PTEN Induces Chemosensitivity in PTEN-mutated Prostate Cancer Cells by Suppression of Bcl-2 Expression*

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The tumor suppressor gene PTEN (MMAC1/TEP1) is lost frequently in advanced prostate cancer (PCa). However, the function of PTEN in tumorigenesis is not understood fully. In this study, we demonstrate that expression of Bcl-2 in prostate tumors correlates with loss of the PTEN protein. This finding was verified by studies in the PCa cell lines DU145, PC-3, LNCaP, and an androgen-refractory subline of LNCaP. Transient transfection of PTEN into the PTEN-null cells resulted in decreased levels of Bcl-2 mRNA and protein. These effects appear to be mediated at the level of gene transcription, since a Bcl-2 promoter-reporter construct was down-regulated by ectopic expression of PTEN in LNCaP cells. The inhibition of Bcl-2 required the lipid-phosphatase activity of PTEN and was blocked by overexpression of a constitutively active form of Akt. Moreover, the transcription-regulatory protein cAMP-response element-binding protein (CREB) may be involved, since decreased phosphorylation of CREB at Ser^{133} was followed PTEN expression, and ectopic expression of CREB repressed completely the PTEN-induced inhibition of Bcl-2 promoter activity. Furthermore, cotransfection of Bcl-2 and PTEN expression vectors rescued PTEN-induced cell death but not G_1 cell cycle arrest. Finally, forced expression of PTEN sensitized LNCaP cells to cell death induced by staurosporine, doxorubicin, and vincristine, and this chemosensitivity was attenuated by exogenous expression of Bcl-2. Taken together, these data demonstrate that loss of PTEN leads to up-regulation of the bcl-2 gene, thus contributing to survival and chemoresistance of PCa cells. These findings suggest that the PTEN gene and its regulated pathways are potential therapeutic targets in prostate cancer.

Prostate cancer (PCa) is the most commonly diagnosed cancer and the second leading cause of male cancer death in the United States (1). The most effective therapy for advanced PCa for the past 50 years has been the removal of testicular androgen

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¶¶ The abbreviations used are: PCa, prostate cancer; CREB, cyclic AMP-response element-binding protein; LNCaP-Rf, androgen-refractory subline of LNCaP; PtdIns, phosphatidylinositol; EGFP, enhanced green fluorescent protein; PBS, phosphate-buffered saline.
and CREB signaling. In addition, we show that PTEN-induced chemosensitivity in PCA cells is achieved, at least in part, by inhibition of Bcl-2 expression.

**EXPERIMENTAL PROCEDURES**

**Cancer Cell Lines and Cell Culture**—The prostate cancer cell lines PC-3, DU145, and LNCaP were purchased from the American Type Culture Collection (Manassas, VA). Cells were maintained in RPMI 1640 medium (BioWhittaker, Walkersville, MD) containing 10% fetal bovine serum (Life Technologies, Inc., Grand Island, NY). Aliquots (1 × 10⁶) were seeded into the 35-mm plates. Following treatments with staurosporine (500 nM), doxorubicin (50 µM), or vincristine (500 ng/ml) (Calbiochem), adherent and nonadherent cells were collected over the time course indicated. Cells were stained with trypan blue exclusion analysis. Cells were transfected with expression vectors for PTEN and/or Bcl-2 along with pEGFP-N1. 12 h after transfection, PTEN or Bcl-2 were selected for EGFP expression. For PTEN expression previously (40), enriched cells (5 × 10⁶) were seeded into the 35-mm plate. Following treatments with staurosporine (500 nm), doxorubicin (500 ng/ml), or vincristine (500 ng/ml) (Calbiochem), adherent and nonadherent cells were collected over the time course indicated. Cells were stained with trypan blue exclusion analysis.

**Tumor Specimens and Immunohistochemistry**—Seventeen cases of prostate adenocarcinoma were selected from the surgical pathology files at the Mayo Clinic from patients who had undergone radical retropubic prostatectomy and bilateral pelvic lymphadenectomy between 1991 and 1994. All patients had clinically localized prostate cancer, and none had received preoperative hormone or radiation therapy. The distribution of tumor stage (1997) was as follows: T2bN0 (four cases), T3aN0 (three cases), and T3bN0 (ten cases). Gleason scores was as follows: Gleason 7 (seven cases), Gleason 8 (four cases), and Gleason 9 (six cases). All of the cases had Gleason scores of 8 or less. A monoclonal anti-β-tubulin (2-28-33) was purchased from Sigma. Antibodies against Bcl-2 (100), Bcl-2XL (H-5), and extracellular signal-regulated kinase 2 (D-2) were purchased from Santa Cruz Biotechnology, Inc. Polyclonal antibodies against Akt, phospho-Akt (Ser473), CREB, and phospho-CREB (Ser133) were purchased from Cell Signaling Technology (Beverly, MA).

**Cell Transfections**—Transient transfections of LNCaP and PC-3 cells were performed by electroporation. Total plasmid DNA (5–20 µg) was kept constant for all transfections by adding empty vectors and pEGFP-N1 plasmid (1–2 µg). Cells were mixed with DNA in 400 µl of growth medium. The DNA-cell mixture was transferred into a 4-mm cuvette (BTX Inc., San Diego, CA) and electroporated with a 150–300 V/10-ms pulse using a BTX T820 square wave electroporator (BTX Inc., San Diego, CA). In each experiment, transfection efficiency was monitored 12 h after transfection by examining aliquots of cells under a Zeiss fluorescence microscope with a wavelength of 488 nm. Transfection efficiency was determined by the percentage of the green cells in the total population. Routinely, 70–95% transfection efficiencies were obtained, and the cells with >75% transfection efficiency were used for further experiments.

**Transcriptional Assays**—Northern blot analysis of Bcl-2 RNA expression was performed as described previously (46). Cells transfected with pCMV-PTEN or a control vector were collected, and total RNA was isolated using the TRIZOL reagent (Life Technologies, Inc., Grand Island, NY). Aliquot (15 µg) of cellular RNA was separated on 1.2% denaturing formaldehyde-agarose gels. A fragment for human Bcl-2 cDNA in pBLC plasmid, purchased from the American Type Culture Collection, was used as a probe for Northern blot hybridization. After stripping, the blot was reprobed with a PTEN cDNA fragment (corresponding to the open reading frame of the PTEN gene) to monitor the ectopic expression of the PTEN gene. Regulation of Bcl-2 promoter activity was determined by using luciferase reporter assays. Cells were harvested 36 h after transfections, and cell lysates were prepared by adding lysis buffer directly to the cells on ice. Firefly luciferase and Renilla luciferase activities were determined using a dual luciferase kit (Promega), and Renilla luciferase activities of cells were used as internal controls.

**Fluorescence-activated Cell Sorting Analysis of Cell Cycle and Apoptosis**—The cells were sorted for EGFP expression 12 h after transfection using FACS Vantage SE (Becton Dickinson, San Jose, CA). Enriched transfected cells were cultured for an additional 48 h. Cells were collected and fixes in freshly prepared ice-cold 70% ethanol for 30 min. After washing with 1 × PBS, the cells were stained with 10 µg/ml propidium iodide and 50 µg/ml RNase A. Cells (1 × 10⁶) were incubated for 30 min. Cell cycle distributions were determined by flow cytometry analysis using a FACS Calibur flow cytometer (Becton Dickinson). Cells were lysed in HPS solution (0.1% sodium citrate, 0.1% Triton X-100, 50 µg/ml propidium iodide), and hypodiploid nuclei were counted by flow cytometry to identify those undergoing apoptosis. Cell cycle and apoptosis data were analyzed with ModFit II and WinMDI software, respectively.

**RESULTS**

**Expression of PTEN and Bcl-2 in Malignant Prostate Tissues and Cell Lines**—A number of studies have reported either loss of PTEN or overexpression of Bcl-2 in advanced prostate tumors (9, 10, 33). However, no study has examined expression of
The Lipid Phosphatase Function of PTEN Is Required for Its Regulation of Bcl-2 Expression—PTEN possesses both protein phosphatase and lipid phosphatase activities (14, 18, 19, 50). The cancer-related mutations of PTEN have been mapped mainly to the phosphatase catalytic domain (7). Several mutated forms of PTEN protein, which lack only the lipid phosphatase function (e.g., PTEN-G129E) or lack both the lipid and protein phosphatase functions (e.g., PTEN-C124S and PTEN-G129R) have been identified (14, 19). Thus, we utilized these constructs to determine whether PTEN-mediated regulation of Bcl-2 requires the activities of protein phosphatase, lipid phosphatase, or both. LNCaP cells were co-transfected with the Bcl-2 promoter-reporter construct and expression constructs containing either the wild-type PTEN or the mutated forms of PTEN (PTEN-G129E, PTEN-C124S, and PTEN-G129R). As shown in Fig. 5A, the restoration of wild-type PTEN into LNCaP cells resulted in a dramatic decrease of Bcl-2 promoter activity. However, none of the PTEN mutants exhibited any inhibition of Bcl-2 promoter-reporter activity, although comparable levels of PTEN protein were detected by Western blot analysis (data not shown). Therefore, we conclude from these experiments that the lipid phosphatase function of PTEN is sufficient for the inhibition of Bcl-2 expression.

Since Akt is an integral part of PTEN signaling, we sought to determine whether Akt could reverse the negative effect of PTEN on Bcl-2 expression. For these studies, we used a myristoylated form of Akt that is constitutively active. As shown in Fig. 5B, expression of the constitutively active Akt attenuated the inhibition of Bcl-2 promoter reporter activity by PTEN.

We were interested to see whether there is a correlation between the activation of endogenous Akt and Bcl-2 expression in prostate cancer cell lines. As shown in Fig. 2, Akt in DU145 cells is underphosphorylated. In contrast, Akt is highly phosphorylated in PC-3, LNCaP, and LNCaP-Rf cell lines, although endogenous levels of Akt protein are similar in all cell lines. A comparison of the four cell lines shows a correlation between Bcl-2 expression and phosphorylated (active) Akt. Taken together, these data suggest that the lipid phosphatase function of PTEN suppresses Bcl-2 expression by inhibiting Akt signaling in PCa cells.

### Table I

| PTEN (-) | PTEN (+) |
|----------|----------|
| % (count/total) | % (count/total) |
| Bcl-2 (+) | 41.2 (7/17) | 11.8 (2/17) |
| Bcl-2 (-) | 5.8 (1/17) | 41.2 (7/17) |

*a* Cases were scored as PTEN-negative when staining was lost in 60% or more of the malignant cells; otherwise they were scored as positive.

*b* Cases were scored as Bcl-2-positive when staining was seen in 30% or more of the malignant cells; otherwise they were scored as negative.

### Summary of PTEN and Bcl-2 protein expression in prostate cancer tissues from radical prostatectomy specimens

| Case | PTEN | Bcl-2 | PTEN | Bcl-2 |
|------|------|-------|------|-------|
| A    | 7/17 | 41.2% | 11.8% | 2/17  |
| B    | 7/17 | 41.2% | 11.8% | 2/17  |

Expression of both PTEN and Bcl-2 proteins was also examined in the PCa cell lines DU145, PC-3, LNCaP, and LNCaP-Rf, an androgen-refractory subline of LNCaP. As shown in Fig. 2A, DU145 cells express PTEN, but not Bcl-2. In contrast, PC-3, LNCaP, and LNCaP-Rf cells express Bcl-2 but not PTEN. Expression levels of PTEN and Bcl-2 proteins in individual cells were further analyzed by immunofluorescence. As shown in Fig. 2B, although PTEN protein was detected, no Bcl-2 protein was evident in DU145 cells. In contrast, Bcl-2 protein was observed, but no PTEN was detectable in PC-3, LNCaP, and LNCaP-Rf cells. Taken together, these studies show that Bcl-2 expression is inversely correlated with PTEN loss in many malignant prostate tissues and cell lines.

### Ectopic Expression of PTEN in PTEN-Null Cells Suppresses Bcl-2 Expression

Although LNCaP and PC-3 cells lack PTEN protein, both cell lines display an intact PTEN signaling pathway (18, 47–49). To determine a causal relationship between the PTEN loss and Bcl-2 overexpression, we transiently transfected a PTEN expression vector into LNCaP and PC-3 cells. As shown in Fig. 3, ectopic expression of PTEN in both LNCaP and PC-3 cells resulted in decreased levels of Bcl-2 protein in a dose-dependent manner. In contrast, PTEN did not affect the expression of Bcl-2 protein, both cell lines display an intact PTEN signaling pathway.

We were interested to see whether there is a correlation between the activation of endogenous Akt and Bcl-2 expression in prostate cancer cell lines. As shown in Fig. 2, Akt in DU145 cells is underphosphorylated. In contrast, Akt is highly phosphorylated in PC-3, LNCaP, and LNCaP-Rf cell lines, although endogenous levels of Akt protein are similar in all cell lines. A comparison of the four cell lines shows a correlation between Bcl-2 expression and phosphorylated (active) Akt. Taken together, these data suggest that the lipid phosphatase function of PTEN suppresses Bcl-2 expression by inhibiting Akt signaling in PCa cells.
PTEN Modulates Bcl-2 Expression via the CREB Transcription Factor—Bcl-2 expression is mediated primarily by the CREB transcription factor (27–30). The phosphorylation of CREB at serine 133 is associated with its transcriptional activity (51). Therefore, we sought to determine whether transfer of PTEN into LNCaP cells would affect the phosphorylation of CREB at serine 133. Fig. 6A shows that ectopic expression of PTEN diminished the phosphorylation of CREB in a dose-dependent manner, whereas it had no effect on the total levels of CREB protein. The function of PTEN was also monitored by Akt phosphorylation at serine 473. As expected, Akt phosphorylation at serine 473 was completely blocked in a dose-dependent manner by PTEN expression (Fig. 6A). In addition, we co-transfected LNCaP cells with PTEN and wild-type CREB expression vectors along with the Bcl-2 promoter-luciferase construct. As shown in Fig. 6B, overexpression of CREB completely reversed the PTEN-induced inhibition of Bcl-2 promoter-luciferase activity. Together, these data suggest that PTEN down-regulates Bcl-2 expression by decreasing the phosphorylation of CREB, thereby modulating its transcriptional activity. PTEN Induces Chemosensitivity in LNCaP Cells by Down-regulating Bcl-2 Expression—Reconstitution of PTEN into PTEN-null cells such as LNCaP can induce both cell cycle

![PTEN Modulates Bcl-2 Expression via the CREB Transcription Factor](image-url)
Transfection efficiencies were observed in these experiments. Data represent the mean values ± S.E. of three independent experiments.

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The constitutively active form of Akt (myristoylated Akt (Myr-Akt)) was co-transfected with the pBcl2-Luc into LNCaP cells (1 × 10⁶). Thirty-six hours after transfection, luciferase activities were measured in each sample. A Renilla luciferase vector was used as an internal control. Data represent the mean values ± S.E. of three independent experiments.

A

B

PTEN Down-regulates Bcl-2 Expression

Our studies demonstrate that reconstitution of PTEN into LNCaP and PC-3 cells, which lack the endogenous PTEN protein, results in decreased levels of Bcl-2 protein and messenger RNA. Our data demonstrate further that PTEN-induced down-regulation of Bcl-2 in these cells requires the lipid phosphatase

FIG. 5. Down-regulation of Bcl-2 is mediated by the lipid phosphatase function of PTEN. A, wild-type PTEN, the mutated forms (PTEN-C124S, PTEN-G129E, and PTEN-G129R), and the control vector pcDNA3.1 were co-transfected with the pBcl2-Luc into LNCaP cells (1 × 10⁶). 36 h after transfection (83 ± 2.2% efficiencies), luciferase activities were measured in each sample. A Renilla luciferase vector was used as an internal control. Data represent the mean values ± S.E. of three independent experiments. B, wild-type PTEN and increasing amounts of a constitutively active form of Akt (myristoylated Akt (Myr-Akt)) were co-transfected with the pBcl2-Luc into LNCaP cells (1 × 10⁶). Thirty-six hours after transfection, luciferase activities were measured in each sample. A Renilla luciferase vector was used as an internal control. Data represent the mean values ± S.E. of three independent experiments.

FIG. 6. PTEN regulates Bcl-2 expression through modulating CREB signaling. A, ectopic expression results in a decrease of CREB phosphorylation at serine 133. LNCaP cells (1 × 10⁶) were transfected with or without the PTEN expression vector. After 48 h, cells were lysed, and 50 μg of protein from each sample was subjected to Western blot analysis for phosphorylated CREB at serine 133 (CREB-p), pan-CREB, Akt, phospho-Akt at serine 473 (Akt-p), and PTEN. Phosphorylated ATF1 (ATF1-p), a protein related to CREB, can also be detected by the phospho-CREB-specific antibody. 97 ± 1.9% transfection efficiencies were observed in these experiments 12 h post-electroporation. Data are representative of the experiment performed three times. B, PTEN-induced inhibition of Bcl-2 promoter-reporter activity can be reversed by ectopic expression of CREB. Wild-type PTEN and increasing amounts of pCMV-CREB expression vector were co-transfected with the pBcl2-Luc reporter construct into LNCaP cells (1 × 10⁶). 36 h after transfection (88 ± 2.5% efficiencies), luciferase activities were measured in each sample. A Renilla luciferase vector was used as an internal control. Data represent the mean values ± S.E. of three independent experiments.

A

B

The decrease in Bcl-2 protein appears to be important for cell viability but not for cell cycling.

Overexpression of Bcl-2 has been associated with chemoresistance in many malignant tumors including those of the prostate (53, 54). Therefore, we were interested in determining whether the PTEN-induced decline of Bcl-2 levels would alter the chemosensitivity of LNCaP cells. We transiently transfected LNCaP cells with an empty vector or expression vectors containing PTEN, Bcl-2, or a combination of PTEN plus Bcl-2 and exposed the cells to staurosporine, doxorubicin, or vincristine. We then analyzed cell viability. As shown in Fig. 8, overexpression of Bcl-2 alone conferred resistance of LNCaP cells to the effects of staurosporine, doxorubicin, and vincristine. In contrast, PTEN expression substantially sensitized LNCaP cells to the killing effects of all three chemotherapeutic agents (Fig. 8). However, this chemosensitivity was alleviated in part by co-transfection of the bcl-2 gene (Fig. 8). DU145 cells that contain functional PTEN were not affected by the ectopic expression of PTEN (data not shown). Thus, we conclude that PTEN-induced chemosensitivity in PCa cells is mediated, at least in part, via the down-regulation of Bcl-2.

DISCUSSION

Loss of PTEN function and overexpression of Bcl-2 occur frequently in PCa, and both events are associated with the development of PCa resistant to androgen deprivation and chemotherapy (33, 35, 36, 54, 55). Our current understanding of the molecular mechanisms underlying the role of Bcl-2 and its overexpression in PCa is very limited. In this study, we examined the expression of PTEN and Bcl-2 in primary prostate tumors and PCa cell lines. The expression status of Bcl-2 or PTEN in DU145, PC-3, or LNCaP cells has been reported previously (11, 37, 48, 56). However, to our knowledge, this is the first study to report that Bcl-2 expression is inversely correlated with PTEN loss in prostate cancer tissues and cell lines.

Our studies demonstrate that reconstitution of PTEN into LNCaP and PC-3 cells, which lack the endogenous PTEN protein, results in decreased levels of Bcl-2 protein and messenger RNA. Our data demonstrate further that PTEN-induced down-regulation of Bcl-2 in these cells requires the lipid phosphatase function of PTEN to regulate Bcl-2 expression through modulating CREB signaling. A, ectopic expression results in a decrease of CREB phosphorylation at serine 133. LNCaP cells (1 × 10⁶) were transfected with or without the PTEN expression vector. After 48 h, cells were lysed, and 50 μg of protein from each sample was subjected to Western blot analysis for phosphorylated CREB at serine 133 (CREB-p), pan-CREB, Akt, phospho-Akt at serine 473 (Akt-p), and PTEN. Phosphorylated ATF1 (ATF1-p), a protein related to CREB, can also be detected by the phospho-CREB-specific antibody. 97 ± 1.9% transfection efficiencies were observed in these experiments 12 h post-electroporation. Data are representative of the experiment performed three times. B, PTEN-induced inhibition of Bcl-2 promoter-reporter activity can be reversed by ectopic expression of CREB. Wild-type PTEN and increasing amounts of pCMV-CREB expression vector were co-transfected with the pBcl2-Luc reporter construct into LNCaP cells (1 × 10⁶). 36 h after transfection (88 ± 2.5% efficiencies), luciferase activities were measured in each sample. A Renilla luciferase vector was used as an internal control. Data represent the mean values ± S.E. of three independent experiments.
PTEN Down-regulates Bcl-2 Expression

Fig. 7. Effects of Bcl-2 rescue on PTEN-induced apoptosis and cell cycle arrest in LNCaP cells. A, Bcl-2 abrogates PTEN-induced apoptosis. pCMV-PTEN (5 μg) was co-transfected with or without pUSE-Bcl-2 expression vector (5 μg) as well as 2 μg of pEGFP-N1 into LNCaP cells (1 × 10^6). 12 h after electroporation, cells were sorted for EGFP expression. Transfected cells were cultured for an additional 60 h and prepared as described under “Experimental Procedures.” Apoptosis was analyzed by measuring hypodiploid cells. B, overexpression of Bcl-2 has no effect on PTEN-induced G1 cell arrest. Cells were transfected as described above and prepared for cell cycle analysis as described under “Experimental Procedures.” Data are from a representative experiment performed three times.

Fig. 8. PTEN induces chemosensitivity in LNCaP cells by inhibition of Bcl-2 expression. LNCaP cells (2 × 10^6) were co-transfected by electroporation with pEGFP-N1 (5 μg), pCMV-PTEN (30 μg), and/or pUSE-Bcl-2 (30 μg). Cells were sorted for EGFP expression 12 h after electroporation. Transfected cells (5 × 10^6) were seeded into 35-mm plates. 12 h later, cells were treated with staurosporine (500 nM), doxorubicin (500 ng/ml), or vincristine (500 ng/ml). Adherent and nonadherent cells were collected over the time course indicated, and viable cells were analyzed using a trypan blue exclusion assay. Cells transfected with empty vector pDNA3.1 or pUSE-Bcl-2 were used as controls. Data represent the mean values ± S.E. of three independent experiments.

function of PTEN as well as inhibition of Akt signaling. These findings support similar results in T cells (57) and PC12 cells (26), indicating that PTEN/Akt-mediated regulation of Bcl-2 expression is a common event in mammalian cells. Therefore, it is likely that frequent loss of PTEN in cancer cells provides a favorable signaling event that induces an increase in Bcl-2 expression, thereby providing advantages for cell survival.

The bcl-2 gene is transcriptionally regulated by many transcription factors including CREB, p53, Wilms’ tumor 1 gene, and estrogen receptor (27, 45, 53, 58). However, the basal activity of Bcl-2 is mediated primarily by CREB (27). In addition, CREB mediates the induction of Bcl-2 by a number of growth factors (28–30). Thus, any signaling that can modulate CREB activity should affect Bcl-2 expression. CREB is one of the downstream targets of Akt (25). In fact, IGF-1-induced up-regulation of Bcl-2 in PC12 cells is mediated by CREB through its activation by Akt (26). Moreover, Akt signaling can be modulated by many intracellular events such as PTEN mutations, integrin-linked kinase overexpression, and phosphoinositide 3-kinase amplification (18, 48, 59–61). As we have demonstrated in this study, PTEN transfer into PTEN-null PCa cells not only abolishes the Akt activation but also diminishes the phosphorylation of CREB and inhibits Bcl-2 expression. These findings suggest a mechanism by which Bcl-2 is up-regulated in the course of PCa progression. Such a mechanism would include loss of PTEN activity, with the concomitant activation of Akt and CREB.

Presently there is no cure for advanced PCa. One of the factors that may contribute to the resistance of PCa to chemotherapy and androgen deprivation is the overexpression of Bcl-2 (36, 62, 63). Treatment of PCa cells with paclitaxel results in the phosphorylation and inactivation of Bcl-2, thereby enhancing chemosensitivity (37, 56, 64). Down-regulation of Bcl-2 levels by genetic approaches, such as the expression of a Bcl-2 antisense cDNA or a ribozyme cRNA, also induces chemosensitivity in prostate cancer cells both in vitro and in vivo (38, 65–68). In this study, we demonstrated that PTEN reconstitution in PTEN-null PCa cells causes a decrease of Bcl-2 protein, which further enhances chemosensitivity of PCa cells.

PTEN overexpression can override the chemoresistance of doxorubicin-resistant bladder cancer cells by inhibiting the activity of Akt (69). However, PTEN gene transfer has no effect on the chemosensitivity of malignant glioma cells that have no functional PTEN, although these cells are sensitized to irradiation- or CD95L-induced apoptosis (70). Accordingly, transfer of PTEN into PC12 cells induces both growth arrest and apoptosis (47, 48). In contrast, reconstitution of PTEN into glioblastoma cells induces only growth suppression (16, 17, 70). Thus, PTEN-induced chemosensitivity in PCa cells may also be cell type-specific. However, whether this specificity is associated with Bcl-2 signaling is not clear.

In summary, we have demonstrated that loss of PTEN and overexpression of Bcl-2 are inversely correlated in both malignant prostate tissues and cell lines. PTEN transfer in the PTEN-null PCa cells induces a decrease of Bcl-2 expression. In addition, the lipid phosphatase function of PTEN is required for the down-regulation of Bcl-2. The effect of PTEN on Bcl-2 expression is accomplished by inhibiting the activities of Akt and CREB. Furthermore, PTEN induces chemosensitivity in PCa cells, which is mediated, at least in part, by its suppression of Bcl-2 expression. Therefore, our results not only define a molecular mechanism that provides an explanation for the overexpression of Bcl-2 in advanced prostate cancer but also suggest that the PTEN gene and its regulated pathway are therapeutic targets for advanced prostate cancer.

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