Positive Cross-Talk between Estrogen Receptor and NF-κB in Breast Cancer

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Abstract

Estrogen receptors (ER) and nuclear factor-κB (NF-κB) are known to play important roles in breast cancer, but these factors are generally thought to repress each other’s activity. However, we have recently found that ER and NF-κB can also act together in a positive manner to synergistically increase gene transcription. To examine the extent of cross-talk between ER and NF-κB, a microarray study was conducted in which MCF-7 breast cancer cells were treated with 17β-estradiol (E2), tumor necrosis factor α (TNFα), or both. Follow-up studies with an ER antagonist and NF-κB inhibitors show that cross-talk between E2 and TNFα is mediated by these two factors. We find that although transrepression between ER and NF-κB does occur, positive cross-talk is more prominent with three gene-specific patterns of regulation: (a) TNFα enhances E2 action on ~30% of E2-upregulated genes; (b) E2 enhances TNFα activity on ~15% of TNFα-upregulated genes; and (c) E2 + TNFα causes a more than additive upregulation of ~60 genes. Consistent with their prosurvival roles, ER and NF-κB and their target gene, BRIC3, are involved in protecting breast cancer cells against apoptosis. Furthermore, genes positively regulated by E2 + TNFα are clinically relevant because they are enriched in luminal B breast tumors and their expression profiles can distinguish a cohort of patients with poor outcome following endocrine treatment. Taken together, our findings suggest that positive cross-talk between ER and NF-κB is more extensive than anticipated and that these factors may act together to promote survival of breast cancer cells and progression to a more aggressive phenotype.

Introduction

The estrogen receptor (ER) is expressed in ~75% of breast tumors and is a major prognostic and therapeutic determinant. Women with ER-positive tumors have a better prognosis and will likely receive endocrine therapy, such as tamoxifen or an aromatase inhibitor. However, not all ER-positive tumors respond to endocrine therapy and, frequently, de novo or acquired resistance occurs. These ER-positive tumors, which tend to retain ER expression but without typical response to tamoxifen, are generally more aggressive with earlier metastatic recurrence (1–3). Gene expression profiling has further delineated the two types of ER-positive tumors, referred to as intrinsic subtypes luminal A and luminal B, with the luminal A subtype associated with good patient outcome and the B subtype with a poor survival rate (4, 5). Interestingly, activation of the proinflammatory transcription factor nuclear factor-κB (NF-κB) may play a role in this dichotomy between ER-positive tumors. Constitutive activation of NF-κB in breast tumors is associated with more aggressive ER-positive tumors (6, 7), the development of resistance to endocrine therapy (8, 9), and progression to estrogen-independent growth (10–12).

Two ER subtypes have been identified, ERα and ERβ, which mediate the biological functions of estrogen primarily through their ability to function as ligand-activated transcription factors. Both ERs can stimulate gene transcription by directly binding to DNA at estrogen response elements or through tethering to other transcription factors (13, 14). ERs can also negatively regulate or repress transcription in either a direct or indirect manner through interaction with other transcription factors (15, 16). In particular, the ability of ERs to repress the transcriptional activity of NF-κB has been well studied. The NF-κB pathway is stimulated by a variety of factors, including proinflammatory cytokines. Following cytokine binding to its receptor, activation of the IκB kinase (IKK) complex occurs, leading to phosphorylation and subsequent degradation of the inhibitory protein, IκB. This allows release of NF-κB family members, p65 and p50, which are sequestered in the cytoplasm by IκB. Once liberated, p65 and p50 can translocate to the nucleus, bind to DNA at cognate NF-κB response elements, and regulate target gene transcription. NF-κB activation can be repressed by ER through several different mechanisms, including prevention of NF-κB binding to DNA (17, 18), recruitment of corepressors into a complex with NF-κB (19), competition for coactivators (20, 21), or prevention of NF-κB nuclear translocation (22). The basis for these different mechanisms has not been fully elucidated but may be related to different cellular backgrounds or to gene-specific mechanisms of cross-talk.

In contrast, very few reports have indicated that positive transcriptional cross-talk can occur between ER and NF-κB (23–26). In each case, the mechanisms for positive cross-talk seem to involve a complex formation containing the ER and NF-κB family members at either an estrogen response element or a NF-κB response element. Previously, we have found that activation of ER and NF-κB in breast cancer cells, via treatment with estradiol (E2) and the proinflammatory cytokine tumor necrosis factor α (TNFα), leads to enhanced transcription of the prostaglandin E2 synthase (PTGES) gene (24). However, the extent to which this positive cross-talk between ER and NF-κB occurs in breast cancer cells is not known. This lack of information prompted us to examine the genome-wide transcriptional cross-talk between ER and NF-κB.
and interestingly, we found that positive cross-talk is predominant compared with repression. We identified a large subset of genes that are synergistically upregulated by the combination of E2 and TNFα in an ER- and NF-κB-dependent manner. This subset of genes is highly enriched in luminal B tumors and may contribute to ER- and NF-κB-dependent breast cancer cell survival. Furthermore, this subset of genes shows a unique expression pattern in breast tumors of women with poor response to tamoxifen and reduced disease-free and overall survival.

Materials and Methods

Materials. 17β-Estradiol (E2) was obtained from Sigma. The cytokines TNFα, interleukin (IL)-1β, and IL-6 were obtained from R&D Systems. IκK inhibitor VII, which inhibits both IKKα and IKKβ, was obtained from Calbiochem. ICI 182,780 was obtained from Tocris. Adenoviral vectors for the dominant-negative form of IκBα (IκBα-DN) were very kindly given to us by Dr. Ruxana Sadikot (University of Illinois at Chicago, Chicago, IL). The inhibitor of apoptosis (IAP) antagonist (SMAC mimetic) was very generously provided by Dr. Xiadong Wang (University of Texas Southwestern Medical Center, Dallas, TX).

Cell culture. MCF-7 cells were obtained from Dr. Benita Katzenellenbogen (University of Illinois at Urbana-Champaign, Urbana, IL) and cultured in MEM containing 5% calf serum as previously described (24). ZR75-1 and T47D cells were obtained from Dr. Debra Tonetti (University of Illinois at Chicago, Chicago, IL) and cultured in RPMI containing 10% fetal bovine serum. All three cell lines express ERα but not ERβ; hence, references to ER in these cell lines indicate ERα. Before E2 treatments, cells were cultured in phenol red-free media containing 5% charcoal-dextran-stripped serum for at least 3 d. Expression of GFP or IκBα-DN was carried out using adenoviral vectors as previously described (24, 27, 28).

Gene expression profiling and analysis. Total RNA was harvested for eRNA labeling, and hybridization to Affymetrix HGU133A GeneChips was carried out as described previously (27, 29, 30), with three replicates for each treatment. Arrays were scanned and analyzed using the GeneChip Operating Software (Affymetrix). CEL files were processed and normalized using “gcrma” function in the R/Bioconductor package (31). Normalized data were further analyzed using GeneSpring software and significantly regulated genes were identified by the following criteria: ≥2.0 fold change and P < 0.05 for at least one treatment condition (E2, TNFα, or E2 + TNFα) compared with untreated control, as previously described (30, 32). After identification of regulated genes, modulation of gene regulation was considered “enhanced” if the fold change by E2 + TNFα was greater than 1.5× the fold change seen with either E2 or TNFα alone. Similarly, gene regulation was considered “reversed” if the fold change by E2 + TNFα was less than 0.67× the fold change seen with E2 or TNFα alone. All microarray data are publicly available through GEO (accession no. GSE11467).

Gene Ontology (GO) term enrichment was carried out using the Functional Annotation Clustering tool in DAVID, as previously described (33). Enrichment of gene expression patterns in human breast tumors was determined using data from a breast tumor compendium and Genomica software as previously described (34). Survival analysis was carried out using gene expression profiles from the Uppsala patient population, as previously described with some modifications (27, 35). A total of 81 ER-positive patients from tumors who subsequently underwent endocrine therapy with tamoxifen only were used to assess the role of E2 + TNFα–regulated genes in patient outcome. Expression profiles of E2 + TNFα–regulated genes were used to cluster patients using average linkage hierarchical clustering. Kaplan-Meier estimates were used to compute survival curves for disease-free survival, distant metastasis–free survival, and disease-specific survival, and the significance of the hazard ratios between the four major patient clusters was determined by the P of the likelihood ratio test, as was described previously (35).

RNA isolation and quantitative PCR. RNA isolation was carried out using Trizol according to the manufacturer’s instructions (Invitrogen). Total RNA (0.5 μg) was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Invitrogen). The resulting product was diluted to 100 μL with double-distilled water and 2 μL were used for each subsequent quantitative PCR reaction. Quantitative PCR was carried out and analyzed as previously described using 36B4 as an internal control (30). Primer sequences are available on request.

Cell survival and apoptosis assays. Cell viability was assessed in MCF-7 cells seeded in 96-well plates using the CellTiter 96 AQueous One Solution (Promega) according to the manufacturer’s instructions. Annexin V-FITC labeling was done following the manufacturer’s instruction (BD Biosciences), and the percentage of positively stained cells was assessed by flow cytometry.

Statistical analysis. Quantitative PCR, survival, and apoptosis data were analyzed by one-way or two-way ANOVA followed by post hoc Tukey or Bonferroni test, as appropriate. Significance for all statistical tests was set at P < 0.05. Data shown are the mean ± SEM from three to six independent determinations.

Results

To examine the genome-wide cross-talk between ER and NF-κB, a microarray study was carried out using RNA from ER-positive MCF-7 breast cancer cells that were treated with E2, TNFα, or both for 2 hours. A total of 395 genes were identified as significantly upregulated or downregulated by at least one of the three treatments (Supplementary Table S1; fold change ≥2.0; P < 0.05). Hierarchical clustering analysis revealed several distinct patterns of regulation (Fig. 1). As previously shown, a large number of genes are downregulated by E2 treatment (cluster A; ref. 30). Interestingly, three distinct subsets of upregulated genes were also identified: genes that are upregulated by E2 (cluster B), genes that are upregulated by TNFα (cluster C), and genes that are upregulated by E2 + TNFα (cluster D). These three subsets of upregulated genes were used to examine cross-talk between E2 and TNFα in more detail.
TNFα modulates gene regulation by E2. To examine how the presence of TNFα influences E2 action, we compared the effects of E2 and E2 + TNFα on the subset of genes that are upregulated by E2 (n = 46). Unexpectedly, we found that TNFα enhances E2 activity on ~30% of these genes (Fig. 2A), which partially accounts for the genes in cluster D (Fig. 1). The ability of TNFα to enhance E2 activity is exemplified by pS2, which is robustly upregulated by E2, not affected by TNFα alone, and upregulated to a greater extent by the combination of E2 + TNFα when compared with E2 alone (Fig. 2B). The ability of TNFα to enhance E2 regulation of pS2 is mediated by the NF-κB pathway because an inhibitor of the IKK complex prevents TNFα action (Fig. 2C).

In addition, we found that TNFα reverses or represses E2 action on <10% of E2-upregulated genes (Fig. 2A), as shown by c-Fos, which is upregulated to a greater extent by E2 alone compared with E2 + TNFα (Fig. 2B). As is the case for enhanced pS2 regulation, the repression of c-Fos by E2 + TNFα is also mediated by the NF-κB pathway because the IKK inhibitor prevented repression of this gene by TNFα (Fig. 2C). In addition, the effect of E2 + TNFα on both pS2 and c-Fos is completely blocked by the ER antagonist, ICI 182,780, indicating that the different effects of TNFα on E2 action require ER activity (Fig. 2C).

On the remaining 60% of the E2-upregulated genes, TNFα seems to have no effect (Fig. 2A), as shown for cluster B (Fig. 1) and as demonstrated by the upregulation of IGFBP4, which is not different between E2 and E2 + TNFα at either the 2- or 3-hour time point (Fig. 2B). However, a slight reduction in E2 activity with the addition of TNFα was seen after 4 hours. ICI 182,780 completely blocked regulation of IGFBP4 by E2 or E2 + TNFα, whereas the IKK inhibitor had no effect (Fig. 2C). Taken together, these findings indicate that the proinflammatory cytokine, TNFα, acting through the NF-κB pathway, can influence E2 activity, either positively or negatively, in a gene-dependent manner. Furthermore, it seems that at the early time points chosen for study, enhancement of E2 activity by TNFα is ~3x more common than repression.

E2 modulates gene regulation by TNFα. We next examined how the presence of E2 influences TNFα activity on genes upregulated by TNFα (n = 39). We found that E2 represses or reverses TNFα activity on ~41% of these genes (Fig. 3A). This finding is expected because the ability of ER to repress NF-κB is well documented. Regulation of the NF-κB target gene, ICAM1, shows the ability of E2 to repress gene regulation by TNFα (Fig. 3B) and that this effect is mediated by ER, as evidenced by the reversal of repression by ICI 182,780 (Fig. 3C). In addition, we found that E2 has no effect...
on the regulation of ∼44% of TNFα-upregulated genes (Fig. 3A), as shown by cluster C (Fig. 1). However, this subset contains several NF-κB target genes that are known to be repressed by ER. For example, our microarray data indicate that E2 treatment has no effect on TNFα upregulation of its own gene, but this is in contrast to several reports in the literature, which show that E2 can repress TNFα autoregulation (19, 36, 37). This conflict was resolved by a time-course experiment, which revealed that E2 can repress regulation of TNFα mRNA but only at the earlier time points examined, and that repression by E2 is lost at 2 hours, the time point at which the microarray was carried out (Fig. 3B). Treatment with ICI at the 2-hour time point had no effect on the autoregulation of TNFα (Fig. 3C). These findings suggest that our microarray data may have underestimated the ability of E2 to repress TNFα activity and that E2 repression of some TNFα-regulated genes may be time dependent.

One particularly unanticipated finding from our study is that E2 can enhance TNFα activity on ∼15% of TNFα-upregulated genes (Fig. 3A and some genes in cluster D of Fig. 1). This enhancement is shown for BIRC3 (c-IAP2), which is an important antiapoptotic gene of the IAP family and is known to be upregulated by NF-κB (38). The upregulation of BIRC3 is greatly enhanced by E2 + TNFα as compared with TNFα alone (Fig. 3B). The effect of E2 on TNFα regulation of BIRC3 is completely prevented by ICI 182,780, indicating an essential role for ER in enhancing NF-κB activity on this gene (Fig. 3C). These findings indicate that although ER represses NF-κB activity on many genes, ER can also enhance NF-κB activity in a gene-specific manner.

**Common E2 and TNFα target genes.** Previous work from our laboratory showed that the PTGES gene is upregulated independently by E2 and TNFα and in a more than additive manner by the combination of E2 + TNFα (24). Our microarray data suggest that a similar regulation occurs on a large subset of common E2 and TNFα target genes (n = 63), which make up the majority of cluster D (Fig. 1). These genes, as represented by PHLD1A1 and IL17RB (Fig. 4A), are significantly upregulated by E2 + TNFα compared with either E2 or TNFα alone across a 4-hour time course.

To examine regulation of PHLD1A1 in more detail, additional studies were carried out using different proinflammatory cytokines and different ER-positive breast cancer cell lines. The combination of E2 + TNFα or E2 + IL-1β, but not E2 + IL-6, led to a significantly greater upregulation of PHLD1A1 in MCF-7 cells compared with E2 or cytokine alone (Fig. 4B). A significant upregulation of PHLD1A1 by E2 + TNFα was also observed in two other ER-positive breast...
cancer cell lines, ZR75-1 and T47D (Fig. 4B). The ER is required for regulation of PHLDA1 by E2 + TNFα because the ER antagonist, ICI 182,780, completely blocked regulation (Fig. 4C). The fact that both TNFα and IL-1β, but not IL-6, can regulate this gene suggests an important role for the NF-κB pathway in the regulation of PHLDA1. Expression of IκBα-DN, which cannot be phosphorylated or degraded and thereby blocks NF-κB activity, confirms that the NF-κB pathway is also required for the enhanced upregulation of PHLDA1 by E2 + TNFα (Fig. 4C). These findings indicate that the scope of positive cross-talk between ER and NF-κB in breast cancer may be broader than expected, rather than being limited to one particular cytokine in a single cell line.

**ER and NF-κB target genes in breast cancer cell survival and human breast tumors.** In total, 80 genes were identified as being upregulated to a greater extent by E2 + TNFα than by E2 or TNFα alone based on three different patterns of regulation: (a) TNFα enhanced E2 activity, (b) E2 enhanced TNFα activity, and (c) upregulation by E2 + TNFα. The top three biological functions associated with these 80 genes were transcriptional regulation, metabolism, and apoptosis/cell death, as identified using functional annotation clustering of enriched GO terms. The overall enrichment score for each cluster ranged from 2.63 to 1.49, but only the transcriptional regulation cluster reached significance (Benjamini Hochberg corrected P = 0.01). The identification of apoptosis-related function for these genes is consistent with the prosurvival roles for both ER and NF-κB. To investigate this further, we took advantage of the fact that treatment of some breast cancer cell lines, including the MCF-7 cells used in these studies, with higher doses of TNFα can induce apoptosis through prolonged activation of the c-jun NH2-terminal kinase pathway (39–41). Treatment of MCF-7 cells with TNFα for 30 to 48 hours caused a >50% reduction in cell viability and a 4-fold increase in the number of apoptotic cells (Fig. 5A and B). The effect of TNFα on cell viability and apoptosis is completely reversed by the combination of E2 + TNFα, indicating that E2 prevents TNFα-induced apoptosis. We find that the ability of E2 to prevent TNFα induced cell death is blocked by ICI 182,780, as well as by an inhibitor of the IKK complex (Fig. 5C), indicating that both ER and NF-κB are required for E2 to promote cell survival in the presence of TNFα. To examine whether BIRC3, an anti-apoptotic factor that is highly upregulated by the combination of E2 + TNFα (Fig. 3B), may be an important effector of ER and NF-κB-mediated cell survival, we used an IAP antagonist that blocks the activity of BIRC3 (42, 43). We found that the IAP antagonist completely prevented an increase in cell survival by E2 + TNFα, which suggests that the ER-negative and NF-κB-dependent upregulation of BIRC3 plays an essential role in breast cancer cell survival (Fig. 5C).

To further examine the significance of the 80-gene signature resulting from positive cross-talk by E2 + TNFα in breast tumor biology, we used a recently described breast tumor compendium that contains gene expression profiles for ~1,200 well-annotated breast tumors (34). We first identified tumors in the compendium that significantly overexpress or underexpress genes from the positive cross-talk gene set by 1.8-fold or more (Fig. 6). We next examined whether this gene expression profile was enriched in tumors with certain attributes or characteristics, including ER status, intrinsic subtype (luminal A, luminal B, Her2, basal, or normal (4)), or grade. As expected, we find that the positive cross-talk gene set is significantly overexpressed in ER-positive tumors and under-expressed in ER-negative tumors (Fig. 6A). In addition, tumors of the luminal B intrinsic subtype, but not those of the luminal A subtype, also significantly overexpress the E2 + TNFα-positive cross-talk genes. Luminal B tumors have previously been shown to be more aggressive ER-positive tumors associated with a worse patient outcome, whereas luminal A tumors are also ER-positive but associated with a better patient outcome (5). These findings suggest that the combination of E2 and proinflammatory cytokines, acting through ER and NF-κB, may coordinately regulate specific genes associated with more aggressive ER-positive tumors. In contrast, grade 3 and basal subtype tumors upregulate the positive cross-talk gene set. This most likely is related to the fact that the majority of basal and grade 3 tumors are ER negative.

The association of the 80 positive cross-talk genes with key clinical parameters suggests that they may be involved in response to endocrine treatments and patient survival. To investigate whether the expression of the 80-gene signature is prognostic of patient outcome, we examined the expression of these genes in a cohort of women with ER-positive breast tumors (n = 81) who had been treated with tamoxifen and followed over time. Hierarchical clustering of patients using expression levels of the 80 genes grouped them into four clusters with distinct expression profiles (Fig. 6B).
Survival analysis of the four patient clusters indicated distinct outcomes for disease recurrence (disease-free survival; Fig. 6C), metastases (distant metastasis-free survival; Fig. 6C), and death from breast cancer (disease-specific survival; Fig. 6C) following tamoxifen treatment. Expression profiles of the 80 genes clearly distinguished patients with good outcomes (Fig. 6B and C, green cluster and curves) from those with poor outcomes (Fig. 6B and C, red cluster and curves). Survival differences between the four patient clusters were statistically significant for disease-free (P = 0.0128) and distant metastasis-free (P = 0.0138) survival and nearly significant for disease-specific survival (P = 0.0782). These findings suggest that the interactions between the ER and NF-κB pathways and their downstream target genes may alter the genetic programming of ER-positive tumors and subsequently affect response to endocrine therapy and patient survival.

Discussion
The major mechanism of cross-talk between ER and NF-κB that has been described is transrepression, whereby ER represses NF-κB activity and NF-κB represses ER activity (44, 45). Our microarray results confirm that transrepression does occur between these two factors in breast cancer cells and that ER repression of NF-κB activity is more common than NF-κB repression of ER activity.

Figure 5. ER- and NF-κB-dependent breast cancer cell survival. MCF-7 cells were treated with 10 nmol/L E2, 50 ng/mL TNFα, or both. Cell viability was assessed after 30 h by MTS assay (A), or apoptosis was measured after 48 h by Annexin V staining (B). *, P < 0.05, compared with TNFα. C, MCF-7 cells were treated with E2, TNFα, or both in the presence or absence of the ER antagonist ICI 182,780 (1 μmol/L), an inhibitor of the IKK complex (1 μmol/L), or an inhibitor of IAP proteins (10 nmol/L), and cell viability was assessed. *, P < 0.05, compared with TNFα.

Figure 6. Genes regulated by E2 + TNFα are enriched in ER-positive and luminal B breast tumors and are associated with response to tamoxifen therapy. A, a set of 80 genes upregulated by E2 + TNFα was assessed in a compendium of breast tumor gene expression profiles. Tumors in which these genes were statistically overexpressed (red) or underexpressed (green) were identified using Genomica software. The ER status, intrinsic subtype, and grade of each tumor are indicated in brown. Enrichment of the 80-gene list in ER-positive and luminal B tumors was observed (red), whereas reduced expression of the 80-gene set was seen in ER-negative, basal, and grade 3 tumors (green). Numbers in parentheses indicate the negative log of the P value for the enrichment of the 80-gene set within each tumor group. B, microarray gene expression data from the tumors of 81 patients treated with tamoxifen following surgery were used to perform hierarchical clustering of patients using the expression profiles of the 80 genes upregulated by E2 + TNFα. Four distinct patient clusters were identified and differentially colored. C, Kaplan-Meier survival analysis was carried out to track disease-free, distant metastasis-free, and disease-specific survival over time for each cluster of patients. Colors of the survival curves correspond with the coloring of the clusters. P values from likelihood ratio tests are shown to indicate differences between the survival curves from each of the patient clusters for each type of outcome examined.
Because NF-κB activation is associated with tumor invasiveness, metastasis, and drug resistance (46), the fact that ER represses NF-κB activity on numerous genes may represent one mechanism by which ER-positive breast tumors are actually less aggressive than ER-negative tumors. This concept has been previously suggested for ER repression of RelB, a member of the NF-κB family, in breast cancer (47). However, results from our microarray analysis clearly show that transrepression is only one of many mechanisms of cross-talk that occur between these two pleiotropic transcription factors.

Three different types of positive cross-talk between ER and NF-κB were identified in our study. First, TNFα can enhance E2 activity on ∼30% of E2-upregulated genes, as shown by ps2. Second, E2 can enhance TNFα activity on ∼15% of TNFα-upregulated genes, such as BIRC3. And third, E2 + TNFα acting together cause a more than additive upregulation of a specific subset of genes, including PHLDA1 and IL17RB. Although repression between ER and NF-κB has been well documented, few instances of a positive interaction between ER and NF-κB have been reported (23–26). For example, ER and NF-κB act together to upregulate prostaglandin E synthase (24), serotonin 1A receptor (26), prolactin in pituitary cells (23), and cyclin D1 in mammary cells (25). Thus, our study adds significant new information to the field by highlighting the extensive degree of positive cross-talk that occurs between ER and NF-κB in breast cancer cells.

The concept that ER and NF-κB can work together to positively upregulate the expression of particular genes could have important implications in breast cancer disease progression. Recent evidence indicates that ER-positive breast tumors with constitutively active NF-κB are more aggressive and less responsive to tamoxifen (7). Our findings suggest that the positive transcriptional cross-talk between ER and NF-κB may enhance the expression of genes involved in the promotion of more aggressive tumors. This is supported by the observation that these genes are enriched in luminal B tumors, a more aggressive subtype of ER-positive breast tumors (5), but not in luminal A tumors, which are also ER-positive but have a better patient outcome. Furthermore, the gene expression profile of tumors from women who failed to respond to tamoxifen and had poor disease-free and overall survival indicates that the expression levels of these genes, including some that are highly expressed in poor outcome patients, are associated with response to endocrine therapy.

One potential reason why these tumors are more aggressive is that both ER and NF-κB are transcription factors with well-characterized prosurvival or antiapoptotic activities. Whereas ER and NF-κB can act individually to upregulate cell survival genes, we find that they can also act together to enhance the expression of additional cell survival genes, including PHLDA1 and BIRC3. PHLDA1 has been shown to be required for survival of cells following serum starvation (48). Furthermore, PHLDA1 has been detected in breast tumors and seems to correlate with better patient outcome in ER-negative tumors and with worse outcome in ER-positive tumors (49). The function and expression of BIRC3, in contrast, have not been well examined in breast tumors. However, the IAP family of apoptosis inhibitors does play an antiapoptotic function in different cancer cells and may contribute to tumor resistance to therapeutic drugs (43, 50, 51). Our findings show that inhibition of BIRC3 using an IAP antagonist (also known as a SMAC mimic), which can bind and inhibit the activity of IAP proteins, completely prevented cell survival in response to E2 and TNFα. We propose that whereas both ER and NF-κB have individual prosurvival functions, they may also act together to regulate the expression of additional antiapoptotic genes, such as BIRC3, which can further contribute to the enhanced survival of breast cancer cells. In addition to BIRC3, other genes may contribute to more aggressive breast tumors. For example, IL17RB has recently been observed to have a prognostic function in breast tumors when its expression is compared with the level of another gene, HOXB13 (52). The ratio of these two genes may also be predictive of tumor responsiveness to tamoxifen (53). Intriguingly, activation of NF-κB is associated with antiestrogen resistance (6, 8, 9), but whether NF-κB activation can alter the HOXB13 to IL17RB ratio and how this influences response to tamoxifen require further investigation.

In summary, our findings indicate that a spectrum of cross-talk between ER and NF-κB can be observed in breast cancer cells, and that, in particular, these two factors act together to enhance the expression of numerous genes. It is possible that the coordinated actions of ER and NF-κB lead to enhanced cell survival, reduced response to therapeutic agents such as tamoxifen, and the development of more aggressive breast tumors. Thus, transcriptional cross-talk between ER and NF-κB represents a potential therapeutic target for breast cancer treatment, and further investigation into this possibility is warranted.

Disclosure of Potential Conflicts of Interest

The authors have no potential conflicts of interest to disclose.

Acknowledgments

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References


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Correction: Positive Cross-Talk between Estrogen Receptor and NF-κB in Breast Cancer

In this article (Cancer Res 2009;69:8918–25), which was published in the December 1, 2009 issue of Cancer Research (1), both instances of "ER-negative and NF-κB-dependent" in the last paragraph of the Introduction, and "ER-negative and NF-κB-dependent" in the Fig. 5 legend, should read as "ER- and NF-κB-dependent". Also, "ER-negative positive" in the first paragraph of the Results should read as "ER-positive". The online article has been changed to reflect this correction and no longer matches the print.

Reference


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