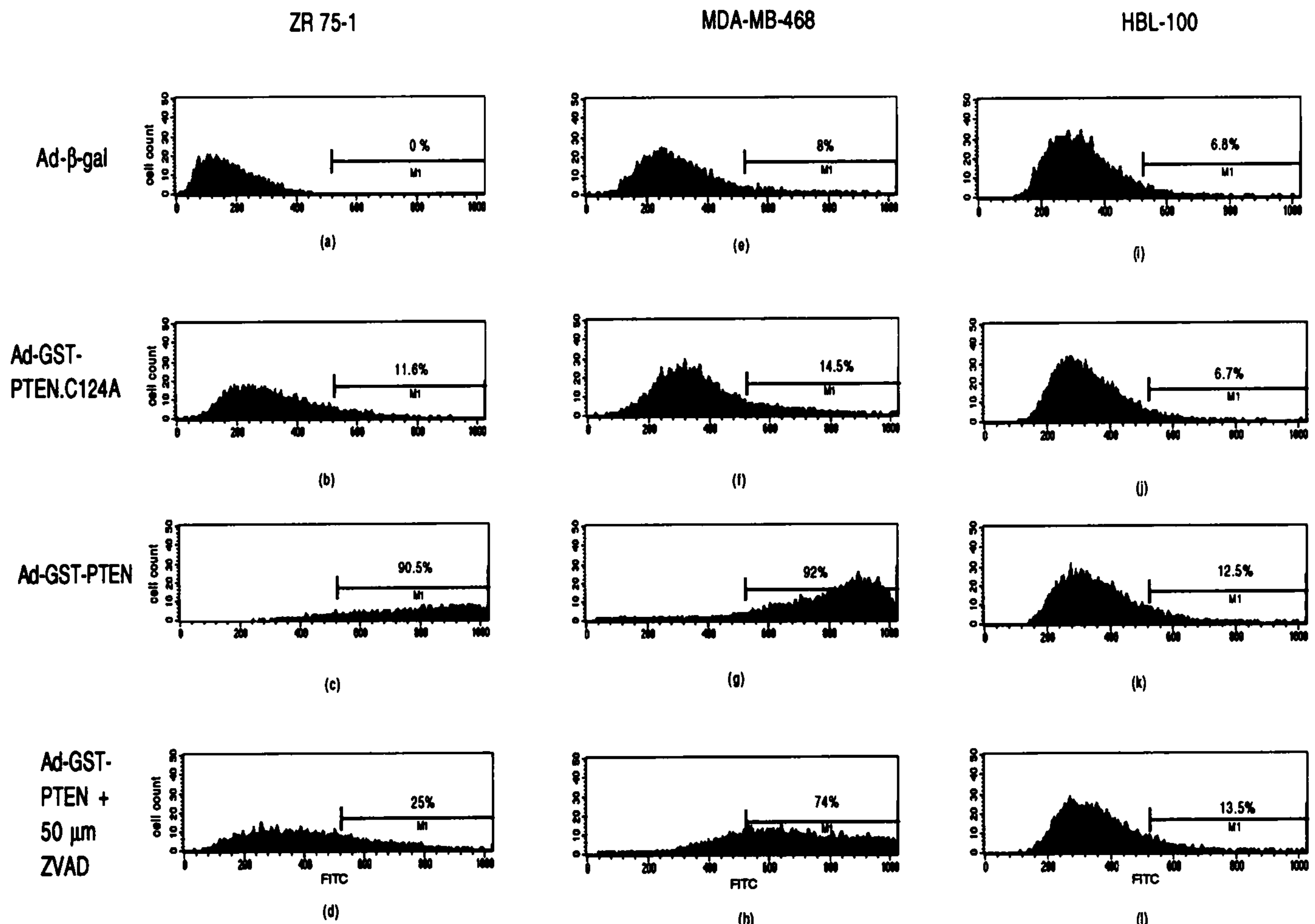

Fig. 2. GST-PTEN induces apoptosis. FACS TUNEL on ZR 75-1 (*a–d*), MDA-MB-468 (*e–h*), and HBL-100 (*i–l*). Cells were seeded at 1×10^5/T25 cm^2 culture flask and infected with three different Ads, Ad-β-GAL (*a, e,* and *i*), Ad-GST-PTEN.C124A (*b, f,* and *j*), and Ad-GST-PTEN (*c, g,* and *k*) at MOI of 20:1. One set of Ad-GST-PTEN-infected cells was treated with 50 μM of ZVAD (*d, h,* and *l*). Total floating and attached cells were collected after 96 h. The cells were labeled with FITC-conjugated anti-dUTP antibody, and samples were analyzed by flow cytometry. *M1,* population of TUNEL-positive cells.

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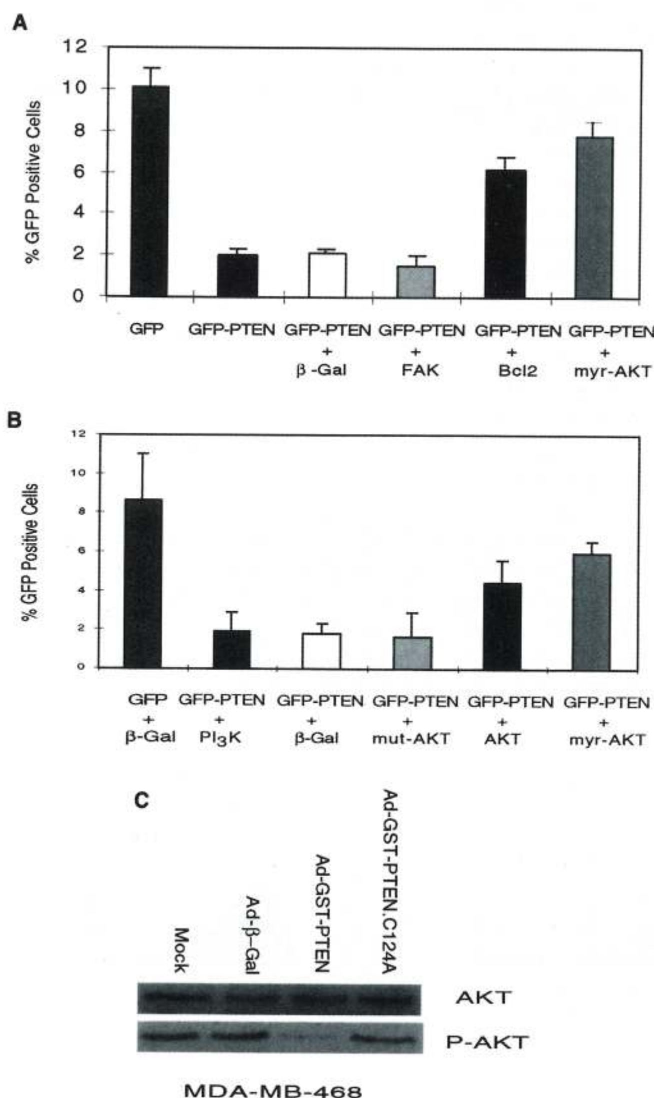


Fig. 3. AKT rescues cells from death and is down-regulated by PTEN. MDA-MB-468 cells were transfected with 1 μg of either GFP or GFP-PTEN and 0.3 μg of potential CMV promoter rescuing plasmids. GFP-positive cells were counted 24 h after transfection over three random fields as a percentage of total cells at ×200. *Columns,* average of three counts; *bars,* SD. All experiments were done in duplicate and then repeated. *A,* comparison of GFP to GFP-PTEN and cotransfection with CMV promoter-driven plasmids expressing β-Gal, FAK, Bcl2, and myr-HA-AKT. Bcl-2 and myr-HA-AKT rescue GFP-positive cells above baseline. In *B,* rescue with AKT requires that the kinase domain is functional. GFP-PTEN is cotransfected with plasmids expressing PI 3′-kinase, β-Gal, mutated HA-AKT (K179M), HA-AKT, and myr-HA-AKT. Only HA-AKT and myr HA-AKT are able to rescue GFP-positive cells. In *C,* PTEN reduces the level of AKT phosphorylation. MDA-MB-468 cells were infected with Ad-β-gal, Ad-GST-PTEN, or Ad-GST-PTEN.C124A at an MOI of 20 for 48 h and then serum starved over night. Attached cells were then lysed in protein sample buffer, subjected to electrophoresis, and probed with antibodies specific for Akt and phosphoserine 473 of Akt. A partial reduction of phosphorylated Akt is detected in the GST-PTEN-infected lane.

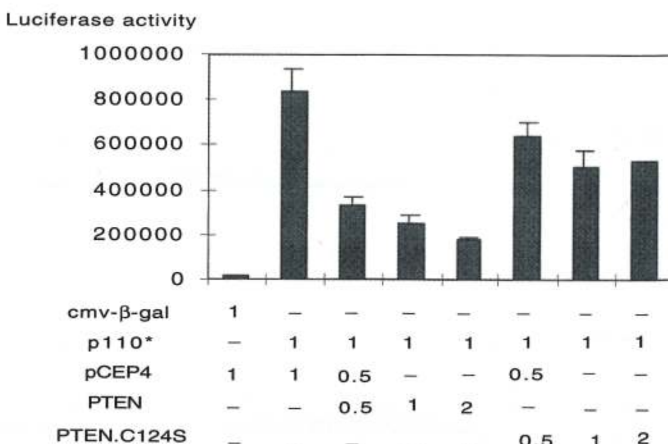
were associated with a greater degree of suppression. Infection with Ad-GST-PTEN.C124A did not result in any noticeable growth suppression relative to Ad-β-Gal, which suggested that the phosphatase activity of PTEN was required for this effect. Only HBL-100 escaped the suppressive effect of Ad-GST-PTEN, which was consistent with the colony suppression data. Analysis of the effect of GST-PTEN was repeated on all of the cell lines, and similar results were obtained.

PTEN Induces Cell Death. We used the adenovirus delivery system to investigate the possible mechanism of growth suppression. Cells of MDA-MB-468, ZR75-1, and HBL-100 were infected for 96 h with Ad-β-Gal, Ad-GST-PTEN, or Ad-GST-PTEN.C124A. The TUNEL assay was performed to detect the genomic degradation of apoptosis, and the stained cells were detected by flow cytometry. For both MDA-MB-468 and ZR 75-1, >90% of the cells were TUNEL positive after 96 h of infection with Ad-GST-PTEN (Fig. 2). Only a slight induction of TUNEL staining was seen with mutant GST-PTEN relative to β-Gal. Ad-GST-PTEN-infected HBL-100 showed only a slight increase in signal relative to control as well. The assay was also performed on these lines *in situ,* and GST-PTEN alone induced TUNEL staining of attached cells (data not shown).

To test whether the observed apoptosis was mediated by caspases, we attempted to inhibit the PTEN-induced apoptosis with the broad spectrum caspase inhibitor ZVAD. At 50 μM, this drug effectively inhibited the appearance of fluorescently labeled nuclei in the TUNEL assay for both ZR75-1 and MDA-MB-468 cells (Fig. 2, *d* and *h*). The shift in the peak of TUNEL staining was strongest in the ZR75-1 line, but a clear shift to the left was also seen in MDA-MB-468. This blockage of apoptosis by ZVAD was not due to a reduction of expression of exogenous GST-PTEN. The expression level of exogenous GST-PTEN was monitored over a 4-day period, and ZVAD-treated samples expressed equivalent amounts of fusion protein when compared with untreated samples (data not shown).

Rescue of Cell Death by AKT and Bcl-2. Recently, PTEN was found to dephosphorylate FAK and PtdIns(3, 4, 5)P_3 (9, 12). Disruption of FAK signaling induces apoptosis in many cell types (14, 15). Similarly, PtdIns(3, 4, 5)P_3 signals cells to stimulate the activity of AKT (protein kinase B), which protects cells from apoptosis (16). Therefore, PTEN may induce apoptosis by interfering with FAK and/or PI phosphate signals that are needed for survival.

To test this hypothesis, we attempted to rescue PTEN-mediated apoptosis with candidate genes that are predicted to function downstream of PTEN signaling. GFP and GFP-PTEN were transfected into MDA-MB-468 cells, and the number of GFP-positive attached cells was counted after 24 h. The percentage of green cells was substantially reduced in the GFP-PTEN-transfected population when compared with GFP alone (Fig. 3*A*). Potential rescuing expression constructs [Bcl-2, myristylated AKT (myr-AKT), FAK, activated PI 3′-kinase (p110*), and β-Gal] were then cotransfected with GFP-PTEN. A 4-fold increase in GFP-PTEN-positive cells was seen in cells cotransfected with myr-AKT. Although Bcl-2 also rescued GFP-PTEN fluorescence, PI 3′-kinase and FAK had no effect (Fig. 3, *A* and



cmv-β-gal	1	–	–	–	–	–	–	–
p110*	–	1	1	1	1	1	1	1
pCEP4	1	1	0.5	–	–	0.5	–	–
PTEN	–	–	0.5	1	2	–	–	–
PTEN.C124S	–	–	–	–	–	0.5	1	2

Fig. 4. PTEN blocks PI 3′-kinase signaling to the c-fos promoter. NIH 3T3 cells were cotransfected with a plasmid containing the c-fos promoter upstream of a luciferase reporter gene and vectors containing p110*, an activated PI 3′-kinase, PTEN, PTEN C124S, and β-Gal. PTEN was able to inhibit p110*-induced promoter activity in a dose-dependent manner. PTEN C124S partially inhibited promoter activity, but the inhibition plateaued. 3T3 cells were transfected with Fugene-6 in the presence of serum-free medium; luciferase activity was quantitated 48 h after transfection. Each *column* represents the mean of three independent experiments; *bars,* SD.

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B). Rescue was also achieved with unmyristylated AKT but not with a mutation inactivating the kinase domain (Fig. 3*B*). To determine whether PTEN was affecting the level of AKT activation in the cell, MDA-MB-468 cells were infected with Ad-GST-PTEN, Ad-GST-PTEN.C124A, and a control virus. Although the total level of AKT was unaffected by wild-type GST-PTEN, the level of phosphorylation at codon 473 was reduced (Fig. 3*C*). Phosphorylation of this site is required for maximal AKT kinase activity and occurs only when AKT is bound to the membrane via PtdIns(3, 4, 5)P_3 (17, 18). No effect was observed with the C124A mutant.

PTEN Blocks PI 3′-Kinase Signaling. The above data suggested that PTEN functions above Akt and Bcl-2 but below PI 3′-kinase. PTEN is known to reduce the level of endogenous PtdIns(3, 4, 5)P_3 in 293 cells (12). However, 293 cells are highly sensitive to the growth-suppressive effects of PTEN, which could potentially lead to alterations in PtdIns(3, 4, 5)P_3 levels because of a nonspecific mechanism (Table 1). To determine whether PTEN blocks PI 3′-kinase signaling, we studied NIH 3T3 cells, which are resistant to PTEN growth suppression (9).[4] A constitutively active PI 3′-kinase mutant, p110*, is known to activate AKT and stimulate the c-fos promoter (19, 20). The PI 3′-kinase signal to the c-fos promoter was inhibited by PTEN, whereas mutant PTEN only partially suppressed reporter activity (Fig. 4). Similar results were obtained with the breast tumor cell line T47D (data not shown). These data suggest that PTEN functions downstream of PI 3′-kinase. They also support the notion that PTEN is able to inhibit PI 3′-kinase signaling by reducing PtdIns(3, 4, 5)P_3 levels in a cell. On the other hand, the ability of PTEN to induce cell death in many lines may indirectly affect fos promoter expression, even in 3T3 cells.

Discussion

The data presented here support the hypothesis that PTEN suppresses cellular growth through the induction of apoptosis. This suppression requires a functionally active phosphatase domain. Wild-type PTEN interfered with two different signals (to AKT and the c-fos promoter) that require PtdIns(3, 4, 5)P_3 for membrane localization of a signaling protein. AKT and its regulatory kinase PDK-1 contain PH domains that must bind PtdIns(3, 4, 5)P_3 for activation (18, 21). The PH domain of SOS regulates the activation of RAC and possibly RAS, which both affect fos promoter activity (22–24). These data, along with the fact that GFP-PTEN-expressing cells could be rescued by AKT, suggest that PTEN functions as a tumor suppressor by inducing AKT-dependent apoptosis because of alterations in PtdIns(3, 4, 5)P_3 signaling. Other effects of altering PI 3′-kinase signaling may also contribute to the tumor suppressor function of PTEN. These include inhibition of fos and the p70/S6 kinase pathway (25).

The rescue of GFP-PTEN-expressing cells was also seen with Bcl-2. AKT is known to induce the expression of Bcl-2 (26). Therefore, the Bcl-2 rescue detected in our hands may be the result of complementation of an AKT defect. On the other hand, it is also possible that Bcl-2 may be blocking apoptosis nonspecifically. The lack of rescue with FAK was surprising; however, FAK and PTEN may coordinate the regulation of cell migration rather than cell death. Another unexpected finding was that cells containing wild-type PTEN were suppressed by exogenous PTEN. This result differs from that of Furnari *et al.* (7), who found that glial cells containing wild-type PTEN were resistant to the suppressive effects of exogenous PTEN. This discrepancy may be the result of differences between epithelial and glial cells with regard to the regulation of PTEN signaling. Alternatively, the discrepancy could be due to differences in the delivery of PTEN.

Growth suppression and/or the induction of apoptosis has been demonstrated for a handful of tumor suppressors, including p53 (reviewed in Ref. 27), BRCA1 (28), and APC (29). In the best understood case, p53 appears to regulate the induction of apoptosis in response to genetic damage (30). Its loss in a tumor can accompany a reduction in the frequency of apoptosis (31). PTEN appears to be physiologically involved in the regulation of apoptosis as well.

[4] J. Li and R. Parsons, unpublished data.

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References

1. Li, J., Yen, C., Liaw, D., Podsypanina, K., Bose, S., Wang, S. I., Puc, J., Miliaresis, C., Rodger, L., McCombie, R., Bigner, S. H., Giovanella, B. C., Ittmann, M., Tycko, B., Hibshoosh, H., Wigler, M. H., and Parsons, R. *PTEN*, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. Science (Washington DC), *275:* 1943–1947, 1997.
2. Steck, P. A., Pershouse, M. A., Jasser, S. A., Yung, W. K., Lin, H., Ligon, A. H., Langford, L. A., Baumgard, M. L., Hattier, T., Davis, T., Frye, C., Hu, R., Swedlund, B., Teng, D. H., and Tavtigian, S. B. Identification of a candidate tumor suppressor gene, *MMAC1*, at chromosome 10q23.3 that is mutated in multiple advanced cancers. Nat. Genet., *15:* 356–362, 1997.
3. Teng, D. H., Hu, R., Lin, H., Davis, T., Iliev, D., Frye, C., Swedlund, B., Hansen, K. L., Vinson, V. L., Gumpper, K. L., Ellis, L., El-Naggar, A., Frazier, M., Jasser, S., Langford, L. A., Lee, J., Mills, G. B., Pershouse, M. A., Pollack, R. E., Tornos, C., Troncoso, P., Yung, W. K., Fujii, G., Berson, A., and Steck, P. A. MMAC1/PTEN mutations in primary tumor specimens and tumor cell lines. Cancer Res., *57:* 5221–5225, 1997.
4. Parsons, R. Phosphatases and tumorigenesis. Curr. Opin. Oncol., *10:* 88–91, 1998.
5. Liaw, D., Marsh, D. J., Li, J., Dahia, P. L. M., Wang, S. I., Zheng, Z., Bose, S., Call, K. M., Tsou, H. C., Peacocke, M., Eng, C., and Parsons, R. Germline mutations of the *PTEN* gene in Cowden disease, an inherited breast and thyroid cancer syndrome. Nat. Genet., *16:* 64–67, 1997.
6. March, D. J., Dahia, P. L., Liaw, D., Parsons, R., Gorlin, R. J., and Eng, C. Germline mutations in *PTEN* are present in Bannayan-Zonana syndrome. Nat. Genet., *16:* 333–334, 1997.
7. Furnari, F. B., Lin, H., Huang, H. S., and Cavenee, W. K. Growth suppression of glioma cells by *PTEN* requires a functional phosphatase catalytic domain. Proc. Natl. Acad. Sci. USA, *94:* 12479–12484, 1997.
8. Cheney, I. W., Johnson, D. E., Vaillancourt, M. T., Avanzini, J., Morimoto, A., Demers, G. W., Wills, K. N., Shabram, P. W., Bolen, J. B., Tavtigian, S. V., and Bookstein, R. Suppression of tumorigenicity of glioblastoma cells by adenovirus-mediated *MMAC1/PTEN* gene transfer. Cancer Res., *58.* 2331–2334, 1998.
9. Tamura, M., Gu, J., Matsumoto, K., Aota, S., Parsons, R., and Yamada, K. M. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. Science (Washington DC), *280:* 1614–1617, 1998.
10. Li, D. M., and Sun, H. TEP1, encoded by a candidate tumor suppressor locus, is a novel protein tyrosine phosphatase regulated by transforming growth factor β. Cancer Res., *57:* 2124–2129, 1997.
11. Myers, M. P., Stolarov, J. P., Eng, C., Li, J., Wang, S. I., Wigler, M. H., Parsons, R., and Tonk, N. K. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. Proc. Natl. Acad. Sci. USA, *94:* 9052–9057, 1997.
12. Maehama, T., and Dixon, J. E. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. J. Biol. Chem., *273:* 13375–13378, 1998.
13. He, T. C., Zhou, S., da Costa, L. T., Yu, J., Kinzler, K. W., and Vogelstein, B. A simplified system for generating recombinant adenoviruses. Proc. Natl. Acad Sci. USA, *95:* 2509–2514, 1998.
14. Xu, L. H., Owens, L. V., Sturge, G. C., Yang, X., Liu, E. T., Craven, R. J., and Cance, W. G. Attenuation of the expression of the focal adhesion kinase induces apoptosis in tumor cells. Cell Growth Differ., *7.* 413–418, 1996.
15. Hungerford, J. E., Compton, M. T., Matter, M. L., Hoffstrom, B. G., and Otey, C. A. J. Inhibition of pp125FAK in cultured fibroblasts results in apoptosis. Cell Biol., *135:* 1383–1390, 1996.
16. Franke, T. F., Kaplan, D. R., and Cantley, L. C. PI3K: downstream AKT ion blocks apoptosis. Cell, *88:* 435–437, 1997.
17. Alessi, D. R., Andjelkovic, M., Caudwell, B., Cron, P., Morrice, N., Cohen, P., and Hemmings, B. A. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J., *15:* 6541–6551, 1996.
18. Stokoe, D., Stephens, L. R., Copeland, T., Gaffney, P. R. J., Reese, C. B., Painter, G. F., Holmes, A. B., McCormick, F., and Hawkins, P. T. Dual role of phosphatidylinositol-3,4,5-triphosphate in the activation of protein kinase B. Science (Washington DC), *277:* 567–570, 1997.

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19. Hu, Q., Klippel, A., Muslin, A. J., Fantl, W. J., and Williams, L. T. Ras-dependent induction of cellular responses by constitutively active phosphatidylinositol-3 kinase. Science (Washington DC), *269:* 100–102, 1995.
20. Klippel, A., Reinhard, C., Kavanaugh, W. M., Apell, G., Escobedo, M. A., and Williams, L. T. Membrane localization of phosphatidylinositol 3-kinase is sufficient to activate multiple signal-transducing kinase pathways. Mol. Cell. Biol., *16:* 4117–4127, 1996.
21. Anderson, K. E., Coadwell, J., Stephens, L. R., and Hawkins, P. T. Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B. Curr. Biol., *8:* 684–691, 1998.
22. Chen, R. H., Corbalan-Garcia, S., and Bar-Sagi, D. The role of the PH domain in the signal-dependent membrane targeting of Sos. EMBO J., *16:* 1351–1359, 1997.
23. Qian, X., Vass, W. C., Papageorge, A. G., Anborgh, P. H., and Lowy, D. R. N-Terminus of Sos1 Ras exchange factor: critical roles for the Dbl and pleckstrin homology domains. Mol. Cell. Biol., *18:* 771–778, 1998.
24. Nimnual, A. S., Yatsula, B. A., and Bar-Sagi, D. Coupling of Ras and Rac guanosine triphosphatases through the Ras exchanger Sos. Science (Washington DC), *279:* 560–563, 1998.
25. Downward, J. Lipid-regulated kinases: some common themes at last. Science (Washington DC), *279:* 673–674, 1998.
26. Ahmed, N. N., Grimes, H. L., Bellacosa, A., Chan, T. O., and Tsichlis, P. N. Transduction of interleukin-2 antiapoptotic and proliferative signals via Akt protein kinase. Proc. Natl. Acad. Sci. USA, *94:* 3627–3632, 1997.
27. Hansen, R., and Oren, M. *p53:* from inductive signal to cellular effect. Curr. Opin. Genet. Dev., *7:* 46–51, 1997.
28. Shao, N., Chai, Y. L., Shyam, E., Reddy, P., and Rao, V. N. Induction of apoptosis by the tumor suppressor protein BRCA1. Oncogene, *13:* 1–7, 1996.
29. Morin, P. J., Vogelstein, B., and Kinzler, K. W. Apoptosis and *APC* in colorectal tumorigenesis. Proc. Natl. Acad. Sci. USA, *93:* 7950–7954, 1996.
30. Clarke, A. R., Purdie, C. A., Harrison, D. J., Morris, R. G., Bird, C. C., Hooper, M. L., and Wyllie, A. H. Thymocyte apoptosis induced by p53-dependent and independent pathways. Nature (Lond.), *362:* 849–852, 1993.
31. Symonds, H., Krall, L., Remington, L., Saenz-Robles, M., Lowe, S., Jacks, T., and Van Dyke, T. p53-dependent apoptosis suppresses tumor growth and progression *in vivo*. Cell, *78:* 703–711, 1994.

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