PED Mediates AKT-Dependent Chemoresistance in Human Breast Cancer Cells

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Abstract
Killing of tumor cells by cytotoxic therapies, such as chemotherapy or gamma-irradiation, is predominantly mediated by the activation of apoptotic pathways. Refractoriness to anticancer therapy is often due to a failure in the apoptotic pathway. The mechanisms that control the balance between survival and cell death in cancer cells are still largely unknown. Tumor cells have been shown to evade death signals through an increase in the expression of antiapoptotic molecules or loss of proapoptotic factors. We aimed to study the involvement of PED, a molecule with a broad antiapoptotic action, in human breast cancer cell resistance to chemotherapeutic drugs–induced cell death. We show that human breast cancer cells express high levels of PED and that AKT activity regulates PED protein levels. Interestingly, exogenous expression of a dominant-negative AKT CDNA or of PED antisense in human breast cancer cells induced a significant down-regulation of PED and sensitized cells to chemotherapy-induced cell death. Thus, AKT-dependent increase of PED expression levels represents a key molecular mechanism for chemoresistance in breast cancer. (Cancer Res 2005; 65(15): 6668-75)

Introduction
PED (known also as PED/PEA-15) is a death effector domain (DED)-family member of 15 kDa with different effects on cell growth and metabolism (1–3). The protein consists of an NH2-terminal DED of 80 amino acids and a COOH-terminal region in which are located the phosphorylation sites for protein kinase C (Ser104), calcium calmodulin kinase II and AKT (Ser116; refs. 4–6). PED inhibits the formation of a functional death-inducing signaling complex and caspase 3 activation following treatment with different apoptotic cytokines including CD95/Fasl, tumor necrosis factor-α (TNF-α) and TNF-related apoptosis-inducing ligand (TRAIL; refs. 2, 7, 8). At least in part, the antiapoptotic action of PED is accomplished through its DED domain, which likely acts as a competitive inhibitor for proapoptotic molecules during the assembly of the death-inducing signaling complex (2, 9). Protein kinase C and AKT play an important role in enabling the antiapoptotic function of PED (5, 6, 9) by regulating its specific interactions and localization within the cell. In order to be recruited into the TRAIL receptor death-inducing signaling complex in TRAIL-resistant gliomas, PED has to be doubly phosphorylated (9). Furthermore, PED inhibits the induction of different stress-activated protein kinases triggered by growth factor deprivation, hydrogen peroxide, and anisomycin (10). PED has been found overexpressed in a number of different tumors, including human gliomas (7) and squamous carcinoma (11). However, the meaning and the molecular mechanisms of deregulation of PED expression in cancer cells are presently unknown. We have recently shown in vitro as well as in intact cells that PED is a substrate of AKT that phosphorylates it on Ser116 and that, in turn, phosphorylation of S116 increases PED protein stability (6). AKT plays a key role on cell survival (12–14). Furthermore, up-regulation of AKT activity has been implicated in a number of human cancers (15, 16), including breast cancer (17). Converging evidences suggest that deregulation of PED expression levels may play an important role in breast cancer (18–20). The 3’ untranslated region of the 2.5-kb spliced isoform of PED contains the mammary transforming (MAT1) proto-oncogene. This sequence, isolated from an experimental mouse mammary tumor, was reported to induce the oncogenic transformation of NIH-3T3 cells and the mammary epithelial cell line TM3 (18). The MAT1-containing PED mRNA is expressed weakly during certain physiologic circumstances, such as pregnancy and lactation, but becomes strongly expressed in murine mammary tumors (19). Furthermore, ped/pea-15 gene maps at 1q21-q22 between the markers D1S2635 and D1S4841 (1) a region frequently gained (up to 50%) in breast cancer (20) and rearranged in leukemia (21). We have previously shown that in the breast cancer cell line MCF-7, PED overexpression induces resistance toward TNF-α and Fasl/CD95-induced apoptosis (2).

We therefore investigated if PED could play a role in the resistance to cell death in breast tumors. We additionally studied the contribution of AKT in PED overexpression and in breast cancer resistance to chemotherapy.

Materials and Methods
Cell culture. Human MCF7, MDA-MB-231, MDA-MB-131, ZR-78 breast cancer cell lines were grown in RPMI 1640 (MDA) or DMEM (MCF7 and ZR-78; Life Technologies, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum supplemented with 2 mmol/L L-glutamine and 100 units/mL penicillin-streptomycin.

Human SK Br-3 and MDA-MB-468 were kindly provided by Dr. Normanno (Istituto Pascale, University of Naples, Naples, Italy) and grown in RPMI. MCF-10A cell line was kindly provided by Dr. Normanno and grown in 50% MF12-50% DMEM (Life Technologies) containing 5% horse serum supplemented with 2 mmol/L L-glutamine and 100 units/mL penicillin-streptomycin, 10 μg/mL insulin, 0.5 μg/mL hydrocortisone, 0.001 μmol/L 17β-estradiol and 0.01 μmol/L dexamethasone.

Note: G. Stassi and M. Garofalo contributed equally to this work.

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10 µg/mL epidermal growth factor (Sigma, St. Louis, MO). Cells were kept in a 5% CO₂ atmosphere and routinely passaged when 80% to 85% confluent.

**Breast cell purification from human tissue.** Specimens were manually cut in small pieces and digested for 4 hours at 37°C in a shaking incubator with 0.5 mg/mL collagenase (Type II, Life Technologies) in DMEM/F12 (Life Technologies) supplemented with 10% fetal bovine serum. Following enzymatic digestion, cells were centrifuged at 1,500 × g and plated in 75 cm² culture flasks coated with 5 µg/mL type I collagen (Calbiochem-Novabiochem, Darmstadt, Germany). Purified cells were allowed to grow in monolayer and detached with trypsin plus EDTA for functional and protein expression analyses.

**Materials.** Media, sera, and antibiotics for cell culture were from Life Technologies. Protein electrophoresis reagents were from Bio-Rad Laboratories (Richmond, VA) and Western blotting and enhanced chemiluminescence reagents from Amersham (Arlington Heights, IL). All other chemicals were from Sigma.

**Flow cytometry.** Cytometric staining was analyzed on a FACSCalibur Instrument (Becton Dickinson, Franklin Lakes, NJ) and data were analyzed with Cell Quest software (Becton Dickinson).

**Protein isolation and Western blotting.** Breast tissue specimens were collected from surgical mastectomy of 20 patients affected by adenocarcinoma, in accordance with the ethical standards of the institution responsible (Committee on Human Experimentation). From the same patient, we collected both neoplastic and adjacent normal tissue. The tissues were homogenized in a homogenizer (Calbiochem-Novabiochem, Darmstadt, Germany). Samples derived from human tissue were precipitated by adding 4 volumes of cold acetone. Briefly, the samples were kept for 30 minutes at −20°C in the presence of cold acetone and centrifuged at 15,000 × g. The pellets were dried and resuspended in Harvest buffer. Fifty micrograms of sample extract were resolved on 12% SDS-polyacrylamide gels using a mini-gel apparatus (Bio-Rad Laboratories) and transferred to Hybond-C Extra Nitrocellulose (Amersham Pharmacia Biotech, Piscataway, NJ). Membranes were blocked for 1 hour with 5% nonfat dry milk in TBS containing 0.05% Tween 20 and incubated for 2 hours with specific antibodies. The following antibodies were used for immunoblotting: anti-AKT (Cell Signaling Technology, Inc., Charlottesville, VA); anti-actin (clone A5441, Vector Laboratories, Burlingame, CA); rabbit polyclonal IgG, Cell Signaling Technology); anti-PED serum previously described (1) and anti-β-actin (Ab-1, mouse IgM, Oncogene, Darmstadt, Germany) anti-p53 from (Santa Cruz Biotechnology, Santa Cruz, CA; sc-6243). The washed membranes were then incubated for 45 minutes with horseradish peroxidase–conjugated anti-rabbit or anti-mouse secondary antibodies (Amersham Pharmacia Biotech) and visualized by using a chemiluminescence detection system (Amersham Pharmacia Biotech).

**Immunohistochemistry.** Immunohistochemical staining was done on 5-µm-thick human breast cancer and normal paraffin sections. Deparaffinized sections were heated in a microwave oven for antigen unmasking in 10 mM sodium citrate (pH 6.0) for 1 minute at 450 W followed by 5 minutes at 100 W. The slides were then cooled for 20 minutes washed in distilled water and PBS, and permeabilized with 0.1% Triton X-100/PBS for 5 minutes. After rinsing with PBS, sections were incubated with 3% hydrogen peroxide for 3 minutes and then with blocking solution for 10 minutes. The sections were exposed overnight at 4°C to rabbit anti-PED antibody (1:100) in 3% bovine serum albumin/PBS and then for 30 minutes at room temperature with a biotinylated secondary antibody. Finally, sections were rinsed and incubated with streptavidin for 30 minutes. Nuclei were counterstained using hematoxylin.

**Cell death and cell proliferation quantification.** Cell viability was evaluated with the CellTiter 96 AQueous One Solution Cell Proliferation

| Table 1. Clinical features of breast cancer patients and PED and p-AKT expression levels |
|---|---|---|---|---|---|---|---|
| Patient no. | Age | Histologic type | TNM stage | Estrogen receptor (%) | Progesterone receptor (%) | erbB2 receptor (%) | PED expression levels | p-AKT |
| 1 | 50 | CDI, G3 | pT2aN1B | 20 | — | — | +++ | — |
| 2 | 58 | CDI, G3 | pT2aN1B | 40 | — | 100 | +++ | +++ |
| 3 | 43 | CDI, G3 | pT1pN1 | 70 | 80 | — | ++ | ++ |
| 4 | 51 | CLI | pT2aN0 | 55 | 25 | — | ++ | ++ |
| 5 | 79 | CDI, G2 | pT1aN0 | 50 | 35 | — | +++ | +++ |
| 6 | 49 | CDI, G1 | pT1aN0 | 50 | 50 | — | +++ | +++ |
| 7 | 60 | CDI, G1 | pT1aN0 | 20 | 40 | — | — | — |
| 8 | 59 | CDI, G2 | pT1aN1 | 60 | 10 | — | ++ | ++ |
| 9 | 56 | CDI, G2 | pT1aN1B | 65 | — | 30 | +++ | +++ |
| 10 | 67 | CDI, G3 | pT1bN1B | 80 | 80 | — | ++ | ++ |
| 11 | 66 | CDI, G2 | pT1bN1B | 70 | 65 | — | +++ | +++ |
| 12 | 65 | CLI | pT2aN0 | 30 | 45 | — | — | — |
| 13 | 47 | CDI, G1 | pT1aN0 | 60 | 50 | — | + | + |
| 14 | 70 | CDI, G3 | pT2aN1B | 70 | 40 | — | +++ | +++ |
| 15 | 65 | CDI, G1 | pT1aN0 | 20 | — | — | — | — |
| 16 | 55 | CDI, G2 | pT1bN1B | 60 | — | 60 | +++ | +++ |
| 17 | 49 | CDI, G1 | pT1aN0 | 70 | 40 | — | +++ | +++ |
| 18 | 52 | CDI, G2 | pT1aN0 | 70 | 50 | — | ++ | ++ |
| 19 | 60 | CDI, G1 | pT1aN1B | 80 | 45 | — | +++ | +++ |
| 20 | 55 | CDI, G2 | pT1bN1B | 60 | — | 50 | +++ | +++ |

NOTE: PED and phospho-AKT expression levels were estimated by densitometric scanning and normalized for the corresponding actin expression levels. Data represent fold increase of PED and p-AKT in cancer samples compared with normal. Abbreviations: CDI, ductal infiltrating carcinoma; CLI, lobular infiltrating carcinoma (49); (−) no increase; (+) 1- to 3-fold increase; (+++) 4- to 6-fold increase; (++++) 6- to 10-fold increase; (++++) >10-fold increase.
Assay (Promega, Madison, WI), according to the manufacturer's protocol. Cells were plated in 96-well plates in triplicate, stimulated and incubated at 37°C in a 5% CO₂ incubator. Etoposide (7 μmol/L), vincristine (1 μmol/L), paclitaxel (5 μmol/L), cisplatin (300 ng/mL), and doxorubicin (5 μmol/L) were used in vitro at doses compatible with the levels reached in vivo during cancer treatment for 24 hours. Metabolically active cells were detected by adding 20 μL of MTS to each well. After 2 hours of incubation, the plates were analyzed on a Multilabel Counter (Bio-Rad).

Transduction of breast cells with lentiviral vectors. Gene transfer was done using a variant of the third-generation lentiviral vector (the advanced generation) recently described (22). In order to simultaneously transduce both reporter and target gene, a new lentiviral vector, Tween, was generated by engineering pRRLsin.cPPT.hCMV.hPGKGFP.Wpre. In this vector, the original hCMV.GFP cassette was substituted with hCMV.hPGK.GFP. A multiple cloning site was inserted downstream of hCMV.

PED antisense cDNA was obtained by PCR amplification of the human PED cDNA using the following primers: 5'-CCCCGCTAGCCGCTCAATGTAG-GAGAGGTG-3' and 5'-CCCTTCGAGGCCCAGGCCGCGGCGGTCGTG-3' containing the XhoI and Xhol cloning sites, respectively (7). The amplified fragment was subcloned in the XhoI site of the Tween vector. Myc-tagged PED cDNA (1) was subcloned in the Xhol site of the Tween lentivector.

AKT E40 K cDNA (constitutively active AKT cDNA, HA-AKTDP) and AKT K179M cDNA (dominant-negative AKT cDNA, HA-AKTDN) with an HA-tag were a kind gift from Prof. G.L. Condorelli (University of Rome "La Sapienza") and were subcloned in the Tween vector.

Lentiviral supernatants were produced by calcium phosphate transient cotransfection of a three-plasmid expression system in the packaging human embryonic kidney cell line, 293T. The calcium-phosphate DNA precipitate was removed after 14 to 16 hours by replacing the medium. Viral supernatant was collected 48 hours after transfection, filtered through 0.45-μm pore nitrocellulose filters and frozen in liquid nitrogen. On the same day of transfection, MDA MB-231 and MDA MB-468 and in the immortalized human mammary cell line MCF10-A (A). Loading control was assessed on the same blot with anti-β-actin antibody.

To evaluate PED expression levels, Western blot analysis was first done on a panel of human breast cancer–derived cell lines with anti-PED antibodies (Fig. 1A and B). We used the MCF-10A cell line, an immortalized human mammary epithelial-derived cell line that retains many characteristics of the normal mammary gland in culture, such as the ability to form acinar structures. PED was clearly detected in all the transformed cell lines analyzed, but not in MCF-10A lysates, thus indicating that PED protein expression is restricted to breast tumor–derived cell lines.

**PED expression in human breast cancer.** We next determined the expression levels of PED in human breast cancer specimens added to the viral supernatant to improve the infection efficiency (22). Cells were then centrifuged for 45 minutes at 1,800 rpm and incubated for 75 minutes in a 5% CO₂ atmosphere. Following the infection cycles, cells were washed twice and fresh medium was added. Infection efficiency was evaluated after 48 hours by flow cytometry.

**c-Jun-NH₂-kinase phosphorylation.** The cells were kept in medium supplemented with 1% serum for 18 hours and then incubated with 0.3 μg/ml paclitaxel as indicated in the description of the experiment. The cells were harvested and the lysates immunoblotted as described above. Anti-phospho (Thr183/Tyr185) c-Jun-NH₂-kinase (JNK; Cell Signaling Technology) or anti-JNK (Santa Cruz Biotechnology) antibodies were used.

**Results**

**PED expression in breast cancer cell lines.** To evaluate PED expression levels, Western blot analysis was first done on a panel of human breast cancer–derived cell lines with anti-PED antibodies (Fig. 1A and B). We used the MCF-10A cell line, an immortalized human mammary epithelial-derived cell line that retains many characteristics of the normal mammary gland in culture, such as the ability to form acinar structures. PED was clearly detected in all the transformed cell lines analyzed, but not in MCF-10A lysates, thus indicating that PED protein expression is restricted to breast tumor–derived cell lines.

**PED expression in human breast cancer.** We next determined the expression levels of PED in human breast cancer specimens.
from 20 patients. All the patients did not receive any medical treatment at the time of the operation. Immunoblot analysis showed that PED was strongly up-regulated in breast cancer cells compared with cells from the adjacent normal tissue (Fig. 2A; Table 1). However, in four tumor specimens—out of the 20 analyzed—(patients no. 1, 7, 12, and 15), we did not detect any difference in PED protein expression compared with the corresponding normal tissues (Table 1; Fig. 2B). Interestingly, these four patients did not exhibit erbB2 expression or strong expression of estrogen receptors (Table 1) at histologic analysis. These data indicate that PED is preferentially overexpressed in breast tumors compared with normal epithelium and that this overexpression is correlated with either erbB2 or estrogen receptor levels. Immunohistochemistry analysis confirmed the presence of PED in breast cancer tissue (Fig. 3A).

**Up-regulation of AKT in breast cancer correlates with overexpression of PED.** We have recently shown that PED is a substrate of AKT, which may regulate its levels by increasing the protein’s stability upon phosphorylation of Ser116 (6). AKT has been frequently found activated in breast cancer (17, 23, 24). Therefore, it is plausible that AKT is responsible for the accumulation of PED protein. In order to address this issue, we analyzed AKT activation in breast tumor samples in which PED was found to be overexpressed. As expected, Western blot and immunohistochemical analysis with anti-phospho-AKT (Ser473) antibodies revealed that AKT was phosphorylated to a greater extent in tumor-derived samples which expressed a higher amount of PED (Table 1; Figs. 2A and 2B). In samples from patients no. 1, 7, 12, and 15, we could not detect any difference in AKT activity and PED expression levels between cancerous areas of the specimen (Table 1; Fig. 2B). Thus, AKT activation in human breast cancer, as revealed by both Western blot and immunohistochemistry, correlates to the increase of PED protein levels.

**PED mediates breast cancer cell death resistance.** If AKT activity is a major determinant for the increased stability of PED in breast cancer, interfering with AKT signaling should modulate PED levels. We therefore tested this hypothesis evaluating PED expression and cell viability following exposure to different chemotherapeutic agents in primary breast tumor cultures and in the MDA-MB231 cell line infected with a lentiviral vector that expresses the HA-tagged dominant-negative or the dominant-active mutant of AKT (HA-AKTDN, HA-AKTDP). Western blot analysis revealed that viral infection with HA-AKTDN resulted in a significant expression of the kinase and in a reduction of PED expression levels (Fig. 4A and B). Furthermore, infection of with HA-AKTDN significantly increased breast cancer cell sensitivity to drug-induced cell death (Fig. 4C and D). Interestingly, infection of MDA cells with HA-AKTDP increased PED expression levels (Fig. 4B) and significantly decreased the sensitivity to drug-induced cell death (Fig. 4D). Finally, to prove that overexpression of PED is responsible for resistance to apoptosis in breast cancer, a PED antisense cDNA was cloned into the Tween lentiviral vector carrying a green fluorescent protein (GFP)-fusion protein and then transduced into the human breast cancer cell line, MDA MB-231. Fluorescence-activated cell sorting analysis of MDA MB-231 cells transduced with PED antisense-containing or a control lentiviral vector, showed that the infection produced a virtually pure population of GFP-positive cells as indicated by the homogenous right-shifted peak (Fig. 5A). The left-shifted peak represents noninfected cells. Transduction of MDA MB-231 with PED antisense considerably reduced PED expression as revealed by immunoblot analysis (Fig. 5B). Accordingly, MDA MB-231 transduced with PED antisense showed an increase in drug sensitivity (Fig. 5C). These data further suggest that PED protects breast cancer cells from chemotherapy-induced apoptosis.

**Inhibition of PED expression in MDA cells reestablishes c-Jun-NH2-kinase activation.** One of the mechanisms of chemotherapy-induced cell death is activation of the JNK pathway (25, 26). We have recently shown that in 293 cells overexpressing PED, JNK activation following different stress stimuli is impaired (10). To test whether this may also occur in breast cancer, we incubated MDA-MB231 cells expressing PED antisense cDNA (MDA PEDc) in the presence of paclitaxel for different lengths of time. JNK activation was evaluated by Western blotting with an

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**Figure 3.** Immunohistochemical analysis of PED expression and AKT activity in breast cancer. **A,** immunohistochemical analysis of paraffin-embedded normal or breast cancer sections labeled with control preimmune IgG (NC), cytokeratin (CK), or anti-PED antibody (1:100) and revealed by AEC (red staining). One representative of two independent experiments is shown. **B,** immunohistochemical analysis of phospho Akt (Ser^473^) done on paraffin-embedded sections of normal or cancer breast tissues (red staining). Hearts from AKT transgenic mice (48) were used as positive controls. One representative of two independent analyses using specimens from different patients is shown.
anti-phospho-JNK antibody that recognizes two JNK isoforms. As shown in Fig. 6, MDA PEDs, different from MDA control cells, had a consistent and sustained increase in JNK phosphorylation. As revealed with Western blot analysis, in both cell types, treatment with paclitaxel at longer time points induced a slight decrease in PED expression levels.

Thus, in breast cancer, PED overexpression may determine increased resistance to chemotherapeutic drug-induced cell death through an inhibition of the stress kinase pathway.

Effect of PED overexpression in normal mammary cells. In order to study the role of PED in normal breast tissue, Myc-tagged PED cDNA, cloned into the Tween lentiviral vector, was transduced into the human breast primary cells obtained from normal tissue and into MCF-10A cells (Figs. 7 and 8, respectively). We also transduced these cells with the HA- AKTDP lentiviral vector, previously described. Fluorescence-activated cell sorting analysis of transduced cells showed that the infection produced a virtually pure population of GFP-positive cells (data not shown). Western blot analysis with anti-PED antibody or with anti-HA antibody revealed that viral infection resulted in a significant expression of the proteins (Figs. 7A and 8A).

First, we studied the effect of PED on expression and activation of AKT, JNK, and p53 in normal conditions or under stress (paclitaxel treatment). AKT, p-AKT (Figs. 7B and 8B), p53, and JNK levels (Figs. 7C and 8C), were not affected by PED overexpression either in the presence or in the absence of paclitaxel. This strongly suggests that in breast cancer, activation of AKT is responsible for the increase in the expression of PED and not vice versa. The overexpression of PED in epithelial cells from normal breast induced a slight but significant increase in the rate of cell proliferation, as revealed in Figs. 7D and 8D, indicating that PED may also participate in the regulation of proliferation beside that of cell death. Interestingly, in MCF-10A cells, PED and AKT overexpression induced an increase in cell death resistance upon 24 hours incubation in serum deprivation conditions (Fig. 8E).

Discussion
The selective induction of cell death by drugs or cytokines is the final goal of any new therapeutic strategy for cancer. Apoptosis is believed to be the main mechanism of cell death induced by chemotherapeutics in cancer cells (27—29). However, in a number of patients, tumor cells evade death signals generated by drugs through the activation of effective antiapoptotic mechanisms, such as increased levels of caspase inhibitors or proteins of the Bcl-2 family (30, 31).

Increased expression of DED family members with antiapoptotic functions such as c-FLIP and PED could also represent a mechanism of apoptosis resistance in cancer cells (2, 3, 32—34). Both these molecules act primarily by preventing the interaction between the adaptor molecule FADD and procaspase-8. PED is a recently identified protein featuring a broad antiapoptotic function (6—9). We have previously shown that PED inhibits the anti-apoptotic signal of CD95/Fasl, TNF-α, and TRAIL in many different cell types, including breast carcinoma (2, 7, 8). PED interacts in vitro and in intact cells with the serine-threonine kinase AKT (6). AKT is also able to phosphorylate PED at Ser116 (6). AKT-mediated phosphorylation of PED increases its stability, thus determining an increase in the content of this protein within the cell (6).

In this work, we show that the differential activation of AKT in normal and transformed breast tissues represents the main mechanism responsible for PED overexpression and thus contributes to the relative increase in the resistance to chemotherapy-induced...
apoptosis observed in breast cancer. In most of the patients analyzed, we identified a tight correlation between AKT activation and PED levels. Furthermore, in this work, we show that exogenous expression of a dominant-negative AKT cDNA or of PED antisense cDNA in human breast cancer cells induced a significant down-regulation of PED and sensitized cells to chemotherapy-induced cell death. Thus, the level of PED seems to be directly correlated to apoptosis resistance in breast cancer.

Genetic and biochemical evidence suggests that activation of the phosphoinositide-3-kinase/AKT pathway contributes to breast cancer tumorigenesis. Up-regulation of AKT activity in breast cancer could be the result of mutations in the AKT-phosphatase
PTEN (35–39). Furthermore, overexpression of the epidermal growth factor receptor family member erbB2 (HER-2/neu) receptor (40–42), and overexpression of estrogen receptors have been shown to increase AKT activity (43, 44). 17β-Estradiol reduces apoptosis both in vitro and in animal models through estrogen receptors and phosphoinositide-3-kinase/AKT-dependent pathways. Treatment of cells with the estrogen receptor antagonist, ICI, blocked the increase in phospho-AKT after stimulation with 17β-estradiol, supporting the hypothesis that AKT activation may occur through an estrogen receptor–dependent mechanism (43). In addition, several compelling evidences support a key role played by AKT in determining tumor cell resistance to chemotherapy (45–47). Thus, one of the mechanisms of apoptosis resistance observed in human cancer characterized by constitutive AKT activation might involve an increase of PED levels.

PED overexpression was also observed in non–small cell lung cancer, in thyroid cancer and in B-lymphocytes from patients affected by B cell chronic leukemia thus suggesting that PED may play a general role in cancer development.26 Whether the increase in PED protein level is also mediated by AKT phosphorylation in these other tumors remains to be determined.

Chemotherapeutics induce cell death by activating JNK and p38 kinases. Activation occurs in a dose- and time-dependent manner (25, 26). Resistance to cell death may implicate an impairment in the activation of these stress kinase pathways. Indeed, the inhibition of JNK activity greatly reduces paclitaxel-induced cell death in cancer cells (25). We have previously shown that PED overexpression inhibits the induction of different stress-activated protein kinases triggered by growth factor deprivation, hydrogen peroxide and anisomycin (10). Interestingly, in this work, we show that in breast cancer, inhibition of PED expression results in sustained JNK activation.

Because expression levels of PED are a focal point for apoptosis regulation, PED represents an attractive target for therapeutic intervention in cancer treatment.

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6 G. Condorelli, M. Garofalo, and C. Zanca, unpublished data.

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Figure 8. Effect of PED overexpression in MCF-10A cells. A, immunoblot analysis of freshly purified primary breast epithelial cells transduced with empty vector (Twee), with Myc-Tagged PED cDNA (Myc-PED) or with (HA)-tagged dominant-active AKT mutant (AKTDP). Twenty micrograms of proteins were loaded. AKT expression was assessed by blotting for HA. B, Western blot analysis of AKT, phospho-AKT and (C) JNK and p53 expression in cells infected with PED cDNA in basal conditions (NT) or treated with 0.3 μg/mL paclitaxel for 12 hours (T). Loading control was assessed by β-actin staining. D, proliferation rate of MCF-10A cells transduced with empty vector or PED as indicated. Cells were plated at a density of 10,000 cells per well and growth rate was evaluated by the cell titer up to 4 days. E, experiments were done in triplicate and were repeated thrice (*, P < 0.005). Percentage of apoptotic cells in MCF-10A vector, MCF-10A PED, or AKTDP transduced cells in the absence of serum for 24 hours. One of three representative experiments is shown.


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