Estrogen Receptor Inhibits c-Jun-dependent Stress-induced Cell Death by Binding and Modifying c-Jun Activity in Human Breast Cancer Cells*  

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Abstract

c-Jun, a major component of the AP-1 transcription factor, is either pro- or anti-apoptotic with cellular determinants unknown. Nuclear estrogen receptor (ER), on the other hand, regulates gene expression through both estrogen response elements and AP-1. Here we show that stress stimulates c-Jun phosphorylation and AP-1 activity in both ER+ and ER− human breast cancer cells and only induces cell death in ER− cells, indicating a determinant role of ER in c-Jun/AP-1 activity. The inhibitory effect of ER in stress-induced cell death is confirmed by ER transfection into ER− cells. Furthermore, inhibition of c-Jun activation by a dominant negative c-Jun blocks AP-1 activity in ER+ cells and attenuates stress-induced cell death but not AP-1 activity in ER− cells, suggesting that the c-Jun/AP-1 activity has distinct properties depending on ER status. ER was shown to inhibit stress-induced cell death through its physical interaction with c-Jun. This is because ER binds c-Jun in breast cancer cells, stress treatment further increases the ER-bound phosphorylated c-Jun, and the c-Jun binding-deficient ER mutant fails to protect stress-induced cell death. Together, our studies reveal a novel function of ER in stress response by modification of c-Jun activity.

Keywords: Estrogen; c-Jun; AP-1; Apoptosis; Breast cancer

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erch of the promoter was assayed with a dual luciferase kit (Promega) to determine luciferase activity. After being permeabilized in a buffer containing 0.5% Nonidet P-40, cells were blocked in 3% bovine serum albumin in phosphate-buffered saline. A rabbit polyclonal phosphor-c-Jun antibody (Ser-63, Cell Signaling) at 1:100 and a mouse monoclonal anti-ER (F10, Santa Cruz) at 1:50 in 3% bovine serum albumin in phosphate-buffered saline was used for Western blot (and immunostaining in some cases) and two of these were chosen for our experiments.

Cell Death Assay, Immunostaining, Immunoprecipitation, and Immunoblotting—For cell death assay, breast cancer cells were infected with either ad-MKK6 or the vector control for 5 h in serum-free medium, followed by an overnight incubation in 10% serum-containing medium, as described previously (5). After an additional 24-h incubation, viable cells were determined with a hemocytometer by trypan blue exclusion assay, and the cell viability was calculated by dividing the viable cell number by that of total cells (dead + viable) (31). Portions of these cells after infection or ARS treatment also were washed with phosphate-buffered saline and resuspended in cold ethanol for flow cytometric analyses (FACS) to determine the apoptotic sub-G1 population. To determine the long-term effects of stress treatment on cell survival, cells were incubated for 2 weeks for colony formation, as previously reported (32). For immunostaining, cells were plated on coverslips 1 day prior and fixed in 3.7% formaldehyde immediately after stress treatment. After being permeabilized in a buffer containing 0.5% Triton X-100 and 0.5% Nonidet P-40, cells were blocked in 3% bovine serum albumin in phosphate-buffered saline. A rabbit polyclonal phosphor-c-Jun antibody (Ser-63, Cell Signaling) at 1:100 and a mouse monoclonal anti-ER (F10, Santa Cruz) at 1:50 in 3% bovine serum albumin in phosphate-buffered saline were used for phosphor-c-Jun and ER staining, respectively. After a 1-h incubation with the second antibody at room temperature (anti-rabbit Cy3 for phosphor-c-Jun, and anti-mouse Alk Phos Red for ER, both at 1:100), coverslips were mounted with a mounting medium from Vector Laboratories (containing 4,6-diamidino-2-phenylindole) and examined under a fluorescence microscope (Leica) for phosphor-c-Jun or ER signal. For immunoprecipitation, cells were washed with cold phosphate-buffered saline and lysed in modified radiolabeled precipitation assay buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% Nonidet P-40, 0.5% SDS, 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM EGTA, 10 mM NaF, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, and 1 μg/ml aprotinin, leupeptin, and pepstatin). The same amount of cell lysates of each group (corresponding to about 30 μg of protein) was incubated overnight at 4 °C with different antibodies for immunoprecipitation. For Western blot analyses, cells were directly lysed in SDS-PAGE loading buffer and separated by SDS-PAGE, as described previously (5, 6).

Experiments in Athymic Mice—4- to 6-week-old female athymic mice were purchased from Harlan Sprague-Dawley, Inc. (Madison, WI). ER-488 human breast cancer cells were infected with ad-vector or ad-
MKK6 as described above for 5 h in non-serum-containing medium at 37 °C and 5% CO₂. Thereafter, cells were washed and trypsinized, and 10⁶ cells in 0.1 ml of medium were inoculated subcutaneously to the front flanks of mice, as described previously (33, 34). Each mouse received two injections; the left site was injected with ad-MKK6-infected cells, and the right site was injected with the same amount of cells infected with ad-vector as a control. Beginning 2 weeks after injection, tumor volume was measured weekly with a caliper for the next 2 months (data not shown). The picture shown in Fig. 1 was taken 2 months after tumor inoculation.

RESULTS

Stress Induces Cell Death in ER− but Not in ER+ Human Breast Cancer Cells—To examine whether endogenous ER expression may affect stress-induced cell death, ER− human breast cancer 468 and ER+ MCF-7 cells were infected with adenovirus expressing a constitutively active p38 stress kinase activator MKK6 or the adenovirus vector. The adenovirus-mediated MKK6 delivery has been shown to activate both p38 and JNK stress pathways in these two cell lines (5). Cell death was assessed by morphological alterations, trypan blue staining, and flow cytometric analysis, as described previously (31). Infection with ad-MKK6 but not the vector induced significant cell death in ER− 468 cells at 48 h, whereas, surprisingly, ER+ MCF-7 cells remained unresponsive to the ad-MKK6 (Fig. 1A). A similar selective cell death inducing effect was also observed by treatment with a chemical JNK/p38 activator arsenite (Fig. 1A) (5, 35). Because the morphological alterations by either ad-MKK6 or ARS are associated with an increase of cells in sub-G₁ populations by FACS (Fig. 1A, the number to the right of each panel), these results indicated that the cell death induced in ER− cells is likely apoptotic.

To rule out cell line specific effects, the ER+ T47D and ER− 251 human breast cell lines were included to extend this observation. Again, ARS treatment has no substantial effects on the viability of ER+ cell lines (MCF-7 and T47D) but reduces the viability by about 80 and 50% in ER− 468 and ER− 231 cell lines, respectively (Fig. 1B). To further examine the in vivo therapeutic potentials of adenovirus-mediated MKK6 gene delivery in ER− tumor, an ex vivo tumor growth assay using ER− 468 cells was performed in Balb/c nude mice as described previously (34). After infection in the cell culture, the 468 cells were collected and subcutaneously injected into the left flank (ad-MKK6 cells) or right flank (ad-vector cells) of the animal with each mouse as its own control. As shown in Fig. 1C, injection of the control virus-infected cells produced a noticeable tumor in all cases about 2 months later, whereas no ad-MKK6-infected cells produced a tumor. ER+ cells were not included in these ex vivo assays as ER+ cells require supplemental estrogen. Together, these results demonstrated that stress selectively induces cell death and inhibits cell growth in ER− but not ER+ human breast cancer cells.

Stress Phosphorylates c-Jun and induces Cell Death in a c-Jun- and ER-dependent Manner—Because c-Jun activation is known to be apoptotic in many systems, we sought to examine whether the selective cell death induced by stress in ER− cells was because of a specific c-Jun activation event in these cells. Following the adenovirus infection, cell lysates were prepared and examined for c-Jun phosphorylation by Western blot using a specific antibody. As shown in Fig. 2A, ad-MKK6 increased c-Jun phosphorylations in both ER− 468 and ER+ MCF-7 cells with the amount of phosphorylated c-Jun generally correlated with the level of MKK6 expression. To examine the effects of ARS on c-Jun phosphorylation, ER+ and ER− cells were treated with ARS for 30 min, and c-Jun phosphorylation was examined by Western blot and indirect immunostaining analysis (Fig. 2, B and C). ARS again induced c-Jun phosphorylation in all cell lines examined regardless of their ER status.

These results indicate that c-Jun phosphorylation appears to be a general response to stress signaling (at least to the active MKK6 and ARS) in human breast cancer cells, independent of ER expression. The opposite cell death-inducing effect of stress in ER+ and ER− cells, therefore, is apparently not because of a difference in the c-Jun activation.

To assess whether c-Jun activation is responsible for the cell death induction by stress in ER− cells, a dominant negative c-Jun, Tam67, coding for c-Jun minus its activation domain, provided by Birrer and co-workers (27), was stably expressed in breast cancer cells (Fig. 2D). Tam67 has been widely applied to inhibit c-Jun activity in variety of systems (7, 36–38) presum-
ably through inhibition of transcription-competent endogenous c-Jun. The endogenous c-Jun at 39 kDa in both vector and Tam67-overexpressed cells was not detectable under these experimental conditions, consistent with limited c-Jun concentrations in these breast cancer cells (18). To determine effects of c-Jun inhibition on cell death, Tam67-expressing (468/Tam) and vector-transfected (468/Neo) cells were infected with ad-vector or ad-MKK6, and cell death was analyzed by colony formation assay. As shown in Fig. 2E, the growth inhibitory effect of MKK6 was significantly reduced in 468/Tam cells in comparison with 468/Neo cells consistent with the cell death inhibitory activities of Tam67 previously observed in neural, vascular, and breast cancer cells (39–41). Although stress activates multiple signaling cascades, the results with Tam67 strongly supported the notion that it is c-Jun activation that leads to cell death in these ER−468 human breast cancer cells.

To further explore whether the selective stress-induced cell death in ER−breast cancer cells is because of the lack of the endogenous ER expression (Fig. 3A), ER cDNA was cloned into a pcDNA4/TO/myc-His vector and was cotransfected with 231 cells by using a T-Rex inducible system (Invitrogen). The stress-induced cell death was analyzed in the same cells with and without inducible ER expression in two transfected clones. As shown in Fig. 3B, both 231 clones expressed higher levels of ER proteins upon the addition of tetracycline (Tet), and the result from clone 1 was further confirmed by immunostaining showing a typical nuclear ER signal in every Tet-treated cell (Fig. 3C). Importantly, in both clones inducible ER expression protects ARS-induced cell death, and this preventive effect remains similar with or without the addition of estrogens (E2) (Fig. 3, D and E), indicating a ligand-independent cell death preventive effect of the ER protein. These results thus provided direct evidence for ER inhibitory activity in stress-induced cell death in breast cancer cells.

Stress Activates AP-1 Activity in Both ER+ and ER−Cells, but c-Jun Inhibition Inhibits AP-1 Activity in ER+ but Not ER−Cells—Although c-Jun inhibition by Tam67 has been reported to block stress-induced cell death in many cell lines (7, 38, 41), it is not known whether this effect couples to its AP-1 regulatory activity. This is especially important for breast cancers, as the coordinating role of ER and c-Jun could result in dramatically different effects on AP-1 regulation in ER+ and ER−cells in response to stress signaling. To assess the effects of Tam67 on AP-1 binding activity, nuclear lysates from the vector or Tam-expressing cells were prepared and incubated with the 32P-labeled AP-1 oligonucleotide. Infection with ad-MKK6 increased AP-1 DNA binding in ER−468/Neo cells, which is completely inhibited by incubation with anti-c-Jun antibody (Fig. 4B) as observed in MCF-7 cells (5). These results indicated that activated c-Jun represented an essential component of MKK6-induced AP-1 activity in both ER+ and ER−human breast cancer cells. Stable Tam67 expression completely inhibited MKK6-induced AP-1 binding in MCF-7 cells (Fig. 4A), but ad-MKK6 still increased the binding in 468/Tam cells (Fig. 4B). Consistent with this result, transient Tam67 expression significantly blocked MKK6-induced AP-1 reporter luciferase activity (AP-1 Luc) in ER+ MCF-7 but not ER−468 cells (Fig. 4, C and D). The different AP-1 regulation by Tam67 could not be because of a higher level of AP-1 Luc activation by MKK6 in ER−468 cells (14.4 in 468 versus 4.4 in MCF-7 cells). This is because a 6.4-fold AP-1 stimulation by a constitutively active MEK1 was significantly inhibited by Tam67 in 468 cells, whereas the 1.8-fold activation was unresponsive to the inhibition in MCF-7 cells (Fig. 4, C and D). Furthermore, a similar AP-1 Luc activation by ARS in both lines was also suppressed.
FIG. 3. Restoration of ER expression in ER− 231 cells inhibits ARS-induced cell death independent of estrogens. A, the status of ER expression in ER+ and ER− human breast cancer cells as shown by direct Western blot. B and C, Tet-inducible ER expression in 231 cells. Human ER cDNA was cloned into a pCDNA4 vector and transfected into ER− 231 cells that already expressed the repressor construct pCDNA6/TR. Cell lysates from two stably transfected clones were examined for ER expression by Western blot (B) and immunostaining (C, only Clone#1) after 24 h of incubation with or without tetracycline (Tet). D and E, ER expression inhibits ARS-induced cell death in 231 cells. Two ER-inducible expression clones of 231 cells were incubated with or without Tet and then treated with ARS for an additional 24 h for cell death assay. To assess estrogen (E2) effects, a portion of these cells was incubated in the absence of serum in phenol red-free medium and in the presence of 10 nM E2 for the last 24 h. Results are means of four separate experiments (bars, S.E.; p < 0.05 in all cases for the cell viability in ARS group between the absence and the presence of Tet. DAPI, 4,6-diamidino-2-phenylindole; GADPH, glyceraldehyde-3-phosphate dehydrogenase.

FIG. 4. The dominant negative c-Jun Tam67 inhibits stress-induced AP-1 activity in ER+ MCF-7 but not in ER− 468 breast cancer cells. A and B, MKK6 induction of AP-1 binding inhibited by Tam67 in MCF-7 but not in 468 cells. Nuclear lysates from stable Tam67-expressing cells with or without MKK6 infection were incubated with 32P-labeled AP-1 consensus oligonucleotide, and EMSA was performed as described (5). Portions of the results in A were reported previously (5) and are presented here for comparison with those from ER− 468 cells. C and D, transient expression of Tam67 inhibits MKK6-induced AP-1 luciferase promoter activity in ER+ MCF-7 but not in ER− 468 cells. AP-1 luciferase reporter (AP-1 luc) was transfected with an active MKK6 (MKK6/2E) or an active MEK1 (MEK1/2E) in the presence or absence of Tam67. Results shown are means of four experiments (bars, S.E.; p < 0.05 for MKK6 versus MKK6 + Tam67 in MCF-7 but not in 468 cells, but p < 0.05 for MEK1 versus MEK1 + Tam67 in 468 but not in MCF-7 cells).

by Tam67 in ER+ MCF-7 but not in ER− 468 cells (data not shown). These results revealed an ER-dependent AP-1 inhibition by Tam67 in response to MKK6 and ARS. This, together with the cell death inhibitory effect of Tam67 in ER− 468 cells, suggests that stress-induced c-Jun-dependent AP-1 activity would have distinctly different biological effects dependent on the ER status of the breast cancer cells.

c-Jun Binds to ER through Its Activation Domain and ER Has Higher Binding Affinity Toward Phosphorylated c-Jun—The results described above suggested that two events are required for stress-induced cell death in human breast cancer cells, c-Jun phosphorylation and lack of ER protein expression. Because previous studies showed that transfected ER binds to overexpressed c-Jun in COS cells (42), it is possible that ER may inhibit c-Jun-induced cell death by a complex formation, leading to sequestering activated c-Jun in response to stress. To test this possibility, HA-tagged c-Jun or Tam67 was transfected into MCF-7 cells (cotransfected with ER in ER− 468 cells), and the anti-HA precipitates were examined for the presence of ER by Western blot. As shown in Fig. 5A, ER was detected in the HA-c-Jun but not in the HA-Tam67 precipitates, indicating that c-Jun is able to bind to both endogenous (MCF-7) and ectopically expressed ER (468) through its N terminus. Because the N-terminal activation domain of c-Jun contains several key phosphorylation residues, these results indicated that c-Jun phosphorylation may play an important role in its interaction with ER.

To directly examine whether ER-c-Jun binding is regulated by c-Jun phosphorylation, V5-tagged ER was transiently expressed in 3T3 cells with or without HA-c-Jun cotransfection. Anti-V5 precipitates were analyzed for the presence of phosphorylated c-Jun with an antibody that recognizes Ser(P)-63-c-Jun and by a second antibody that assays total c-Jun (phosphorylated and unphosphorylated). Mouse NIH 3T3 cells were used because ER expression in this cell line increases Ras-transforming efficiency (43), a scenario similar to ER+ MCF-7 cells in which Ras transfection increases the tumor malignancy (44). As shown in Fig. 5B, anti-V5 antibody failed to pull down c-Jun without HA-c-Jun transfection, indicating a limiting amount of endogenous c-Jun in these cells. If HA-c-Jun was coexpressed, however, the c-Jun signal was detected, but only a small portion of the transfected c-Jun was phosphorylated. In
response to ARS, however, phosphorylated c-Jun was increased in the ER immunoprecipitates, with the ratio increasing up to 6.5-fold in comparison with that of 1.4-fold for ER-bound total c-Jun (Fig. 5, B and E). These results suggest that ER protein may have a higher binding affinity to phosphorylated c-Jun. To test this possibility, a constitutively active c-Jun was used in the binding assay with a dominant negative c-Jun and the WT c-Jun as controls. A constitutively active c-Jun was generated by changing Ser-63, Ser-73, Thr-91, and Thr-93 to aspartic acid (D) (c-Jun(ST/D)) to mimic the negatively charged phosphate, whereas a non-phosphorylation inert mutant c-Jun was made by replacing these residues with alanine (A) (c-Jun(ST/A)) (45).

The results in Fig. 5C show that after transfection of equal amounts of WT and mutant c-Jun plasmids, the ER precipitates showed minor amounts of coprecipitated WT c-Jun, no coprecipitation of the non-activated c-Jun(ST/A), and a strong band for the activated c-Jun(ST/D) coprecipitated with ER. Thus, the c-Jun phosphorylation status may be the determinant for strong binding to ER.

To assess whether or not endogenous ER binds to endogenous c-Jun and if this binding is regulated by stress in human breast cancer cells, ER H11001 MCF-7 cells were treated with or without ARS in the absence of DNA transfections. Another c-Jun activator, 12-O-tetradecanoylphorbol-13-acetate (TPA), was included to strengthen our conclusion about possible relationships between the c-Jun phosphorylation and ER-c-Jun binding under physiological conditions. To assess ER-c-Jun binding, the cell lysates were incubated with an anti-ER antibody, and the precipitates were examined for the presence of c-Jun and phosphorylated c-Jun by Western blot. The results in Fig. 5D show that the endogenous ER forms a complex with the endogenous c-Jun without treatment, and portions of this c-Jun protein in the ER complex were phosphorylated. In response to ARS, however, ER-bound phosphorylated c-Jun increased by 10-fold (Fig. 5D and E, 0.43 versus 4.50). ER-bound total c-Jun also increased by 8-fold. Because there was a significant decrease in the mobility of the total c-Jun band after ARS, this band may also represent phosphorylated c-Jun (45). These results, for the first time, demonstrated a direct binding of endogenous ER to endogenous c-Jun in human breast cancer cells, which is further increased in response to stress. The ER-c-Jun complex formation could play an important role in ER protection against stress-induced and c-Jun-dependent cell death.

An Essential Role of Direct ER-c-Jun Binding in ER Protection against Stress-induced Cell Death—If ER inhibits stress-induced cell death dependently on c-Jun binding, a c-Jun binding-deficient ER should lose this activity. To test this possibility, a c-Jun binding-deficient ER mutant with a deletion at the N terminus (ER(315–595)) was made by a PCR-based technique, according to a previous GST pull down study (21) (Fig. 6A). To assess whether ER requires its transcription...
activity to inhibit cell death and thereby distinguish its transcriptional regulation from the cell death inhibitory effect, a transcription-inactive ER with a point mutation at the C terminus (L540Q) (30) was also generated by site-directed mutagenesis. Transcription activity of the WT and mutant ER constructs was assayed by cotransfection with an ERE-dependent luciferase reporter (28) in 468 cells, and their effect on AP-1 Luc activity was also analyzed for comparison. Results in Fig. 6B show that both mutants lose the ERE-dependent transcription activity compared with the wild-type ER in these cells, and a similar result was also obtained in ER+ MCF-7 cells (data not shown). An increase in AP-1 reporter activity was observed after transfection of WT ER (p < 0.05 versus control), as previously described (22). ER(L540Q) also increased the AP-1 activity (p < 0.05 versus control), and this effect is slightly higher than the WT ER (p > 0.05 versus WT ER). Mechanisms for this moderate increase in AP-1 activity by ER(L540Q) over the WT ER are not clear at this time but may involve differences in their protein stability under certain conditions (46). A decreased ER activity and an increased AP-1 activity was also observed for another ER mutant (ER(K206A)) (47). To determine whether these mutations affect their c-Jun binding activity their c-Jun binding activity in breast cancer cells, the WT and mutant ER constructs were stably expressed in ER− 468 cells through G418 selection (Fig. 7A), and ARS- and ad-MKK6-induced cell death was determined. Results in Fig. 7B show that both ARS and ad-MKK6 significantly decreased cell viability in the vector-transfected 468 cells, and these effects were significantly reduced in cells expressing wild-type ER (p < 0.01, for MKK6 and ARS in 468/ER cells versus MKK6 and ARS in 468/Neo cells). A similarly significant cell death-protective effect was also observed in cells expressing the transcription-inactive ER(L540Q) (p < 0.01 versus those in 468/Neo cells). The cell death inhibitory activity, however, is significantly lost in cells expressing the c-Jun binding-deficient ER-(315–595) versus (p < 0.01 for MKK6 and ARS in 468/ER-(315–595) cells versus those in 468/ER cells, Fig. 7B). The effects of these ER transfectants on ad-MKK6-induced cell death were further confirmed by FACS (Fig. 7C). These results indicate that the cell death protective effect of ER is, at least in part, dependent on its ability to bind to c-Jun. This conclusion is further supported by results with ER(L540Q) in which the cell death protective activity of this mutant was positively linked to its c-Jun binding ability but not to its transcriptional activity. These results together demonstrated that ER inhibits stress-induced cell death independent of its transcriptional activity but dependent on its c-Jun binding.

**DISCUSSION**

Estrogen-ER signaling has a well established activity in inhibition of cell death in neuron cells (48, 49), but surprisingly this effect has not been documented in human breast cancer cells. Furthermore, mechanisms involved in this cell death protective effect of ER signaling are not known. A study by Manolagas and co-workers (50) recently showed that sex hormones inhibit cell death in osteoblast cells independently of transcriptional activity. The overexpression of transfected ER

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construct and high levels of steroid sex hormones added to the culture may activate additional mitogenic pathways such as ERK (50, 51) and phosphatidylinositol 3-kinase (52) cascades. This potentially could lead to a nonspecific anti-apoptotic response resulting in a dominant survival signal over a stress insult. Consistent with that study (50), the anti-apoptotic effect of ER was found in our present study to be transcription-independent, as observed with the transcriptionally inert ER (L540Q). In contrast to the earlier report (50), our results demonstrate that the cell death inhibitory effect of ER in breast cancer cells is independent of estrogen. More importantly, our study reveals that the cell death inhibitory activity of ER in human breast cancer cells is because of its c-Jun binding ability.

Accumulating evidence suggests that the estrogen-ER signaling may provide a redundant mitogenic pathway in parallel to the Ras/ERK/c-Jun/AP1 pathway to ensure a proliferative response of breast cancer cells to growth factors and estrogens (14). Our results, for the first time, demonstrated a distinct biological difference between c-Jun-dependent AP1 activity in ER+ and ER− human breast cancer cells in response to stress. The direct ER-c-Jun binding may result in recruiting additional cofactors to an ERE-AP1 DNA complex in response to stress, leading to distinct gene activations different from those activated by AP1 in the absence of ER. Although our results with the dominant negative ER suggested that this may occur independently of ERE-dependent transcription, an AP1-dependent gene regulation may be involved in this process, as both the WT ER and ER (L540Q) stimulate AP1 activity (Fig. 6B). Potential target genes involved in this process may include cyclin D1 (53) and Bcl-2 (54). This assumption is supported by the fact that ER is expressed in 80% of the Bcl-2-positive mammary tumors but only in 30% of the Bcl-2-negative tumors (55). Thus, ER expression may positively contribute to breast cancer growth by a dual mechanism. In normal situations ER is activated by ligand estrogen (14) and other mitogenic signals (56) to promote breast cancer cell proliferation primarily through ERE-dependent transcriptional regulation. In stressful conditions, ER may facilitate breast cancer growth by inhibiting cell death independently of ER transcription but likely involving a c-Jun binding-dependent AP1 transcriptional regulation.

Selective cell-killing effects of stress-induced c-Jun activation in ER− breast cancer cells may have a therapeutic potential. Although ER+ breast cancer patients have benefited for decades from anti-estrogen therapy such as tamoxifen citrate, so far there have been no valid therapeutic targets for ER− human breast cancer (57). Our experimental model predicts that stress reagents such as etoposide, taxol, and vinblastine, which strongly activate the JNK/c-Jun pathways (31, 58, 59), may be selectively effective in treatment of ER− but not ER+ human breast cancers. Along with this approach, inhibition of ER activity in ER+ breast cancer may correspond to a sensitization strategy to stress stimuli.

Demonstration of ER cell death inhibitory activity through c-Jun binding will contribute significantly to understanding the signaling specificity of c-Jun activation. In response to stress, a burst of c-Jun phosphorylation within a short time will activate cell death in ER− breast cancer cells. In ER+ cells, the phosphorylated c-Jun will be absorbed by ER through their direct binding, which will, through a slow release process thereafter, result in ER-modified c-Jun-dependent AP1 tran-
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