hPEBP4 Resists TRAIL-induced Apoptosis of Human Prostate Cancer Cells by Activating Akt and Deactivating ERK1/2 Pathways*

Hongze Li†, Xiaojian Wang‡, Nan Li§, Jianming Qiu¶, Yuanyuan Zhang†, and Xuetao Cao‡∥

From the †Institute of Immunology, Zhejiang University, Hangzhou 310031, China, the ‡1Institute of Immunology and National Key Laboratory of Medical Immunology, Second Military Medical University, Shanghai 200433, China, the §1Institute of Immunology and National Key Laboratory of Medical Immunology, Second Military Medical University, Shanghai 200433, China, the ¶1Institute of Immunology, Zhejiang University, Hangzhou 310031, China, the ‡1Institute of Immunology and National Key Laboratory of Medical Immunology, Second Military Medical University, Shanghai 200433, China

The treatment options available for prostate cancer are limited because of its resistance to therapeutic agents. Thus, a better understanding of the underlying mechanisms of the resistance of prostate cancer will facilitate the discovery of more efficient treatment protocols. Human phosphatidylethanolamine-binding protein 4 (hPEBP4) is recently identified by us as an anti-apoptotic molecule and a potential candidate target for breast cancer treatment. Here we found the expression levels of hPEBP4 were positively correlated with the severity of clinical prostate cancer. Furthermore, hPEBP4 was not expressed in TRAIL-sensitive DU145 prostate cancer cells, but was highly expressed in TRAIL-resistant LNCaP cells, which show highly activated Akt. Interestingly, hPEBP4 overexpression in TRAIL-sensitive DU145 cells promoted Akt activation but inhibited ERK1/2 activation. The hPEBP4-overexpressing DU145 cells became resistant to TRAIL-induced apoptosis consequently, which could be reversed by PI3K inhibitors. In contrast, silencing of hPEBP4 in TRAIL-resistant LNCaP cells inhibited Akt activation but increased ERK1/2 activation, resulting in their sensitivity to TRAIL-induced apoptosis that was restored by the MEK1 inhibitor. Therefore, hPEBP4 expression in prostate cancer can activate Akt and deactivate ERK1/2 signaling, leading to TRAIL resistance. We also demonstrated that hPEBP4-mediated resistance to TRAIL-induced apoptosis occurred downstream of caspase-8 and at the level of BID cleavage via the regulation of Akt and ERK pathways, and that hPEBP4-regulated ERK deactivation was upstream of Akt activation in prostate cancer cells. Considering that hPEBP4 confers cellular resistance to TRAIL-induced apoptosis and is abundantly expressed in poorly differentiated prostate cancer, silencing of hPEBP4 suggests a promising approach for prostate cancer treatment.

Prostate cancer is the most common malignancy and one of the leading causes of cancer mortality in men. The development of human prostate cancer has been viewed as a multistage process, involving the onset as small latent carcinoma of low histological grade to large metastatic lesion of higher grade. Unfortunately, chemotherapy and radiation therapy are not effective for prostate cancer patients. Therefore, a better understanding of molecular mechanisms for the resistance of prostate cancer cells to therapeutic agents will be useful to explore more efficient treatment protocols. Multiple signaling pathways govern the progression of prostate cancer, and the two most significant and well investigated pathways are the phosphatidylinositol 3-kinase (PI3K) and MAPK pathways (1, 2). The PI3K/Akt pathway mediates survival signals in androgen-dependent and -independent prostate tumor cell lines, and controls the progression of prostate cancer to an androgen-independent state (1). The activation of ERK1/2 is detectable in premalignant lesions and/or early stage cancers; hence, it may play a role in the pathogenesis or early progression of prostate cancer (3, 4). It has been shown that activation of Akt and inactivation of ERK in high Gleason-grade prostate cancer, and importantly, increased Akt activation, alone or together with decreased ERK activation, is an excellent predictor of poor clinical outcome in prostate cancer (6). Therefore, overactivation of the Akt pathway is regarded as one of the reasons for resistance of prostate cancer to therapeutic agents, and targeting to inhibit Akt activation may be beneficial in order to increase the therapeutic efficacy of human prostate cancer.

Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) acts through the pro-apoptotic TRAIL-R1/DR4 and TRAIL-R2/DR5 receptors, which leads to the cleavage and activation of caspase-8, resulting in BID cleavage, a Bcl-2 inhibitory protein, and triggering mitochondrial depolarization in tumor cells (7–9). TRAIL is currently being tested in clinical trials as an anticancer agent for barely detectable systemic cytotoxic effects. Recent studies have shown that Akt and ERK are all important regulators of TRAIL sensitivity in many cancers including prostate cancer (10–12). Akt phosphorylates specific

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† These authors contributed equally to this work.

‡ To whom correspondence should be addressed: Inst. of Immunology and National Key Laboratory of Medical Immunology, Second Military Medical University, 800 Xiangyin Road, Shanghai 200433, China. Fax: 86-21-6538-2502; E-mail: caot@public3.sta.net.cn.

† The abbreviations used are: PI3K, phosphatidylinositol 3-kinase; Akt/PKB, protein kinase B; ERK, extracellular signal-regulated kinase; PEBP, phosphatidylinosolamine-binding protein; PI, propidium iodide; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; TNF, tumor necrosis factor; FACS, fluorescent-activated signal sorting; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase.
targets such as Bad, caspase-9, and transcription factor FKHRL1, thus promoting cell survival and blocking apoptosis (13–15). DR4 and DR5 can also activate MAPK signaling pathways, and the activation is required for sensitization of prostate cancer cells to TRAIL-induced apoptosis (16, 17). So, regulation of Akt and ERK pathways may affect the sensitivity or resistance of TRAIL-induced apoptosis.

Human phosphatidylethanolamine-binding protein 4 (hPEBP4) is a novel member of the PEBP family identified by us as an anti-apoptotic molecule (18). hPEBP4 is preferentially expressed in breast cancer and ovarian cancer cells (18). We demonstrate that silencing of hPEBP4 can potentiate TNFα-induced apoptosis in MCF-7 breast cancer cells via ERK1/2 and JNK activation, indicating hPEBP4 is a candidate target molecule for breast cancer treatment (19). Our preliminary data showed that hPEBP4 was also highly expressed in prostate cancer cells; therefore, the primary aim of this study is to investigate the implication of hPEBP4 as a therapeutic target in human prostate cancer. We selected several human prostate cancer cell lines including TRAIL-resistant LNCaP prostate cancer cells and TRAIL-sensitive DU145 cells (20, 21) as cellular models to analyze the relation between hPEBP4 expression and TRAIL-induced apoptosis. Interestingly, hPEBP4 was found to be highly expressed in TRAIL-resistant LNCaP prostate cancer cells, but not in TRAIL-sensitive DU145 cells. Importantly, we found hPEBP4 expression is positively associated with the severity of prostate cancer. Furthermore, we demonstrate that hPEBP4 resists TRAIL-induced apoptosis by promoting Akt activation and ERK1/2 deactivation, and the effector process occurs downstream of caspase-8 and at the level of BID cleavage. These data suggest silencing of hPEBP4 may represent a promising approach for the treatment of human prostate cancer.

**EXPERIMENTAL PROCEDURES**

Reagents and Cell Culture—TRAIL was obtained from PeproTech (Rocky Hill, NJ). Lipofectamine reagent was from Invitrogen (Carlsbad, CA). Antibodies specific to phospho-AktSer473, phospho–ERK1/2, Bcl-2, Bcl-xL, and BID were from Santa Cruz Biotechnology (Santa Cruz, CA). The PI 3-kinase inhibitors, wortmannin and LY-294002, and the MEK1 inhibitor U0126 were obtained from Calbiochem. The arrays contain 105 dots in total and areas of normal prostatic epithelium, benign prostatic hyperplasia were also available for review along with infiltrating cancer. The arrays were fixed with formalin, embedded in paraffin, and immunostained with anti-hPEBP4 antibody (18) using avidin-biotin peroxidase complex method.

The proportion of carcinoma and benign prostatic hyperplasia staining, and the intensity of staining seen were analyzed according to criteria described by Malik et al. (5). The intensity is designated as 0 when no tumor cells stain (negative), 1 when 10–20% of cells stain (weak staining), 2 when 20–50% of cells stain (moderate staining), and 3 when >50% of cells stain (strong staining). For statistical analyses, groups scoring 2+ and 3+ were combined (“strong staining”), the group scoring 0 was designated as negative, and the group scoring 1+ was weak staining. Statistical analysis was performed by using χ² analyses with Kappa and McNemar statistics in contingency tables for agreement and disagreement of specific comparisons (5).

**Cell Transfection**—The expression vectors hPEBP4-B (the hPEBP4 expression vector) and p75PEBP4-B (the PE-binding domain-truncated vector) were constructed as described previously (18), and transfected into DU145 cells using Lipofectamine reagent with pcDNA3.1/Myc-His (–) B as a mock control. 48 h after transfection, the cells were screened under 0.8 mg/ml G418 (Merck, Darmstadt, Germany) for 20 days. Individual G418-resistant colonies were subcloned as DU145/hPEBP4-B and DU145/p75PEBP4-B. The hPEBP4 expression was determined by RT-PCR and Western blot analysis.

**hPEBP4 RNA Interference Assay**—For stable silencing of hPEBP4 expression in human prostate cancer LNCaP cells, the cells were transfected with the hPEBP4-RNAi or Neo plasmid using Lipofectamine reagent as described previously (19). 48 h after transfection, the cells were screened under 0.6 mg/ml G418 for 25 days. Individual G418-resistant colonies were subcloned as LNCaP/Neo and LNCaP/hPEBP4-RNAi, and hPEBP4 silencing was determined by RT-PCR and Western blot analysis.

**Apoptosis Assay**—Cells were washed, resuspended in the staining buffer, and examined with Vybrant Apoptosis kit (Invitrogen) according to the manufacturer’s instructions. Stained cells were analyzed by fluorescence-activated cell sorting (FACScalibur, Becton Dickinson, Mountain View, CA).

**Western Blot Analysis**—BCA Protein Assay Reagent Kit (Pierce,) was used to measure protein concentration. Samples containing equal amounts of protein were separated by 12% SDS-PAGE and transferred to Protran nitrocellulose membranes. Blots were probed with the indicated antibodies with appropriate horseradish peroxidase-conjugated antibodies as secondary antibodies (Cell Signaling Technology). Supersignal West Femto Maximum Sensitivity substrate (Pierce) was used for the chemiluminescent visualization of proteins (19).

**Co-precipitation and Immunoprecipitation Assay**—Stably transfected DU145 cells were serum-starved for 24 h, then stimulated with 50 ng/ml TRAIL for 10 min, and lysed in lysis buffer (Cell Signaling Technology). The cell lysates were mixed with Ni-nitritolactiic acid beads (Qiagen, Hilden, Germany), gently agitated overnight at 4 °C, and washed four times in lysis buffer containing 10 mM imidazole and 0.1% Triton X-100, and Western blot was performed as previously described (19). For immunoprecipitation (IP) of endogenous hPEBP4 from
TRAIL-stimulated LNCaP cell lysates with anti-hPEBP4 polyclonal antibody (18), LNCaP cell lysates were precleared with protein A-Sepharose beads (Sigma), and IP performed using anti-hPEBP4 polyclonal antibody cross-linked to protein A-Sepharose beads as previously described (19).

**Statistical Analysis**—The correlation between hPEBP4 expression and pathologic grade of human prostate cancer was evaluated using Cochran-Mentel-Haenszel test performed with the computer program SPSS Version 10.2.

**RESULTS**

**Increased Expression of hPEBP4 in Poorly Differentiated Prostate Cancer**—Our previous study showed that hPEBP4 was highly expressed in human breast cancer tissues, and hPEBP4 was also highly expressed in human breast cancer, ovarian cancer, and prostate cancer cell lines (18). To address the relationship between hPEBP4 expression and the progression of prostate cancer, here we examined the expression pattern of hPEBP4 in human prostate cancer tissues by tissue microarrays. hPEBP4 expression was shown to be present in a very high percentage of prostate cancer tissue (93%, 68/73), compared with the very low percentages in matched benign lesion (11%, 1/9; \( p < 0.001 \), Table 1) and in normal prostate tissue (8.7%, 2/23; \( p < 0.001 \)). The data demonstrate the preferential expression pattern of hPEBP4 in human prostate cancer tissues. Moreover, increased staining intensity of hPEBP4 was associated with loss of differentiation of the primary tumors (Table 1). None of the well-differentiated tumors (grade I) exhibited strong staining intensities and only 28.6% in moderately differentiated adenocarcinomas (grade II); however, about 60% of poorly differentiated (grade III, 10/17) and >90% of undifferentiated adenocarcinomas (grade IV, 29/32) exhibited strong staining for hPEBP4 (\( p < 0.001 \); Table 1, Fig. 1A). These results revealed a positive correlation of hPEBP4 expression with the severity of human prostate cancer and suggest that hPEBP4 may be involved in the development and progression of prostate cancer. The expression pattern of hPEBP4 in prostate cancer cell lines was also examined. To our interest, hPEBP4 was found to be not expressed in TRAIL-sensitive DU145 prostate cancer cells, but highly in TRAIL-resistant LNCaP cells (Fig. 1B), indicating hPEBP4 might also be involved in the resistance of human prostate cancer to TRAIL-induced apoptosis.

**hPEBP4 Activates Akt and Inhibits TRAIL-induced Apoptosis**

**hPEBP4 Activates Akt and Inhibits ERK Activation in Human Prostate Cancer Cells**—It is demonstrated that Akt and ERK pathways are important in regulating survival of prostate cancer cells, and increased Akt activation and ERK deactivation strongly predict poor clinical outcome in prostate cancer (6). Because hPEBP4 was selectively expressed in prostate cancer cells, especially in LNCaP cells, which show high levels of constitutively activated Akt, taken together with our previous data showing inhibition of ERK1/2 by hPEBP4, so the effects of hPEBP4 preferential expression in human prostate cancer cells on the activation of Akt and ERK1 pathways was investigated.

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**TABLE 1**

<table>
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<th>Pathologic grade</th>
<th>n</th>
<th>Negative</th>
<th>Weak staining intensity</th>
<th>Strong staining intensity</th>
<th>Positive</th>
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<tr>
<td>Normal</td>
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<td>21(91.3)</td>
<td>2(8.7)</td>
<td>0(0.0)</td>
<td>2(8.7)</td>
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<tr>
<td>Hyperplasia</td>
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<td>8(88.9)</td>
<td>1(11.1)</td>
<td>0(0.0)</td>
<td>1(11.1)</td>
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<tr>
<td>Cancer tissue</td>
<td>73</td>
<td>7(6.8)</td>
<td>25(34.2)</td>
<td>43(58.9)</td>
<td>68(93.2)</td>
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<tr>
<td>Grade I</td>
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<td>2(20.0)</td>
<td>8(80.0)</td>
<td>0(0.0)</td>
<td>8(80.0)</td>
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<tr>
<td>Grade II</td>
<td>14</td>
<td>2(14.3)</td>
<td>8(57.1)</td>
<td>4(28.6)</td>
<td>12(85.7)</td>
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<tr>
<td>Grade III</td>
<td>17</td>
<td>1(5.9)</td>
<td>6(35.3)</td>
<td>10(58.8)</td>
<td>16(94.1)</td>
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<tr>
<td>Grade IV</td>
<td>32</td>
<td>0(0.0)</td>
<td>3(9.4)</td>
<td>29(90.6)</td>
<td>32(100.0)</td>
</tr>
</tbody>
</table>

\( ^* p < 0.001 \) vs. normal.

\( ^a p < 0.001 \) vs. hyperplasia.

\( ^b p < 0.001 \) grade IV vs. other grades.

\( ^c p < 0.05 \) grades III and IV vs. grades I and II.

**FIGURE 1.** Increased expression of hPEBP4 in poorly differentiated prostate cancer. A, representative results of immunohistochemical staining of hPEBP4 protein (yellow) in one sample with no expression of the normal prostate tissue (panel a) and samples with weak to strong staining in prostate cancer tissues (panels b–e). Photos were taken under \( \times 200 \) magnifications. B, expression pattern of hPEBP4 in prostate cancer cell lines. RT-PCR with hPEBP4 and human \( \beta \)-actin-specific primers were performed for 30 and 23 cycles, respectively.
First, TRAIL-sensitive DU145 cells with almost no expression of hPEBP4 were transfected with hPEBP4-B (the hPEBP4 expression vector), p75PEBP4-B (the PE-binding domain-truncated vector), or control vector. RT-PCR (data not shown) and Western blot analysis confirmed the overexpression of hPEBP4 in DU145 stable transfectants (Fig. 2A). Compared with mock or p75PEBP4-B transfectants, DU145/hPEBP4-B transfectants exhibited higher level of active Akt not only in their baseline levels but also following TRAIL stimulation. Simultaneously, hPEBP4-B transfectants displayed lower ERK1/2 activation upon TRAIL stimulation; however, the baseline ERK1/2 activation of the transfectants was almost equal (Fig. 2, B and C). Second, TRAIL-resistant LNCaP cells with high expression of hPEBP4 were stably transfected with hPEBP4-RNAi or Neo plasmids. RT-PCR and Western blot analysis confirmed the complete silencing of hPEBP4 expression in LNCaP cells (Fig. 2D). Silencing of hPEBP4 in LNCaP cells reduced the spontaneously high levels of constitutively active Akt and also inhibited TRAIL-induced Akt activation, but promoted TRAIL-induced ERK1/2 activation (Fig. 2, E and F). However, the TRAIL-induced activation of JNK and p38, the other two signaling molecules in MAPK pathway, was barely affected by hPEBP4 expression (Fig. 2G). These data suggest hPEBP4 promotes Akt activation and inhibits ERK activation in human prostate cancer cells.
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wortmannin or LY-294002 completely inhibited TRAIL-mediated Akt activation (data not shown) and almost reversed the inhibitory effect of hPEBP4 on TRAIL-induced apoptosis in DU145 cells (Fig. 3C). Considering hPEBP4-silenced LNCaP cells exhibited higher ERK activation following TRAIL stimulation, next we explored whether the enhanced ERK1/2 activation contributes to the apoptosis sensitization induced by hPEBP4 interference. hPEBP4-silenced LNCaP cells were pre-incubated with MEK1 inhibitor (U0126) for 1 h and then treated with TRAIL. As shown in Fig. 3D, U0126 partially reversed the promoting effect of hPEBP4-silencing on TRAIL-induced apoptosis of LNCaP cells, indicating that potentiation of TRAIL sensitivity by hPEBP4 silencing requires ERK1/2 activation in LNCaP cells. We also measured the protein expression of TRAIL receptors, DR4 and DR5 in the hPEBP4-silenced LNCaP cells, and found that the expression levels of both receptors remained almost unchanged (Fig. 3E), thus excluding the possibility that hPEBP4 regulates TRAIL sensitivity by affecting the expression of TRAIL receptors. Collectively, these findings suggest that hPEBP4 confers TRAIL resistance in prostate cancer cells by promoting Akt activation and ERK1/2 deactivation.

hPEBP4 Confers TRAIL Resistance by Regulating Expression of Apoptosis-related Proteins through Akt Activation and ERK1/2 Deactivation—It is well known that TRAIL-induced apoptosis is mediated through a caspase cascade (22, 23). To investigate whether hPEBP4 affects TRAIL-induced caspase activation in human prostate cancer cells, we examined the activation of caspase-3 and caspase-8 in DU145 transfectants treated with TRAIL. Overexpression of hPEBP4 in DU145 cells had no effect on the activation of caspase-8 (Fig. 4A), but inhibited TRAIL-induced caspase-3 activation, which could be rescued by wortmannin or LY-294002 (Fig. 4B). Because TRAIL induces apoptosis of prostate cancer cells through activation of caspase-8 which may cleave BID (a Bcl-2 inhibitory protein) ultimately resulting in apoptosis (24, 25), we then examined the effects of hPEBP4 on BID cleavage. Overexpression of hPEBP4 in DU145 cells inhibited TRAIL-induced BID cleavage, which could be reversed by LY-294002 treatment, suggesting that hPEBP4-mediated TRAIL resistance via PI3K/Akt pathway in DU145 cells occurs downstream of caspase-8 and at the level of BID cleavage. We further demonstrated that overexpression of hPEBP4 inhibited TRAIL-induced down-regulation of Bcl-2 and Bcl-xL in DU145 cells (Fig. 4C). However, the protein level of other apoptosis-related proteins such as BAX and BAK remained unchanged (data not shown). Cyclin-dependent kinase inhibitors, p21 and p27 play roles in induction of apoptosis (26, 27) and Akt can inhibit p27 transcription as a mechanism through which PI3K/Akt can block apoptotic signals (14). We found that overexpression of hPEBP4 inhibited TRAIL-induced up-regulation of p27 whereas the protein level of p21 remained unchanged (Fig. 4C). Consistent with apopto-
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**FIGURE 4. hPEBP4 regulates the expression of apoptosis-related proteins and its requirement for Akt and ERK1/2 pathways.** Stable transfectants of DU145 cells were preincubated with PI3K inhibitor for 1 h or not and then stimulated with 50 ng/ml TRAIL for 24 h. Cell lysates were subjected to Western blot analysis and probed with anti-caspase-8 (A), anti-caspase-3 and anti-BID antibody (B), which can recognize BID and its cleavage. C, cells treated as in A were subjected to Western blot and probed with anti-p27, anti-p21, anti-Bcl-2, anti-Bcl-xL, D and E, stable transfectants of LNCaP cells were preincubated with MEK1 inhibitor for 1 h or not, and then stimulated with 200 ng/ml TRAIL for 48 h. Cell lysates were subjected to Western blot and probed with the indicated antibodies.

ERK1/2 deactivation, at least in part, contributes to the increased Akt activation in prostate cancer cells.

**DISCUSSION**

Better understanding of the molecular alterations that facilitate human prostate cancer progression and possible mechanisms involved in the apoptotic response will contribute to the design of novel strategies for the treatment of human prostate cancer. TRAIL offers promising therapeutic potential based on its ability to induce apoptosis in various cancer cell lines with little toxicity toward normal cells. However, not all cancer cells undergo apoptosis when treated with TRAIL. Several mechanisms have been described that may control sensitivity to TRAIL-mediated apoptosis (30). Two of the well studied signaling pathways that mediate TRAIL resistance are Akt and MAPK pathways. In general, significantly increased Akt activation was detected in more than 50% of primary carcinomas of prostate, especially hormone-refractory prostate carcinoma (31), and ERK1/2 activity declines with prostate cancer progression although elevated levels of phosphorylated ERK1/2 have been reported in advanced disease (3, 4). Akt enhances cell proliferation and inhibits apoptosis in cancer cells, and highly constitutively activated Akt had been demonstrated to be directly correlated with cell survival and resistance to TRAIL in prostate carcinomas of both hPEBP4-overexpressing DU145 cells and LNCaP cells with TRAIL stimulation, but not in those of unstimulated cells (Fig. 5, A and B). However, the association of hPEBP4 with Akt or PI3K was not detected (data not shown).

Because the cross-talk between the MEK1/ERK1/2 and PI3K/Akt signaling pathways has been shown to regulate their activity bidirectionally (28, 29), we investigated the possibility that the regulation of ERK1/2 activation by hPEBP4 might be responsible for the regulation of Akt activation or vice versa. hPEBP4-silenced LNCaP cells were pretreated with U0126 to inhibit ERK1/2 activation, then we found that U0126 completely inhibited TRAIL-mediated ERK1/2 activation and partially reversed the decrease of Akt activation by hPEBP4 interference. We further used PI3K inhibitor to assess the effect of increased Akt activation on the ERK1/2 phosphorylation in hPEBP4-overexpressing DU145 cells. Interestingly, we found that LY294002 had no significant effect on TRAIL-induced ERK1/2 activation (Fig. 5, C and D). Taken together, these data indicate the hPEBP4-mediated down-regulation of Bcl-2 and Bcl-xL (Fig. 4, J). The data indicate that hPEBP4 confers TRAIL resistance by regulating the expression of apoptosis-related proteins, which occurs downstream of caspase-8 and at the level of BID cleavage and is required for Akt activation.

On the other hand, silencing of hPEBP4 expression in LNCaP cells further potentiated TRAIL-induced activation of caspase-3, cleavage of BID (Fig. 4D), up-regulation of p27, and down-regulation of Bcl-2 and Bcl-xL (Fig. 4E). Moreover, the MEK1 inhibitor, U0126, could partially inhibit the hPEBP4 silencing-induced potentiation of apoptosis-related protein expression by TRAIL stimulation. So, hPEBP4-conferred TRAIL resistance by regulating expression of apoptosis-related proteins also requires ERK1/2 deactivation.

hPEBP4-mediated ERK1/2 Deactivation Is Required for the Increased Akt Activation in Human Prostate Cancer Cells—We previously showed that hPEBP4 can transfer to the cell membrane and bind to Raf-1 and MEK1, thus inhibiting MEK1/ERK1/2 activation in MCF-4 breast cancer cells following TNF-α stimulation (18, 19). Here, we want to know whether hPEBP4 has the same binding to Raf-1 or MEK1 upon TRAIL stimulation in DU145 cells and LNCaP cells. The results showed that Raf-1 and MEK1 were detected in the immunoprecipitates of both hPEBP4-overexpressing DU145 cells and LNCaP cells with TRAIL stimulation, but not in those of unstimulated cells (Fig. 5, A and B). However, the association of hPEBP4 with Akt or PI3K was not detected (data not shown).
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In prostate cancer cells, TRAIL induced apoptosis via activating caspase-8, which mediated cleavage of BID; generating a proteolytic fragment, tBID, which is capable of inducing mitochondrial cytochrome c release and providing a function link between death receptors and the mitochondria (24, 25). hPEBP4 inhibited TRAIL-induced BID cleavage and caspase-3 activation but had no effect on caspase-8 activation. Thus, the hPEBP4-mediated inhibition of TRAIL-induced apoptosis occurs downstream of caspase-8, at the level of BID cleavage. Simultaneously, hPEBP4 regulated other apoptosis-related proteins, which can be reversed by LY294002 and U0126.

Previous studies showed that hPEBP4 can bind to MEK1 and Raf-1 upon TNF-α stimulation. Here the hPEBP4 association with both MEK1 and Raf-1 after TRAIL stimulation was observed in DU145 cells overexpressing hPEBP4 or LNCaP cells with endogenous hPEBP4 expression. However, we have not found the association of hPEBP4 with Akt or PI3K. Several reports have addressed the cross-talk between PI3K/Akt and ERK1/2 pathways. But there have been conflicting reports on the role of PI3K/Akt in the regulation of ERK1/2 activation as well as the role of ERK1/2 in the Akt regulation (28, 29). In this study, U0126 completely inhibited TRAIL-mediated ERK1/2 activation and partially reversed the decreased activation of Akt by hPEBP4 interference in LNCaP cells. However, LY294002 had no significant effect on TRAIL-induced ERK1/2 activation in hPEBP4-overexpressing DU145 cells. Although we did not find evidence of physical association between hPEBP4 and PI3K or Akt, this does not rule out the possibility that hPEBP4 also promoted Akt activation directly in an unknown way. Further investigation will be required to clarify the mechanism of hPEBP4-mediated Akt activation in prostate cancer cells.

Based on the findings here, we propose the working model of hPEBP4-conferred TRAIL resistance in human prostate cancer cells: hPEBP4 transfers to cell membrane and binds to Raf-1 or MEK1, then inhibit Raf-1/MEK1/ERK1/2 activation following TRAIL stimulation. Subsequently, the ERK1/2 deactivation may facilitate to activate Akt, consistent with the report that

cancer (10–12). So, direct inhibition of the activation of Akt, such as targeting Akt activation by a small molecule inhibitor, may represent an attractive therapeutic strategy for prostate cancer (32). In this study, we showed, by the tissue microarray examination of human prostate cancer that the severity of human prostate cancer is associated with increased expression of hPEBP4, which was recently identified as an anti-apoptosis molecule by us (18). Furthermore, hPEBP4 was highly expressed in TRAIL-resistant LNCaP cells, which show highly active levels of Akt, but not in TRAIL-sensitive DU145 cells. What is more, overexpression of hPEBP4 in DU145 cells promoted Akt activation but inhibited ERK1/2 activation. Accordingly, the hPEBP4-overexpressing DU145 cells became resistant to TRAIL-induced apoptosis, which could be reversed by PI3K inhibitors. In contrast, silencing of hPEBP4 in TRAIL-resistant LNCaP cells could reduce the highly constitutively active Akt but increase ERK1/2 activation, resulting in their sensitivity to TRAIL-induced apoptosis. The MEK1 inhibitor could restore TRAIL resistance in hPEBP4-silenced LNCaP cells. Thus, hPEBP4-mediated regulation of ERK1/2 and Akt activation appears to be responsible for the observed inhibition of TRAIL-induced apoptosis. Collectively, the abundant expression of hPEBP4 in poorly differentiated prostate cancer might lead to higher activation of Akt and lower activation of ERK1/2; thus resulting in failure of treatment for prostate cancer. The silencing of hPEBP4 may represent a promising approach for the treatment of cancers that express high levels of hPEBP4.
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ERK1/2 may activate Akt directly (27). hPEBP4 may also enhance Akt activation directly in unknown way. Activated Akt then intervenes in the apoptosis cascade upstream of cytochrome c release (BID cleavage level) and downstream of caspase-8 activation, leading to the suppression of apoptosis induction (Fig. 6).

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REFERENCES