Regulation of ERα-mediated transcription of Bcl-2 by PI3K-AKT crosstalk: Implications for breast cancer cell survival

MELLYSSA R. BRATTON1,2*, BICH N. DUONG3*, STEVEN ELLIOTT2,4, CHRISTOPHER B. WELDON5, BARBARA S. BECKMAN1,2,7, JOHN A. McLACHLAN1,2,7 and MATTHEW E. BUROW1,2,4,6

1Tulane University School of Medicine, Department of Pharmacology, 1430 Tulane Ave.; 2Tulane/Xavier Center for Bioenvironmental Research, Health & Environmental Research Bldg, 1324 Tulane Ave., SL-3, New Orleans, LA 70112; 3University of Illinois at Chicago, Department of Anatomy and Cell Biology, 808 S Wood St., Rm 578 M/C 512, Chicago, IL 60612; 4Tulane University School of Medicine, Department of Medicine, Hematology/Oncology Section, SL-78, 1430 Tulane Ave., New Orleans, LA 70112; 5Department of Surgery, Children's Hospital Boston, 300 Longwood Ave., Boston, MA 02115; 6Tulane Cancer Center, 1430 Tulane Avenue, SL-68, New Orleans, LA 70112; 7Department of Environmental Health Sciences, Tulane University School of Public Health and Tropical Medicine, 1440 Canal St., New Orleans, LA 70112, USA

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Abstract. Both estrogen, through the estrogen receptor (ER), and growth factors, through the phosphatidylinositol-3-kinase (PI3K)-AKT pathway, have been shown to independently promote cell survival. Here, we investigated the role of ER/PI3K-AKT crosstalk in the regulation of cell survival in MCF-7 breast carcinoma cells. The ER inhibitor ICI 182,780 was used to determine the requirement of the ER for estrogen in the suppression of tumor necrosis factor-α (TNFα) induced apoptosis. Gene reporter assays and Western blot analyses were used to determine the involvement of the pro-survival factor Bcl-2 and the coactivator GRIP1 in this survival crosstalk. We demonstrated that an intact ER signaling pathway was required for estrogen to suppress apoptosis in the suppression of tumor necrosis factor-α (TNFα) induced apoptosis. Gene reporter assays revealed that ERα, not ERβ, was targeted by AKT, resulting in transcriptional potentiation of the full-length Bcl-2 promoter, ultimately leading to increased Bcl-2 protein levels. AKT targeted both activation function (AF) domains of the ERα for maximal induction of Bcl-2 reporter activity, although the AF-II domain was predominately targeted. In addition, AKT also caused an upregulation of GRIP1 protein levels. Finally, AKT and GRIP1 cooperated to increase Bcl-2 protein expression to a greater level than either factor alone. Collectively, our study suggests a role for ER/PI3K-AKT crosstalk in cell survival and documents the ability of AKT to regulate Bcl-2 expression via differential activation of ERα and ERβ as well as regulation of GRIP1.

Introduction

Homeostasis in normal breast tissue is maintained by a balance between cell survival and cell death (1). Hence, disregulation of this homeostasis in favor of cell survival can lead to cell proliferation and cancer (2). Estrogen (E2) is an endogenous factor that plays a critical role in normal mammary functions (3). However, E2 has been implicated in breast cancer due to its pro-survival effects (4,5). The phorbol ester insulin-like growth factor 1 (IGF-1), is also involved in cell survival and its effects are largely mediated by the phosphatidylinositol 3 kinase (PI3K)-AKT signaling cascade (6). While there are many downstream targets of PI3K, the serine/threonine kinase AKT is the primary mediator of PI3K survival signaling due to AKT's ability to regulate cellular components that affect cell survival decisions, such as Bad and Forkhead transcription factors (7).

The actions of E2 are mediated by the estrogen receptor (ER). The two isoforms of the ER, ERα and ERβ, belong to the steroid/thyroid hormone superfamily of nuclear receptors that function as ligand-activated transcription factors (8). Transcriptional activities of the receptors are mediated by two distinct activation function (AF) domains: the AF-I domain in the amino terminus exhibits constitutive ligand-independent activity, and the AF-II domain in the carboxy-terminus requires ligand binding for activity (9). To achieve full transcription potential, the ER must also recruit histone modifying coactivators to overcome the steric hindrances imposed on tightly packed chromatin (10,11). Glucocorticoid receptor-interacting protein 1 (GRIP1), a member of the p160...
family of coactivators, has been shown to interact with both AF domains of the ER (12-14), increasing the transcriptional activity of the receptor (15). Several coactivators have been implicated in breast cancer etiology (16-18), and researchers have recently begun to study peptide growth factor regulation of coactivator function to better understand cancer development (19-21).

Both E2 (22-24) and IGF-1 (25-27) enhance cancer cell survival in part through their abilities to upregulate Bcl-2 expression. A Camp response element (CRE) within the Bcl-2 promoter is targeted by IGF-1 signaling via AKT phosphorylation of the cAMP response element binding protein (CREB) (26,27). Likewise, the ER has been shown to play an integral role in E2 regulation of Bcl-2 expression (28-30). However, the exact role of the ER at the Bcl-2 promoter remains unclear. A survival crosstalk between the ER-E2 and IGF1-PI3K-AKT signaling pathways (31-33) may protect cancer cells from apoptosis induced by the chemotherapeutic drug tamoxifen (34) and the microbial product wortmannin (35). In these cases, peptide growth factor activation of the PI3K-AKT signaling cascades potentiated ER expression. A cAMP response element (CRE) within the cAMP response element binding protein (CREB) (26,27). Likewise, the ER has been shown to play an integral role in E2 regulation of Bcl-2 expression (28-30).

The endogenous cytokine tumor necrosis factor α (TNFα) has pleiotropic biological functions (36) and is produced by a variety of cell types in both normal (37) and breast cancer tissue (38). TNFα induces apoptosis in normal breast tissue during involution (39) and in breast cancer cells (40). TNFα-induced apoptosis is inhibited by Bcl-2 (41). Hence, factors that control Bcl-2 expression may increase cell survival and contribute to breast cancer growth, even in the presence of TNFα (36,42).

Given the roles of estrogen and peptide growth factors in breast cancer biology, we hypothesize that PI3K-akt-ERα-E2 crosstalk enhances cancer cell survival. We evaluated the ability of ER/PI3K-AKT crosstalk to affect MCF-7 cells at both the biological and molecular levels. Our results indicate that activation of the PI3K-AKT pathway contributes to breast cancer cell survival through transcriptional activation of the Bcl-2 gene product in an ERα-dependent manner, which may increase breast cancer cell survival in the presence of TNFα (43). Given the potential of TNFα use in breast cancer therapy (36,42), a mechanistic study of this PI3K-AKT-ERα-E2 survival crosstalk was investigated.

Materials and methods

Reagents. Dulbecco's modified Eagle's medium (DMEM), phenol-red free DMEM, fetal bovine serum (FBS), BME amino acids, MEM amino acids, L-glutamine, penicillin, streptomycin, and sodium pyruvate were obtained from Gibco-BRL (Gaithersburg, MD). Porcine insulin was purchased from Sigma (St. Louis, MO), and charcoal stripped (CS) FBS was obtained from HyClone (Logan, UT). Lipofectamine and Effectene were purchased from Gibco-BRL (Grand Island, NY) and Qiagen (Valencia, CA), respectively. ICI 182,780 was obtained from Tocris (Ellisville, MO). TNFα was obtained from R&D systems (Minneapolis, MN). 17β-estradiol (E2) and all protease inhibitor cocktails were purchased from Sigma; NuPAGE 4-8% Bis-Tris gel was obtained from Invitrogen (Carlsbad, CA); Bio-Rad protein assay reagent was purchased from Bio-Rad (Hercules, CA). The primary antibodies, mouse anti-human Bcl-2, rabbit anti-GRIP1, and rabbit anti-actin, were obtained from BD PharMigen (San Diego, CA), Upstate Biotechnology (Lake Placid, NY) and Sigma, respectively. The secondary antibodies, horseradish peroxidase (HRP) conjugated goat anti-mouse and anti-rabbit, were purchased from Transduction Laboratories (Lexington, KY) and Cell Signaling (Beverly, MA), respectively. ECL chemiluminescence system was obtained from Amersham (Buckinghamshire, England), and Biomax film was purchased from Kodak (Rochester, NY). The inverted fluorescence microscope was purchased from Leica (Wetzlar, Germany), and the Monolight 2010 luminometer was obtained from Analytical Luminescence Laboratory (Ann Arbor, MI).

Plasmids. pGL3-Con-Luc, pEGFP-N1, and dominant negative AKT (AKT-DN) were obtained from Promega (Madison, WI), Clontech (Palo Alto, CA), and Upstate Biotechnology, respectively. The following expression vectors have been previously described: constitutive active AKT (AKT-CA) (44), pCDNA3.1-ERα and 8 (45). pRST7-ERα and pRST7-ERβ constructs (AF-I with alanine substituting for amino acids at positions 436, 440, and 443; AF-II containing amino acids 90-477) were generously provided by Dr Donald McDonnell (46). Bcl-2-Luciferase reporter construct (-3934 to -1287 basepairs upstream from the transcription start site) was a gift of Dr Martin P. Smith and Dr Linda Boxer (47).

Cell culture. Estrogen receptor positive MCF-7 human breast carcinoma (48) and estrogen receptor negative human embryonic kidney (HEK) 293 cells (49) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS, BME amino acids, MEM amino acids, L-glutamine, 100 U/ml penicillin, 100 U/ml streptomycin, sodium pyruvate, and 1x10^-6 M porcine insulin under myco-supplement-free conditions at 37°C in humidified 5% CO2 and 95% air. For described studies, MCF-7 cells were grown for 48 h in plasma-free conditions at 37˚C in humidified 5% CO2 and 95% air. For described studies, MCF-7 cells were grown for 48 h in plasma-free conditions at 37°C in humidified 5% CO2 and 95% air. For described studies, MCF-7 cells were grown for 48 h in plasma-free conditions at 37°C in humidified 5% CO2 and 95% air. For described studies, MCF-7 cells were grown for 48 h in plasma-free conditions at 37°C in humidified 5% CO2 and 95% air. For described studies, MCF-7 cells were grown for 48 h in plasma-free conditions at 37°C in humidified 5% CO2 and 95% air. For described studies, MCF-7 cells were grown for 48 h in plasma-free conditions at 37°C in humidified 5% CO2 and 95% air. For described studies, MCF-7 cells were grown for 48 h in plasma-free conditions at 37°C in humidified 5% CO2 and 95% air.

Transient transfection and luciferase assay. MCF-7 and HEK 293 cells were transfected as previously described (23). Briefly, cells were plated in 5% CS-DMEM for 48 h prior to plating onto 24-well plates at 1x10^4 cells/well and allowed to attach overnight. The next day, the cells were transfected for 5 h in the serum/supplement-free DMEM using either Lipofectamine or Effectene according to the manufacturers' protocols. For reporter-based luciferase assay, MCF-7 and HEK 293 cell were transfected with the reporter (Bcl-2-Luc), along with vector control (Vec), AKT-CA, or AKT-DN, with 293 cells additionally transfected with various ERα or ERβ constructs. After 5 h, the transfection medium was replaced with 5% CS-DMEM, and treated with chemicals (vehicle control or 1 nM E2, with or without 100 nM ICI 182,780).
After 24 h, the treatment-containing medium was removed and 1X lysis buffer (100 μl) was added per well and gently shaken for 30 min at room temperature. The cell debris was then pelleted by centrifugation at 15,000 x g for 5 min. Luciferase activity for 30 μl of cell extract was determined using luciferase assay substrate in a Monolight 2010 luminometer.

Viability assay. Viability assay using crystal violet (CV) was performed as previously described (44). Briefly, MCF-7 cells (1x10⁶/well) in 24-well plates with 5% CS-DMEM were treated with 10 ng/ml TNF-α, with or without 1 nM E₂, with or without 100 nM ICI 182,780 for 24 h, stained using 0.5% crystal violet (20 μl) for 15 min, washed twice with phosphate buffered saline (PBS), lysed in 1% SDS solution (600 μl), transferred to a 96 well plate, and absorbency (ABS) at 550 nm was measured. ABS of treated samples was normalized to untreated control (100%). For reporter gene viability assay, cells were transfected with pGL3-Luc reporter and empty vector (Vec) or constitutive active AKT (AKT-CA), treated for 48 h with 10 ng/ml TNF-α, with or without 1 nM E₂, with or without 100 nM ICI 182,780 and harvested for luciferase assay with data represented as percent viability normalized to untreated control (100% ± SEM). Apoptosis analysis with fluorescence microscopy was performed as previously described (50) with the use of green fluorescence protein (GFP) instead of LacZ as a marker of transfection. Cells were transfected with pEGFP (100 ng) and 500 ng empty vector (Vec) or constitutive active AKT (AKT-CA) using Effectene, followed by treatment with 10 ng/ml TNF-α, with or without 1 nM E₂, with or without 100 nM ICI 182,780 and harvested for fluorescence microscopy analysis. Newells were used to normalize fluorescence microscope.

Immunoblot analysis. MCF-7 and HEK 293 cells were seeded at 5x10⁶ cells/100 mm² plate. MCF-7 cells were transfected with a total of 5 μg DNA of empty vector (Vec), constitutive active AKT (AKT-CA) using Effectene, followed by treatment with 10 ng/ml TNF-α, with or without 1 nM E₂, with or without 100 nM ICI 182,780 and harvested for immunoblot analysis for protein levels. Immunoblot analysis was performed as described (51). A viability assay was performed in MCF-7 cells according to the manufacture's instructions. The membrane was blocked with PBS/0.05% Tween/5% nonfat dry milk solution at 4°C overnight with gentle shaking. The blot was washed in PBS/0.05% Tween solution and incubated with 1:5,000 dilution of HRP-conjugated goat anti-mouse or 1:2,000 dilution of HRP-conjugated anti-rabbit antibody for 30 min at room temperature with gentle shaking. Following four washes with PBS/0.05% Tween solution, immunoreactive proteins were detected using ECL chemiluminescence system and recorded by fluorography on Kodak Biomax film according to the manufacturer's instructions. The membrane was then stripped in stripping solution (2% SDS, 62.5 mM Tris-HCl, pH 6.7, 7 μl/ml β-mercaptoethanol) for 12 min at 60°C, and the same protocol as above was used to re-probe the membrane with 1:200 dilution of rabbit anti-actin antibody and subsequently with 1:3,000 dilution of anti-rabbit antibody. Fluorograms were quantitated by image densitometry using Quality One program for data acquisition and analysis (Bio-Rad, Hercules, CA).

Statistical analysis. Data were analyzed using one-way analysis of variance (ANOVA) and post-hoc Tukey's multiple comparisons with Origin 7.0 software. Statistically significant changes were determined at the p<0.05 level as indicated for each figure.

Results

The ER is required for cell survival signaling through AKT. Both estrogen, acting through the ER (51) and peptide growth factors, working through PI3K-AKT (52), can suppress apoptosis induced by various agents. To determine whether the previously described crosstalk between these two signaling pathways (31-35) can protect MCF-7 breast carcinoma cells from apoptosis induced by the endogenous cytokine TNFα, cell viability assays were performed. TNFα (10 ng/ml) caused a significant decrease in MCF-7 cell survival compared to untreated control cells (Fig. 1A). E₂ (1 nM) functioned as a potent cell survival signal by elevating cell survival to the levels of untreated control cells (55.9±5.4% viability with E₂) (Fig. 1A). Not surprisingly, ablation of ER signaling with the pharmacologic inhibitor ICI 182,780 (1 μM) abrogated the anti-apoptotic activity of E₂.

Since AKT is involved in cell survival, the ability of this kinase to mediate cell survival in the presence of TNFα was investigated. A viability assay was performed in MCF-7 cells transfected with either empty vector (Vec) or constitutive active AKT (AKT-CA) using Effectene. In the presence of TNFα (10 ng/ml), AKT-CA elevated cell survival from 55.9±5.4% to 95.2±4.3% viability with E₂ (Fig. 1A). Not surprisingly, ablation of ER signaling with the pharmacologic inhibitor ICI 182,780 (1 μM) abrogated the anti-apoptotic activity of E₂.

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The ER is required for proliferation of Bcl-2 expression by AKT. The transcriptional activity of the ER is important for cell survival (53), since the receptor upregulates the expression of anti-apoptotic Bcl-2 protein (54). Since E₂ has been shown to increase Bcl-2 expression...
(mRNA) via a transcriptional mechanism (24), we investigated the roles of AKT and ER in the regulation of Bcl-2 expression. In contrast to previous results, our initial reporter-based luciferase assays of the full-length Bcl-2 promoter reveal that E2 alone did not induce significant Bcl-2 promoter activation (Fig. 2A). Differences in promoter regions may contribute to
these variations, as our Bcl-2 reporter construct i) lacks the estrogen responsive elements located within the coding region of the gene that is required for E2 induction of Bcl-2 promoter activity (29), and ii) contains \( \pi \) binding sites that have been shown to negatively regulate Bcl-2 expression in reporter gene assays (54). More importantly, our results indicate that in the presence of 1 nM E2, AKT-CA potentiated Bcl-2 expression by 54-fold over Vec (Fig. 2A). Additionally, ICI 182,780 abolished this Bcl-2 upregulation, demonstrating that the ER was required for transcriptional activation of the Bcl-2 promoter by AKT.  

Immunoblot analysis was used to determine whether upregulation of the Bcl-2 promoter translates into greater protein production. Previous studies exploring AKT regulation of Bcl-2 focused on the ability of this kinase to activate the Bcl-2 promoter region with reporter assays (26,27), and Western blot analyses did not account for loading controls (34). Compared to untreated vector control, E2 (1 nM) expectedly enhanced Bcl-2 protein levels, and AKT-CA increased Bcl-2 expression even further (Fig. 2B and C). AKT-CA enhanced ligand-independent expression of Bcl-2, perhaps not surprisingly since peptide growth factor (PGF) signaling itself can increase Bcl-2 expression (26,27). More importantly, both E2 and AKT-CA upregulation of Bcl-2 protein was blocked by ICI 182,780, again demonstrating a requirement for the ER. These results reveal that transcriptional upregulation of the Bcl-2 pro-survival gene may be one of the mechanisms by which the ER and AKT cooperate to enhance cell survival.  

**AKT potentiates Bcl-2 expression through ERs.** Even though ER\( \alpha \) and ER\( \beta \) are co-expressed in mammary tissues and breast carcinomas (55), the two receptors are differentially influenced by growth factor signaling (56). To determine the specificity of
PI3K-AKT crosstalk with each ER (α or β), human embryonic kidney (HEK) 293 cells lacking both ERα and ERβ were used (49). Since our earlier results suggest a requirement for the ER in Bcl-2 promoter activation, similar experiments were used to determine the involvement of ERα and ERβ. In the presence of E2 (1 nM), AKT potentiation of Bcl-2 promoter activation was shown to require ERα but not ERβ (Fig. 3A). Although ERα potentiated Bcl-2 reporter activity by 5-fold over Vec alone, this increase was much less than the 54-fold potentiation observed in Fig. 2A, suggesting that both ERs may be required for the robust potentiation seen in the MCF-7 cells that contain both ER isoforms.

ERα contains two activation function domains (AF-I and AF-II) that recruit coactivator proteins and are responsible for the transcriptional activities of the receptor. Here, expression vectors for ERα that have been mutated to functionally lack either AF domain were used to examine the specificity of AKT potentiation of ERα activity. AKT-CA potentiated Bcl-2 expression through the full-length ERα, the ERα-AF-I and AF-II domains, indicating that AKT-CA may target both activation domains of ERα (Fig. 3B). AKT-CA potentiated Bcl-2 expression to the highest levels through the full-length ERα. Since the AF-I and AF-II domains synergize for maximal ER transcriptional activity (15), the ability of AKT to target both AF domains of the ERα is likely necessary for maximal potentiation of Bcl-2 expression.
Although Bcl-2 expression is enhanced with ERα, AKT-CA did not significantly different from vector (data not shown). However, in the presence of E2, AKT-CA and GRIP-1 potentiated Bcl-2 expression to higher levels than either factor alone. This potentiation is seen with all ERα constructs, although the effects were lowest with the AF-I mutant (Fig. 5B) and highest with the full-length (Fig. 5A). Collectively, these data suggest that AKT-CA and GRIP-1 together may preferentially target the AF-II domain to potentiate Bcl-2 expression.

To determine whether AKT-CA and GRIP1 potentiate Bcl-2 protein expression in the presence of E2, we used immunoblot analyses. Both AKT-CA and GRIP1 were able to potentiate Bcl-2 protein expression to similar levels, but not significantly different from vector (data not shown). However, in the presence of E2, AKT-CA and GRIP1 together were able to significantly enhance Bcl-2 protein expression to a greater level than either factor alone (Fig. 6A and B), suggesting that these two factors may cooperate to transcriptionally activate the Bcl-2 promoter region.

**Discussion**

Crosstalk between the ER-E2 and the IGF1-PI3K-AKT signaling pathways has been shown to promote cancer cell survival. While growth factor signaling pathways have been shown to activate the ER, the precise role of the receptor in this survival crosstalk remains unclear. Previously, Campbell et al demonstrated the ability of AKT to rescue cells from apoptosis induced by the chemotherapeutic drug tamoxifen (34). However, since tamoxifen is an ER antagonist (57), the contribution of ER to AKT-induced cell survival could not be completely assessed. Indeed, Campbell’s studies suggest that AKT survival mechanisms may be ER-independent. Recently, Boland’s group demonstrated that E2 may protect murine skeletal muscle cells from H2O2-induced apoptosis through ERα and ERβ, possibly involving PI3K/AKT signaling (58). Our studies with a physiological inducer of apoptosis, TNFα (43), reveal that the ER is required for AKT-mediated cell survival in breast carcinoma cells (Fig. 1B), suggesting that the PI3K-AKT and ER-E2 signaling pathways converge to regulate cell survival decisions.

The ER's ability to regulate transcription of target genes, such as Bcl-2, has been linked to its ability to protect breast cancer cells from TNFα-induced apoptosis (24,53). Here, we show that ERα, both AF-I and AF-II domains, is targeted by AKT to bring about potentiation of Bcl-2 expression at both the transcriptional and translational levels (Figs. 2 and 3). These results re-enforce the role of the ERα as a mediator of cell survival in vitro (59) and in vivo (60). Even though ERβ was

**AKT increases GRIP1 protein expression.** Coactivators are integral components in ER-mediated transcriptional signaling (10). Here, we investigated AKT regulation of GRIP1 protein levels using immunoblot analyses of MCF-7 cells (Fig. 4A and B). E2 alone was not able to enhance GRIP1 protein expression in vector control cells. However, AKT-CA did enhance GRIP1 protein expression by 1.8-fold and by 2.14-fold in the presence of 1 nM E2. These results suggest that AKT plays a role in expression of the coactivator GRIP1.

**AKT and GRIP-1 cooperatively enhance Bcl-2 expression.** Since AKT was able to enhance GRIP1 protein expression, we investigated the possibility that GRIP1 may potentiate Bcl-2 expression in the presence of E2. As previously shown, AKT-CA alone was able to potentiate Bcl-2 expression through ERE (Fig. 3B and C). Overexpression of GRIP1 alone was not able to achieve this potentiation (Fig. 5A). However, AKT-CA and GRIP1 potentiated Bcl-2 expression to higher levels than either factor alone. This potentiation is seen with all ERE constructs, although the effects were lowest with the AF-I mutant (Fig. 5B) and highest with the full-length (Fig. 5A). Collectively, these data suggest that AKT-CA and GRIP1 together may preferentially target the AF-II domain to potentiate Bcl-2 expression.

**To confirm the crosstalk between ERα and AKT leads to an increase in Bcl-2 protein levels,** Western blot analyses were performed in HEK 293 cells transfected with AKT-CA and ERα (Fig. 3C and D). E2 (1 nM) induction of Bcl-2 protein expression was enhanced with ERα, although the increase was not significant compared to vector control cells. In these cells containing only ERα, AKT-CA did potentiate Bcl-2 protein expression in the absence of E2 (Fig. 3D), as seen in Fig. 2C. However, the presence of E2 had little effect on Bcl-2 expression. Because these cells do not contain ERβ, Bcl-2 potentiation may not be maximized. Collectively, our results suggest that AKT regulation of cell survival decisions require ERα, although the ERβ may also be important.

**Figure 6. AKT and GRIP1 cooperatively enhance Bcl-2 protein expression.** (A) MCF-7 cells were transfected with 5 μg vector control (Vec), constitutive active AKT (AKT-CA), pSG5-GRIP1-HA (GRIP1), or 2.5 μg each AKT-CA and GRIP1. Cells were treated with E2 (1 nM) for 24 h prior to harvesting. Whole cell extracts (50 μg) were subjected to Western blot analysis using a Bcl-2 antibody. The blots were then stripped and re-probed with an actin antibody as an internal loading control. (B) Densitometry of the Western blot analysis. Data are represented as Bcl-2 protein level relative to actin (Bcl2/actin) and are normalized to vector control. Data are the results of two independent experiments. Asterisk denotes statistical significance from vector E2, and two asterisks denotes statistical significance from AKT-CA E2 and GRIP-1 E2 (p<0.05).
not able to activate the Bcl-2 promoter in response to AKT, this receptor isoform may play a secondary supportive role, since AKT was able to potentiate Bcl-2 promoter activity much more potently in cells containing both receptor isoforms (MCF-7) than in cells with only ERα (HEK 293). Recently, we showed that ERβ is targeted by AKT signaling (21), as demonstrated by the ability of AKT to potentiate ERβ transcriptional activity at a consensus ERE promoter. Hence, AKT regulation of ERβ function in cell survival decisions may be more important in cells or tissues where ERβ expression predominates, such as in the prostate (55).

The exact role of the ER at the Bcl-2 promoter remains to be determined. Since the ER is involved in both long-term transcriptional regulation of genes (genomic effects) (61,62) and in immediate cytoplasmic signaling events (non-genomic effects) (63-67), these two functions of the ER may converge at the Bcl-2 promoter. The ER-Eα complex upregulates Bcl-2 expression either directly by acting on EREs located within the coding region of the gene (29) or indirectly through interaction with the Sp1 protein (28). In addition, the ER may also complex with components of cytoplasmic signaling pathways, such as PI3K (68).

Previously, we showed that the overexpression of coactivators may provide a survival advantage to breast carcinoma cells in the presence of TNFα (69). Other researchers have found that the coactivator PELP1 may affect breast cancer cell sensitivity to apoptosis induced by TNFα (70). Here, we show for the first time the ability of AKT to upregulate GRIP-1 protein expression (Fig. 4) and the cooperation between AKT and GRIP-1 to enhance Bcl-2 transcriptional and protein expression to levels higher than either factor alone (Fig. 5). These results suggest that AKT potentiation of the Bcl-2 promoter region may result from AKT’s ability to regulate both ERα and factors that interact with the receptor, such as GRIP-1. Once expressed, GRIP-1 may cooperate with AKT to enhance ERα activity in order to potentiate Bcl-2 expression. Other investigators have demonstrated that the coactivator amplified in breast cancer 1 (AIB1/SRC-3) is required for Bcl-2 expression. However, these investigators suggested that the regulation of Bcl-2 protein expression occurs independently of the ER (71). It is possible that GRIP-1 and AIB1 may affect the ER differentially at the Bcl-2 promoter.

Both the ER-Eα and PI3K-AKT signaling pathways have been shown to independently protect breast cancer cells from apoptosis induced by TNFα (44,50,72). In this study, we provide evidence that a survival crosstalk between the ER-Eα and PI3K-AKT pathways protects breast carcinoma cells from TNFα-induced apoptosis. Given the importance of TNFα in cell survival regulation of both normal and malignant breast tissue (39,40), the ability of a survival crosstalk to enhance cell survival may lead to cancer development. A growing area of research explores the potential of TNFα and its family members in cancer therapy (73-77). Hence, molecular studies of survival crosstalk that compromise the apoptotic effects of TNFα may prove therapeutically useful (36).

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