

Gene Expression Preferentially Regulated by Tamoxifen in Breast Cancer Cells and Correlations with Clinical Outcome

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Abstract

The beneficial effect of the selective estrogen receptor (ER) modulator tamoxifen in the treatment and prevention of breast cancer is assumed to be through its ability to antagonize the stimulatory actions of estrogen, although tamoxifen can also have some estrogen-like agonist effects. Here, we report that, in addition to these mixed agonist/antagonist actions, tamoxifen can also selectively regulate a unique set of >60 genes, which are minimally regulated by estradiol (E₂) or raloxifene in ER α -positive MCF-7 human breast cancer cells. This gene regulation by tamoxifen is mediated by ER α and reversed by E₂ or ICI 182,780. Introduction of ER β into MCF-7 cells reverses tamoxifen action on ~75% of these genes. To examine whether these genes might serve as markers of tamoxifen sensitivity and/or the development of resistance, their expression level was examined in breast cancers of women who had received adjuvant therapy with tamoxifen. High expression of two of the tamoxifen-stimulated genes, *YWHAZ/14-3-3z* and *LOC441453*, was found to correlate significantly with disease recurrence following tamoxifen treatment in women with ER-positive cancers and hence seem to be markers of a poor prognosis. Our data indicate a new dimension in tamoxifen action, involving gene expression regulation that is tamoxifen preferential, and identify genes that might serve as markers of tumor responsiveness or resistance to tamoxifen therapy. This may have a potential effect on the choice of tamoxifen versus aromatase inhibitors as adjuvant endocrine therapy. (Cancer Res 2006; 66(14): 7334-40)

Introduction

Tamoxifen, a selective estrogen receptor modulator (SERM), is effective in the prevention and treatment of breast cancer (1–3) and has been the most widely used endocrine therapy for breast cancer. Originally, it was termed an antiestrogen because it was assumed that the positive effects of tamoxifen arise from its ability to antagonize the stimulatory actions of estrogen acting through its receptors. However, tamoxifen is now more accurately called a SERM, reflecting its ability to display estrogen-like agonist effects, as well as antiestrogen-like antagonist effects, in a tissue or cell

type-selective manner. These mixed agonist/antagonist actions of tamoxifen are associated not only with its effectiveness in the prevention and treatment of breast cancer and positive effects on the maintenance of bone mineral density but also with some deleterious side effects, such as an increased risk of endometrial cancer and thromboembolic events (4).

One of the primary functions of the ERs (ER α and ER β) is their ability to regulate gene transcription in a ligand-dependent manner. Recently, we and several other groups have used gene expression profiling in an attempt to identify genes regulated through the ER in breast cancer cells (reviewed in refs. 5–7 and references therein). We have found that estrogen both up-regulates and down-regulates a wide range of genes, many of which would be consistent with stimulation by estrogen of breast cancer cell proliferation and survival (6). In studies using other ER ligands, such as SERMs, we and others have found that tamoxifen acts primarily to antagonize actions of estrogen but can also have an estrogen-like agonist effect on several genes known to be regulated by estradiol (E₂) in breast cancer cells (8, 9). The ability of tamoxifen to have these mixed agonist/antagonist effects is consistent with the mounting evidence that different ligand-receptor complexes are capable of regulating gene expression through differential coregulator recruitment in a gene-specific manner (10, 11). In addition, because ER β is present in ~70% of ER-positive breast cancers (12), it is important to understand the activity of tamoxifen in the presence of ER α plus ER β .

Using gene expression analysis, we have now identified a third aspect of tamoxifen action: its ability to uniquely regulate a set of genes, which are minimally affected by E₂ or other SERMs. This gene regulation by tamoxifen seems to be mediated by ER α in MCF-7 cells, and the introduction of ER β into these ER α -containing cells represses tamoxifen action on a large proportion of these genes. The expression of these tamoxifen preferentially regulated genes was examined in a cohort of women with breast cancer treated with tamoxifen who showed either prolonged benefit or a higher frequency of breast cancer relapse. In fact, the elevated expression of two of these tamoxifen-stimulated genes in breast cancers was found to correlate with disease recurrence following tamoxifen treatment. This preferential regulation of genes by tamoxifen represents a novel dimension in SERM action in breast cancer with potential therapeutic and diagnostic implications.

Materials and Methods

Cell culture, RNA extraction, and real-time PCR. Methods for the culture and treatment of MCF-7 cells, extraction of RNA, and real-time PCR have been described previously (6, 8).

Adenoviral delivery of ER β into MCF-7 cells. Recombinant adenoviruses were constructed and prepared as described (13). MCF-7 cells were

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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infected with either recombinant adenovirus containing full-length (530 amino acid) ER β or control adenovirus containing no insert for 48 hours before ligand treatment. A multiplicity of infection of 10 was used, which results in an ~1:1 ER α to ER β protein ratio as determined from Western blots of whole-cell extracts compared with standard curves generated with known amounts of recombinant ER α and ER β protein from PanVera (Madison, WI).

GeneChip microarray analysis. Affymetrix human GeneChip U133A microarrays (Affymetrix, Santa Clara, CA) were used for expression profiling following infection of MCF-7 cells with adenovirus (empty virus) or AdER β and treatment with 10^{-8} mol/L E $_2$ or 10^{-8} mol/L *trans*-hydroxytamoxifen (Tam) for 4 or 24 hours. cRNA preparation and hybridization have been described previously (6). The array data were preprocessed using the GeneChip Robust Multichip Average package in R/Bioconductor (14).⁶ A false discovery rate of <0.05, as determined by Significance Analysis of Microarray software (15), was used to identify genes significantly regulated by ligand treatment. Expression data were then loaded into GeneSpring software and normalized to the control (adenovirus-infected, vehicle-treated) samples. Genes preferentially regulated by Tam treatment were identified using a fold change cutoff of ≥ 2.0 for Tam and ≤ 1.3 for E $_2$ treatment, and for genes preferentially down-regulated by Tam a change in gene expression from the control level of 1.0 to a level of ≤ 0.5 -fold for Tam and ≥ 0.77 for E $_2$, based on our prior findings (8). The modulation of Tam action by ER β was determined by a 1.5-fold repression or enhancement of Tam action when compared with Tam action in the presence of no ER β (empty adenovirus) because this gave robust and significant differences that were reproducibly validated by quantitative reverse transcription-PCR. Microarray data are based on triplicate arrays and three independent samples for each treatment. The entire data set will be available through National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO)⁷ and accessible through GEO Series accession number GSE4025.

Whole-cell extract preparation and Western blotting. Whole-cell extracts were prepared using radioimmunoprecipitation assay buffer (1 \times PBS, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 10^{-6} mol/L sodium orthovanadate, 10 μ g/mL phenylmethylsulfonyl fluoride, 30 μ L/mL aprotinin). Protein (25-100 μ g) was separated on 10% SDS-PAGE gels and transferred to nitrocellulose membranes. Western blotting was done using antibodies against IEX-1 (Santa Cruz Biotechnology, Santa Cruz, CA), β -actin (Sigma, St. Louis, MO), ER α (HC20, Santa Cruz Biotechnology), or ER β (16). Quantitation of ER α and ER β protein levels in MCF-7 cells before and after adenoviral ER β gene delivery was determined from standard curves we prepared using different known amounts of recombinant ER α or ER β protein from PanVera and ImageQuant TL quantitation software (Amersham Biosciences, Piscataway, NJ).

Tumor specimens and survival analysis. The original patient material consisted of freshly frozen primary breast tumors from a population-based cohort of 315 women representing 65% of all breast cancers resected in Uppsala County from January 1, 1987 to December 31, 1989 (17, 18). After exclusions based on RNA integrity and array quality control, expression profiles of 254 tumors were deemed suitable for further analysis. Clinicopathologic characteristics were derived from the patient records and from routine clinical measurements at the time of diagnosis as described elsewhere (17). Microarray analysis of patient samples was carried out on Affymetrix U133 A and B arrays, and processing of the expression data was described previously (19). We analyzed the 69 ER-positive tumors from patients who subsequently underwent endocrine therapy with tamoxifen only to assess the role of tamoxifen-regulated genes in breast tumor biology. All ER-negative tumors ($n = 37$) and associated expression and survival data were included as controls.

Association of tamoxifen-regulated genes with patient survival and disease recurrence was determined in several ways. First, patients were sorted by disease recurrence within 10 years following tamoxifen treatment and then the expression profiles of the tamoxifen-regulated genes were assessed for

significant differential expression between the recurring and nonrecurring patients by the Wilcoxon rank sum test. Second, genes that showed significant differences ($P < 0.05$) between the two outcome groups were used to group patients using average linkage hierarchical clustering. Kaplan-Meier estimates were used to compute survival curves, and the significance of the hazard ratios between the major patient clusters was determined by the P of the likelihood ratio tests as was described previously (19). We also did Monte Carlo simulations similar to what was done previously (19) to assess the statistical validity of the two tamoxifen up-regulated genes that were found to correlate with poor outcome on tamoxifen.

Results

Tamoxifen preferential regulation of gene expression. In a previous gene expression profiling study, we compared the agonistic and antagonistic activities of several SERMs on estrogen-regulated genes (8). In the current study, we have focused on the identification and characterization of genes that are preferentially regulated by Tam but not by E $_2$ or other SERMs, such as ICI 182,780 (ICI) or raloxifene. In MCF-7 cells treated with 10 nmol/L E $_2$ or Tam for 4 or 24 hours, we have identified a total of 50 genes up-regulated by Tam and 14 genes down-regulated by Tam, with these genes being affected little if at all by treatment with E $_2$ (Fig. 1; Supplementary Table S1). GeneChip array expression data for this set of genes after 24 hours of treatment with E $_2$ or Tam are shown in Fig. 1.

Six of these genes found to be robustly up-regulated by Tam based on microarray analyses were chosen for examination in more detail. The Tam-selective stimulation of expression of these genes (*FAR1*, *IEX-1*, *KRT13*, *PTPRG*, *Rab30*, and *SOCS1*) was verified using real-time PCR. As shown in Fig. 2A, when cells were treated with E $_2$ or the SERMs ICI, raloxifene, or Tam, only Tam elicited strong stimulation. Next, to examine whether Tam regulation of these genes is mediated by the ER, cells were treated with 10^{-8} mol/L Tam in combination with 10^{-6} mol/L (100-fold excess) E $_2$ or the antiestrogen ICI (also known as fulvestrant). For all six genes, both E $_2$ and ICI blocked the stimulatory effect of Tam, indicating that Tam action on these genes is mediated by the ER (Fig. 2B).

To determine whether the effects of Tam represent primary responses, cells were treated with the protein synthesis inhibitor cycloheximide alone or in combination with Tam for 4 hours. All six of the genes examined seemed to be primary responses to Tam because cycloheximide had either no effect or enhanced the effect of Tam on their up-regulation (Fig. 3).

As shown in Fig. 3, IEX-1 up-regulation is superinduced by cycloheximide treatment, which is consistent with the findings that *IEX-1* is an immediate early response gene to many stimuli (20). In addition, because the up-regulation of *IEX-1* in breast cancer cells is associated with an inhibition of breast cancer cell proliferation (21), we decided to further characterize the regulation of this gene. Dose-response and time course experiments were carried out, and the findings are shown in Fig. 4. An increase in IEX-1 mRNA could be detected within 30 minutes, and an increase in IEX-1 protein was detected over 2 to 8 hours of Tam treatment (Fig. 4A and B). Dose-response studies indicate that the maximum response of IEX-1 was observed at 10 nmol/L to 1 μ mol/L Tam (Fig. 4C), whereas raloxifene had no effect across the broad range of doses. We also observed that the RNA synthesis inhibitor actinomycin D caused a rapid down-regulation of IEX-1 mRNA levels (Fig. 4D). The time course for this down-regulation was identical in the presence or absence of Tam,

⁶ <http://www.bioconductor.org>.

⁷ <http://www.ncbi.nlm.nih.gov/geo>.

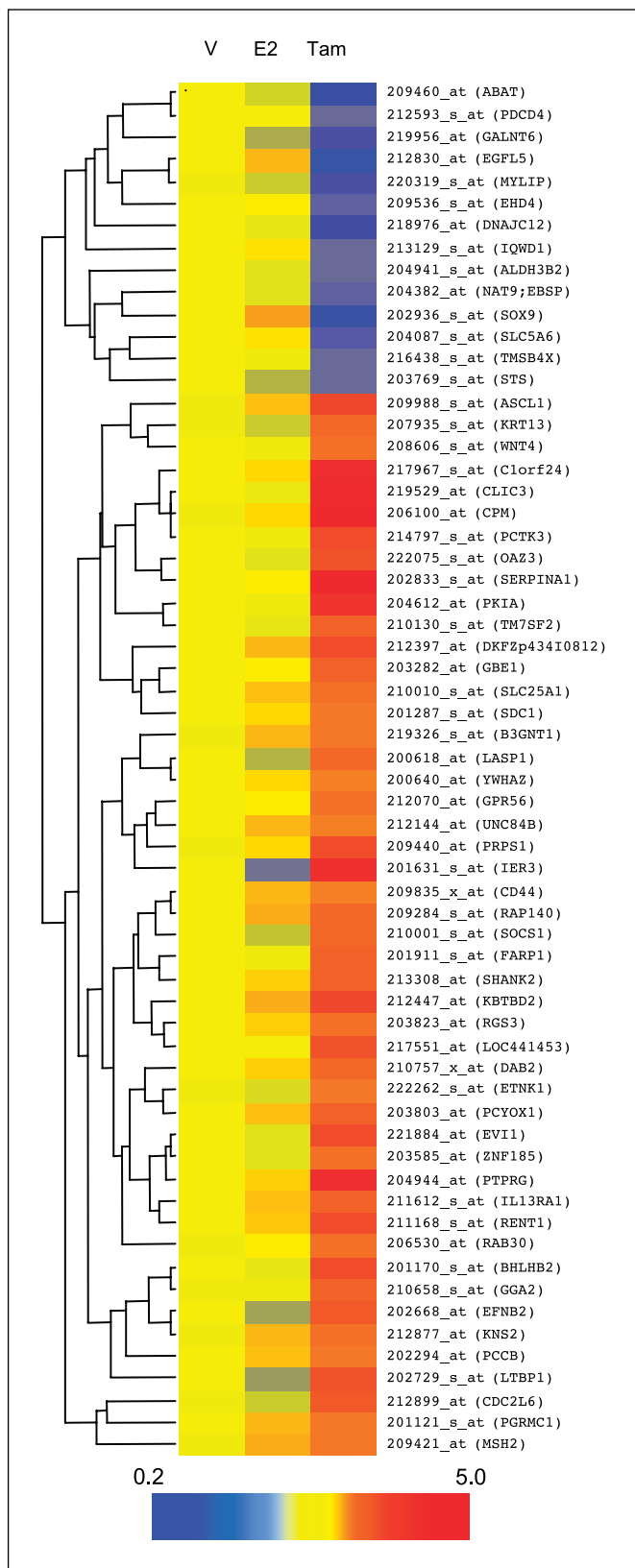


Figure 1. Identification of genes preferentially regulated by tamoxifen. Microarrays were carried out using RNA from MCF-7 cells treated with 10 nmol/L E₂ or Tam for 24 hours. Genes preferentially regulated by Tam were identified (as described in Materials and Methods) and clustered based on their expression profile in response to the two ligands.

indicating that Tam is not acting to regulate IEX-1 mRNA stability and further supporting the suggestion that IEX-1 is a primary transcriptional response to Tam.

Effect of ERβ on tamoxifen regulation of gene expression.

Several lines of evidence indicate that Tam can have different effects through the two ERs, ERα and ERβ (22–24). To examine whether ERβ can influence tamoxifen activity on these preferentially regulated genes in MCF-7 cells, which only express ERα, adenoviral delivery of ERβ was used and additional microarrays were carried out. In cells expressing both ERα and ERβ, we find that the major effect of ERβ was to repress the response of MCF-7 cells to Tam. Specifically, the regulation of 38 of the 50 genes up-regulated by Tam and 7 of the 14 genes down-regulated by Tam was reduced >1.5-fold in MCF-7 cells coexpressing ERβ (Fig. 5A). This inhibitory effect of ERβ on Tam activity was examined and verified for several representative genes (*FARP1*, *IEX-1*, *Rab30*, and *SOCS1*) by real-time PCR (Fig. 5B). Of note, the

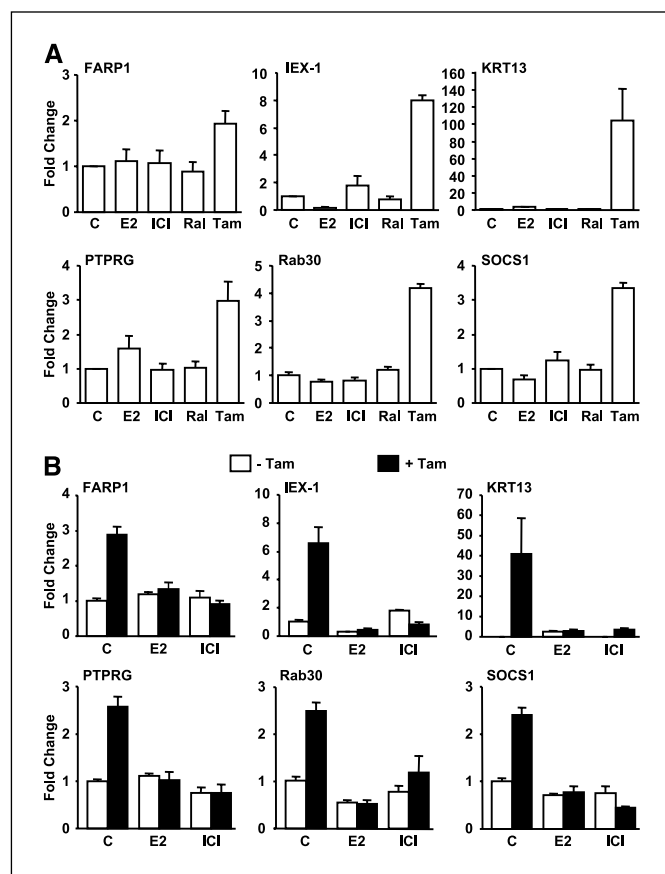


Figure 2. Verification by real-time PCR of the preferential up-regulation of genes by tamoxifen (A) and that this gene regulation by tamoxifen is mediated by the ER (B). A, gene regulation was verified using RNA obtained from MCF-7 cells treated for 24 hours with 0.1% ethanol control vehicle (C), 10⁻⁸ mol/L E₂, 10⁻⁶ mol/L ICI, 10⁻⁶ mol/L raloxifene (Ral), or 10⁻⁶ mol/L Tam. The gene abbreviations used, full gene names, and Genbank accession numbers are as follows: *FARP1* (FERM, RhoGEF ARHGEF and pleckstrin domain protein 1, AI701049), *IEX-1* (immediate early response 3, S81914), *KRT13* (keratin 13, X14640), *PTPRG* (protein tyrosine phosphatase, receptor type G, U46116), *Rab30* (Ras oncogene family member, U57092), and *SOCS1* (suppressor of cytokine signaling 1, AB000734). The primers used for real-time PCR are provided in Supplementary Table S2. Columns, mean of three determinations; bars, SE. B, real-time PCR was carried out on RNA obtained from MCF-7 cells treated for 24 hours with 0.1% ethanol control vehicle, 10⁻⁸ mol/L Tam, 10⁻⁶ mol/L E₂, or 10⁻⁶ mol/L ICI alone or with 10⁻⁸ mol/L Tam in combination with control vehicle or 10⁻⁶ mol/L E₂ or ICI. Columns, mean of three determinations; bars, SE.

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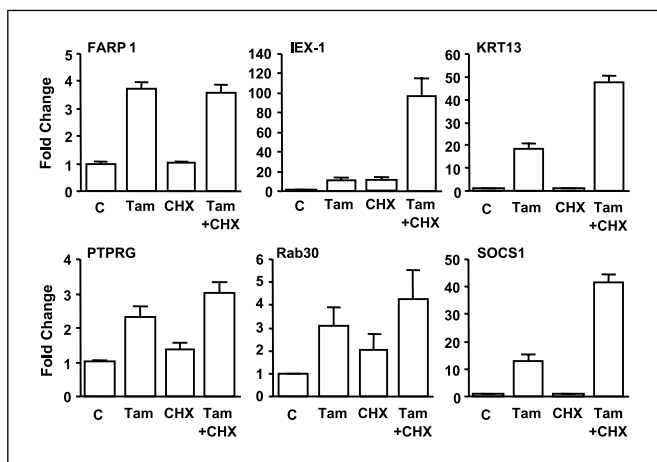


Figure 3. Cycloheximide does not prevent the up-regulation of gene expression by Tam. MCF-7 cells were treated with 0.1% ethanol control vehicle or 10^{-8} mol/L Tam with or without 10 μ g/mL cycloheximide (CHX), a protein synthesis inhibitor, for 4 hours. Real-time PCR was then carried out. These gene stimulations are not blocked by cycloheximide and hence seem to be primary responses to Tam.

presence of ER β in vehicle-treated cells (+ER β , vehicle) elicited changes in expression of some genes similar to that of Tam but often more weakly, consistent with the ligand-independent activity of this receptor form (25). This was seen both by microarray analysis and quantitative PCR (Fig. 5A and B). For KRT13 and PTPRG, the basal level of mRNA expression was enhanced by ER β in the absence of ligand treatment, whereas the stimulation by Tam was not affected (Fig. 5B).

Clinical correlations: expression of tamoxifen preferentially regulated genes in breast cancer patients and association with probability of disease recurrence on tamoxifen. To evaluate the possible prognostic value of these tamoxifen-regulated genes, we examined the expression of these genes in a cohort of women with ER-positive breast tumors ($n = 69$) who had been treated with tamoxifen and followed over time. The expression profiles of the 64 Tam-regulated genes were analyzed in tumor samples from the 69 ER-positive patients treated with tamoxifen following surgery. As a group, there was no significant association of the 64 genes with response to Tam as measured by disease recurrence (i.e., disease-free survival). However, when the 64 genes were ranked (Wilcoxon rank sum) based on their individual association with disease recurrence, 2 genes showed significant association ($P < 0.05$). These 2 genes were *YWHAZ/14-3-3z* ($P = 0.0199$) and *LOC441453* ($P = 0.0327$). When the patient samples were clustered using this two-gene cassette, patients clustered into good and poor outcome groups ($P = 0.000621$), with the higher expression of the two genes associated with a higher probability of recurrence (Fig. 6). Three other tamoxifen selectively regulated genes (*RAP140*, *MYLIP*, and *GPR56*) showed some borderline significance ($P > 0.05 < 0.07$) with outcome to tamoxifen. By Monte Carlo simulations, we estimated the statistical likelihood of finding two random gene probes whose hierarchical clustering could produce a Kaplan-Meier curve likelihood ratio $P \leq 0.000621$. The Monte Carlo P obtained was 0.015 (based on $\sim 100,000$ iterations of Monte Carlo simulation), indicating that it is unlikely that the two tamoxifen-responsive, survival-associated genes (*YWHAZ* and *LOC441453*) were selected by chance.

Consistent with the hypothesis of ER regulation, *YWHAZ* and *LOC441453* were associated with disease recurrence only in ER-positive patients on tamoxifen; no correlation was observed in the 37 women with ER-negative breast cancers. For the ER-negative patients, P s of the "high" and "low" expression groups for disease-specific survival and disease-free survival were at $P = 0.213$ and 0.328 , respectively. A list of all Tam-regulated genes and the Wilcoxon rank sum P s for their association with disease recurrence are presented in Supplementary Table S3.

Discussion

Tamoxifen has been and is still for many patients a cornerstone in the management of hormone-responsive breast cancer. In the adjuvant setting, the recurrence rate is reduced by $\sim 50\%$ (26) and the mechanism of its effectiveness is primarily thought to be due to its ability to antagonize estrogen action through interaction with the ER (1, 4, 8, 11). However, tamoxifen often acts as a partial agonist/antagonist or a full agonist, depending on the gene as well as the cellular background (8–12). Eventually, treatment with tamoxifen can lead to resistance where breast cancer cells no longer see tamoxifen as an antagonist and it may actually stimulate breast cancer cell proliferation (1, 27, 28). Our studies show that, in addition to the antagonistic, partial agonist/antagonistic, or even full agonist activities of Tam on genes regulated by E $_2$, Tam is also capable of regulating the expression of some genes that are not regulated by either E $_2$ or other SERMs. This regulation, like that of the other categories of tamoxifen-modulated gene expression, is mediated via the ER. Although these genes must be viewed within the context of all the genes, on which Tam exerts agonist or antagonistic actions, we investigated whether some of these genes preferentially regulated by Tam might serve as markers of Tam responsiveness or the development of resistance in breast cancer.

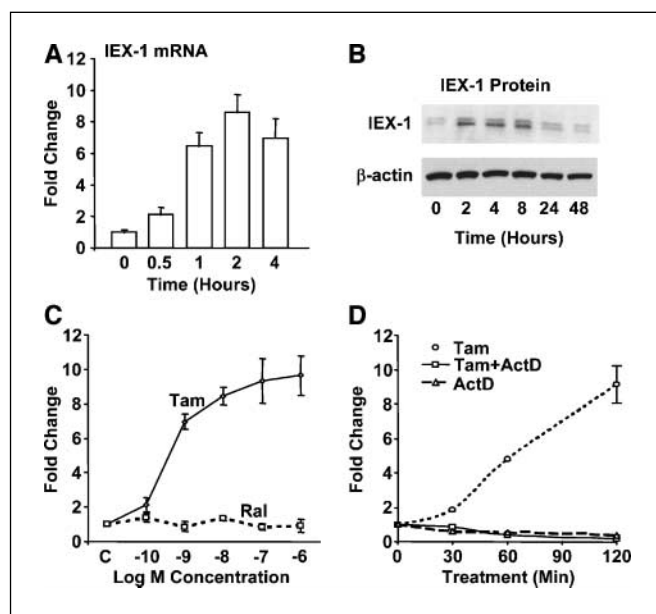


Figure 4. Time- and dose-dependent up-regulation of IEX-1 mRNA and protein levels by Tam. MCF-7 cells were treated with 10^{-8} mol/L Tam for different times (A and B), with different doses of Tam or raloxifene for 4 hours (C), or with 10^{-8} mol/L Tam for up to 120 minutes in the presence or absence of the RNA synthesis inhibitor actinomycin D (*ActD*; D). RNA extraction and PCR analysis (A and C–D) or Western blot (B) was then carried out for IEX-1. β -Actin was also examined by Western blot as a loading control.

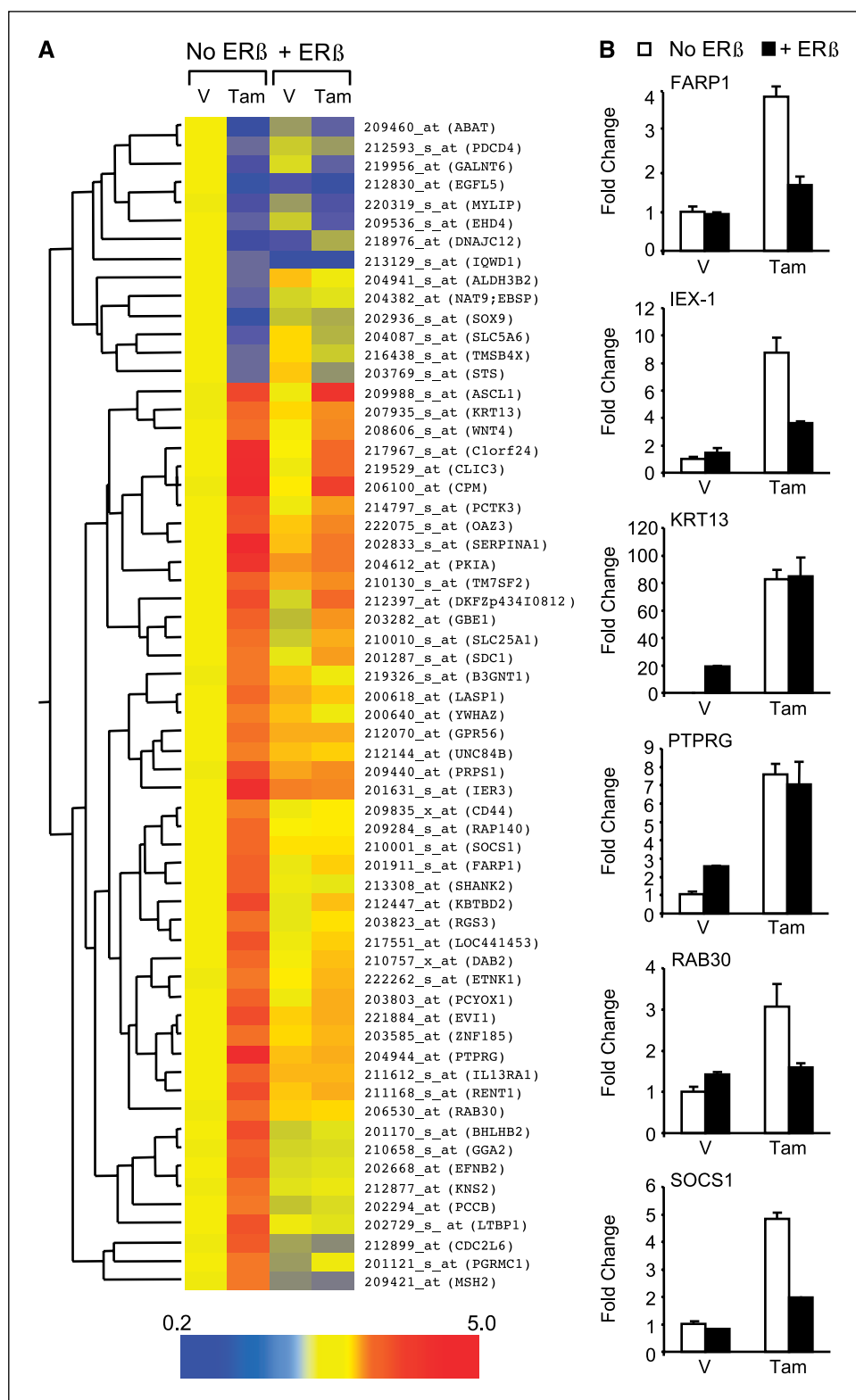


Figure 5. ERβ modulates tamoxifen activity on genes preferentially regulated by tamoxifen. Microarrays were carried out using RNA from MCF-7 cells infected with a control empty adenovirus (*no ERβ*) or with adenovirus expressing ERβ (*+ERβ*) for 48 hours and then treated with 10 nmol/L Tam for 24 hours. **A**, genes preferentially regulated by Tam were clustered based on their expression profile in response to tamoxifen in ERβ-negative or ERβ-positive (*+ERβ*) MCF-7 cells. **B**, verification of ERβ modulation of tamoxifen activity on gene expression. PCR analysis was carried out for six genes preferentially regulated by Tam. The presence of ERβ inhibited responsiveness to Tam for some genes (*FARP1*, *IEX-1*, *Rab30*, and *SOCS1*) but not for other genes (*KRT13* and *PTPRG*). For *KRT13* and *PTPRG*, ERβ altered basal gene expression in the absence of Tam.

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Our data show that Tam action, in preferentially regulating this set of genes, is mediated by the ER because both E₂ and ICI block Tam action. Several lines of evidence, including X-ray crystallography, ER mutagenesis, and peptide mapping, have all indicated that different ER ligands are capable of inducing different ER structures (11, 29–31). In addition, Tam is known to require

different regions of the ER for enhanced transcriptional activity than does E₂ (32) and to differentially recruit coregulatory proteins to estrogen-responsive genes (33). That these genes are stimulated very preferentially by Tam suggests that they might be emblematic of pharmacologic differences between Tam and the other SERMs, raloxifene and ICI.

The fact that ER β could reverse the regulation of ~75% of these genes by Tam, including reversal of the Tam stimulation of *YWHAZ/14-3-3z* and *LOC441453*, is of note because human breast tumors often express ER β along with ER α , and these women are commonly treated with Tam (12). However, the mechanism by which ER β modulates Tam gene activity is not clear. In response to E₂ or other ER ligands, ER α and ER β can have both common and distinctly different gene regulatory activity (24, 34, 35). In addition, heterodimers of ER α /ER β may have distinct activity from either ER α or ER β homodimers (24, 36). For these Tam-regulated genes, it is possible that the activation function-1 (AF-1) domain of ER α , which is essential for Tam agonist activity, may be repressed by the strong AF-1 activity of ER β (36). Other evidence suggests that different surfaces of ER α and ER β may be exposed on Tam binding, which may lead to the recruitment of different coactivator complexes to each receptor in the presence of Tam compared with E₂ (37). The relationship between ER β and response to endocrine therapy still remains unclear and under active investigation (38).

The genes preferentially regulated by Tam encode proteins that have diverse roles in multiple functional pathways and gene networks. For example, *PKIA*, an inhibitor of cyclic AMP-dependent protein kinase A activity, *PTPRG*, a receptor-type protein tyrosine phosphatase, and *SOC1*, an inhibitor of Janus-activated kinase (JAK)/signal transducers and activators of transcription (STAT) signaling, all could potentially alter different cellular signaling pathways and, thus, responsiveness of breast cancer cells to other hormones, growth factors, or cytokines. In addition, IEX-1 has been shown to have growth-inhibitory effects on MCF-7 cells (20, 21), suggestive of a beneficial effect of Tam. In addition, several other Tam preferentially regulated genes might have potential tumor suppressor roles. *SOC1*, a negative regulator of cytokine action through the JAK/STAT pathway, has been proposed to suppress growth of hepatocellular carcinomas (39). Similarly, *PTPRG* was identified as a potential tumor suppressor because it is located in a region rearranged in some cancers and deleted in some breast cancers (40).

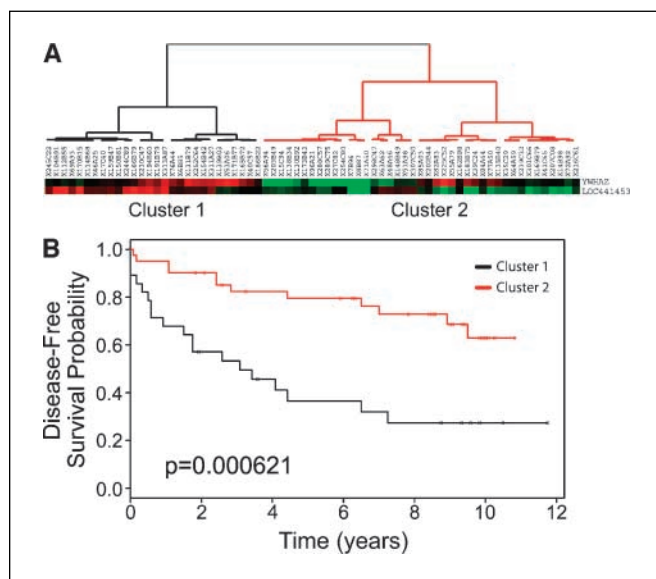


Figure 6. Correlation of tamoxifen-regulated genes with patient outcome. *A*, hierarchical clustering of 69 ER-positive tumor samples from patients treated with tamoxifen into two groups of high (*black dendrogram*; cluster 1) and low (*red dendrogram*; cluster 2) *YWHAZ* and *LOC441453* expression clusters. *B*, Kaplan-Meier plots of disease recurrence for clusters 1 (*black*) and 2 (*red*) patients with the associated *P* from likelihood ratio analysis.

The two genes whose expression we found to correlate with decreased disease-free survival, *YWHAZ* and *LOC441453*, have yet to be implicated in breast cancer progression. Both genes were preferentially up-regulated by tamoxifen in MCF-7 cells as seen in Figs. 1 and 5 and Supplementary Table S1. *YWHAZ*, also known as 14-3-3 ζ , belongs to the highly conserved 14-3-3 family of proteins, which regulate the cell cycle and prevent apoptosis and mediate signal transduction by binding to phosphoserine-containing proteins. *YWHAZ* is a scaffolding protein that is phosphorylated on specific serine residues by protein kinase C and AKT. *YWHAZ* is thought to play a central role in insulin receptor and epidermal growth factor receptor signaling and in cell cycle regulation (41–44). The *YWHAZ* protein binds and stabilizes key signaling proteins, such as IRS1, protein kinase B/AKT1, β -catenin, and RIN1, and activates c-raf, BAD, and cbl (41, 42, 44). High expression of a protein central in cell signaling, such as *YWHAZ*, under the influence of tamoxifen might contribute to the promotion of tumor recurrence. Little is known about *LOC441453*, which is also known as similar to olfactory receptor, family 7, subfamily A, member 17. The protein contains a conserved seven transmembrane receptor motif (NCBI RefSeq, gene ID 441453), but its role in cells is virtually unexplored currently. Further study of the biological roles of both proteins would be of interest.

It is known that some ER-positive breast cancers, even if small and from lymph node-negative patients, can do poorly on tamoxifen. High expression of *YWHAZ/14-3-3z* and *LOC441453* might indicate a more aggressive cancer. *YWHAZ/14-3-3z* is a critical player in numerous cell signaling pathways, so elevated expression is likely to be associated with a more aggressive phenotype. In fact, Oncomine database analysis⁸ of two published clinical studies (45, 46) confirms that high *YWHAZ/14-3-3z* expression is associated with poor outcome on endocrine therapy; there is no information on *LOC441453* available. Therefore, on tamoxifen treatment, which would further increase expression of these two genes, one might predict a poor outcome on tamoxifen. It is possible that women with such breast cancers might do better with aromatase inhibitor therapy to avoid tamoxifen stimulation of such genes. Further work to study the predictive value of these genes would require a separate neoadjuvant therapy study, where tumor biopsies would be obtained before and shortly after the initiation of tamoxifen treatment to measure the effect of tamoxifen on expression of these genes.

In conclusion, we have identified several genes preferentially regulated by Tam in ER-positive breast cancer cells, suggesting a novel dimension in the action of this SERM. The correlation of high expression of some of these genes with disease recurrence on tamoxifen indicates that these genes are likely to be useful in predicting the effectiveness of tamoxifen in treating ER-positive breast cancer and assessing the development of tamoxifen resistance. Such approaches are already proving useful in the prediction of risk of recurrence following tamoxifen treatment (47, 48) and in the molecular classification of tamoxifen-resistant breast cancers (49). However, it is clear that additional genes, such as those observed in this report to be preferentially regulated by tamoxifen and to correlate with a shorter disease-free survival and time to recurrence, might serve as valuable prognostic markers to improve the accuracy of tamoxifen resistance and recurrence prediction that might potentially affect the type of endocrine therapy selected. Aromatase inhibitors are increasingly being used in the adjuvant setting with good results (50). As with tamoxifen, however, there are also side effects of concern with

⁸ <http://www.oncomine.org>.

aromatase inhibitors (i.e., a higher incidence of bone fractures and joint pain; ref. 50). These issues and the findings presented in this article should stimulate researchers to use endocrine therapies in a more tailored way based on a more detailed understanding of the mechanisms of action for tamoxifen, which therefore may still be the drug of choice for many breast cancer patients. Potentially, they might also assist in the development of new SERMs with an optimized complement of positive effects in breast cancer.

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