Interplay between AP-1 and estrogen receptor α in regulating gene expression and proliferation networks in breast cancer cells

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Estrogen receptor α (ERα) is a ligand-dependent transcription factor that plays an important role in breast cancer. Estrogen-dependent gene regulation by ERα can be mediated by interaction with other DNA-binding proteins, such as activator protein-1 (AP-1). The nature of such interactions in mediating the estrogen response in breast cancer cells remains unclear. Here we show that knockdown of c-Fos, a component of the transcription factor AP-1, attenuates the expression of 37% of all estrogen-regulated genes, suggesting that c-Fos is a fundamental factor for ERα-mediated transcription. Additionally, knockdown of c-Fos affected the expression of a number of genes that were not regulated by estrogen. Pathway analysis reveals that silencing of c-Fos downregulates an E2F1-dependent proproliferative gene network. Thus, modulation of the E2F1 pathway by c-Fos represents a novel mechanism by which c-Fos enhances breast cancer cell proliferation. Furthermore, we show that c-Fos and ERα can cooperate in regulating E2F1 gene expression by binding to regulatory elements in the E2F1 promoter. To start to dissect the molecular details of the cross talk between AP-1 and estrogen signaling, we identify a novel ERα/AP-1 target, PKIB (cAMP-dependent protein kinase inhibitor-β), which is overexpressed in ERα-positive breast cancer tissues. Knockdown of PKIB results in robust growth suppression of breast cancer cells. Collectively, our findings support c-Fos as a critical factor that governs estrogen-dependent gene expression and breast cancer proliferation programs.

Introduction

The estrogen receptors (ERs) play key roles in the growth and maintenance of the normal mammary gland and the onset and progression of breast cancer. ERs, including ERα and ERβ, are ligand-activated transcription factors belonging to the nuclear receptor superfamily (1). ERs regulate transcription through direct and indirect interaction with DNA. The direct mode involves estrogen-activated ER binding to estrogen-responsive elements in cis-regulatory genome sequences (2). In the indirect pathways, liganded ERs are tethered to DNA by interacting with other transcription factors, for example, specificity protein 1 and AP-1 (activator protein-1) (3,4). However, the molecular details of the latter interactions and how these relate to transcriptional activities in breast cancer cells remain unclear. Addressing these issues is critical to fully elucidate the mechanism of estrogen-stimulated breast cancer growth.

The AP-1 transcription factor is an important component of multiple signal transduction pathways. The AP-1 complex is composed of homodimers of Jun family members or heterodimers of Jun and Fos family members. Upon induction by a variety of extracellular stimuli, the AP-1 proteins bind to their cognate DNA sequences, known as 12-O-tetradecanoylphorbol-13-acetate (TPA) response elements, in the regulatory regions of target genes and activate transcription (5). It has been shown that Fos/Jun heterodimers are more stable and efficient in driving transcriptional activation than Jun/Jun homodimers (6). A limited number of genes containing AP-1 sites in their promoters have been shown to be regulated by ERα via an AP-1 site, such as the genes encoding collagenease (7), human insulin-like growth factor 1 (8) and the human choline acetyltransferase gene (9). However, whether ERα or AP-1 is recruited to the regulatory regions of these target genes and the potential molecular interplay between these signaling pathways at the level of chromatin is unclear.

AP-1 plays a critical role in regulating breast cancer cell proliferation (10,11). The importance of the ERα/AP-1 cross talk in breast cancer etiology and progression has been demonstrated in many studies. For example, in the ERα-positive breast cancer cell line MCF-7, upregulated AP-1 activity has been associated with tamoxifen resistance and increased invasiveness (12). Clinically, tamoxifen resistance in ERα-positive breast tumors has been associated with upregulated AP-1 activity (13,14). Together, these findings suggest that enhanced AP-1 activity may act to bypass the hormone dependence in tamoxifen-resistant breast tumors.

In breast cancer cells, ERα promotes proliferation by regulating the expression of key cell cycle regulatory genes, such as E2F1 (15). The E2F1 transcription factor is well known for its involvement in the cyclin/cyclin-dependent kinase/retninoblastoma pathway and for controlling the expression of target genes involved in cell proliferation, differentiation and apoptosis (16). Transcriptional activation of E2F1, and subsequently its downstream target genes, has been shown to be critical for estrogen regulation of proliferation in MCF-7 cells (17). Furthermore, increased E2F1 protein levels have been associated with increased breast cancer grade and reduced disease-free and overall survival (18).

In recent years, bioinformatics analysis of the ERα cistrome has provided evidence that AP-1 cis-regulatory motifs are enriched within the regions bound by ERα in breast cancer cells (19,20), suggesting a global cross talk between ERα and AP-1 at the chromatin level. In this report, we have addressed the role of AP-1 in the gene expression programs including those controlled by estrogen-activated ERα.

Materials and methods

RNA extraction

For the microarray analysis, the MCF-7 cells were transfected with control small interfering RNA (siRNA) or with the pool of siRNAs targeting c-Fos for 72 h and then treated with vehicle or 17β-estradiol (E2) for 24 h. Total RNA was extracted using RNeasy Mini Kit (Qiagen, Valencia, CA).

Microarray analysis

The Agilent SurePrint G3 Human GE 8x60K (GEO Platform GPL14550) with 42 405 transcripts, including 29 271 annotated genes, was used for global gene expression profiling analysis. RNA samples from three replicates for each treatment were subjected to the analysis. Target synthesis and hybridizations were performed by the Bioinformatic and Expression Analysis core facility (BEA, www.bea.ki.se, Novum, Karolinska Institutet, Huddinge, Sweden) according to standard protocols. The image analysis was performed using Agilent Feature Extraction Software. Raw data were imported to Partek Genomics Suite (www.partek.com/parteks), and subjects were subjected to quantile normalization. Two-tailed unpaired Student’s t-tests were used to compare sample groups, and q-values were estimated using the qvalue package in R. We applied a filter of P < 0.05 for significantly modulated gene expression and at least a 2.0-fold change in mean differential expression. The expression microarray data have been deposited in the GEO database under accession number GSE36586.
Bioinformatic analyses

For analyses of pathways and overrepresented gene sets Pathway Studio 9.0 (Ariadne Genomics) was used. Overrepresentation analyses were carried out using Fisher’s exact test using the software’s proprietary gene ontology (GO) gene sets as well as the transcription factor target gene sets from MsigDB v3.0, which basically predicts transcription factor binding by use of TRANSFAC motifs (21). Overrepresentation occurs when the number of genes mapping to either a GO term or transcription factor exceeds what could be expected by random sampling from the genome. Pathway analyses and generation of images were carried out within the software.

RNA interference

A pool of siRNAs targeting c-Fos (si_c-Fos) or PKIB (si_PKIB) and negative control siRNA (si_Ctrl) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). siRNA transfections were carried out using a final concentration of 50 nM oligo (at 40–60% confluence) using the interferin transfection reagent (Polyplus) according to the manufacturer’s recommendations.

Quantitative real-time PCR

Real-time PCR was performed in a 7500 Fast Real-Time PCR (Applied Biosystems, Foster City, CA) with Fast SYBR Green Master mix (Applied Biosystems) according to conditions specified by the manufacturer. The specificity of all primer pairs was checked with melting curve analysis. Primer sequences will be provided on request. For real-time PCR analysis, unpaired two-tailed t-test was used to compare differences between two parallel treatment groups.

Chromatin immunoprecipitation

MCF-7 cells were seeded in 150-mm dishes and grown for 72 h in phenol red-free DMEM supplemented with 5% DCC-FCS serum. Cells were then treated with vehicle or 10 nM E2 for 45 min and chromatin immunoprecipitation (ChIP) was performed as previously described (22). The antibodies used were as follows: anti-ERα antibody HC-20 (Santa Cruz, CA), anti-c-Fos antibody H-125 (Santa Cruz, CA) and normal rabbit IgG (Santa Cruz, CA). The immunoprecipitated DNA was amplified by real-time PCR. Primer sequences used for ChIP-PCR are given in Supplementary Table 1, available at Carcinogenesis Online.

Cell proliferation assay

MCF-7 cells were seeded into 24-well plates 24 h before siRNA transfections. At the indicated time points after transfection, cells were washed with phosphate-buffered saline, harvested by trypsinization and then resuspended in growth medium. The number of viable cells was determined using a hemocytometer after staining with trypan blue.

Bromodeoxyuridine-labeling experiments

MCF-7 cells were transfected with control siRNA or a pool of siRNAs targeting PKIB for 72 h. Cells were then pulsed with 10 µM bromodeoxyuridine (BrdU) for 1 h before harvesting. Incorporated BrdU was detected with a mouse anti-BrdU antibody, followed by staining with anti-mouse FITC-conjugated antibody. Finally, cells were resuspended in an appropriate volume of Hoechst 33342 solution (Invitrogen). The number of BrdU-positive cells over total Hoechst 33342 positive cells was counted in an unbiased setup with an inverted microscope (IX70, Olympus, Hamburg, Germany) and ImageJ 1.41 software (http://rsbweb.nih.gov).

Results

We have previously shown that silencing of c-Fos in MCF-7 breast cancer cells caused a reduction in E2-induced cell proliferation (23), in agreement with other studies showing a role for c-Fos in the proliferation of MCF-7 cells (11). The present study focuses on the molecular details of the effects of c-Fos on proliferation in breast cancer cells by assaying genome-wide alterations in gene expression upon c-Fos knockdown in MCF-7 cells. Using gene expression profiling and pathway network analyses, we assess the global impact of c-Fos on the transcriptome with focus on the E2/ERα-regulated transcriptome and identify regulated pathways.

c-Fos knockdown exerts extensive genome-wide transcriptional regulation

We have recently shown that siRNA against c-Fos efficiently eliminates the target mRNA and protein in MCF-7 cells (23). To address the role of c-Fos in estrogen-regulated gene expression, MCF-7 cells were transfected with a control siRNA or with the pool of siRNAs targeting c-Fos for 72 h and then treated with vehicle or E2 for 24 h, whereupon global gene expression profiles were assessed.

We identified 1069 genes that were regulated at least 2-fold by E2 treatment in control siRNA-transfected cells, which agrees with previous findings (24). Knockdown of c-Fos by siRNA affected the expression of 37% of all E2-regulated genes. Specifically, c-Fos knockdown attenuated E2-regulated gene expression for 25% of E2-induced genes and for 47% of E2-repressed genes, as illustrated in Figure 1. In comparison, 5% of the human transcriptome was affected by knockdown of c-Fos alone (Figure 1), with 874 and 656 genes being up- or downregulated by c-Fos knockdown, respectively. Real-time PCR analysis confirmed the microarray data for genes selected from the different groups (Supplementary Figure 1, available at Carcinogenesis Online). Our results demonstrate that ablation of c-Fos results in a substantial decrease of the E2/ERα mediated effect on gene expression and provide strong functional evidence for a role of c-Fos in transcriptional pathways governed by ERα.

We evaluated enrichment, as annotated by GO, of specific gene functions for the groups of genes whose estrogen regulation was impacted by c-Fos knockdown. Table I shows that E2-induced genes that were affected by c-Fos contained a significant overrepresentation of genes involved in the G1-S transition of the mitotic cell cycle, cell proliferation, regulation of the cell cycle and DNA replication. Among the E2-repressed genes that were affected by c-Fos, we noted an enrichment of genes involved in negative regulation of cell proliferation, cell–cell signaling and induction of apoptosis. These findings are consistent with c-Fos contributing to the mitogenic effect of E2 in ERα-positive breast cancer cells. A detailed list of overrepresented gene sets is shown in Table I.

Table I. Overrepresented GO biological processes

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<th>Gene list</th>
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<th>P-value</th>
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<tr>
<td>E2-repressed genes affected by c-Fos knockdown</td>
<td>Negative regulation of cell proliferation</td>
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<td>Genes induced by c-Fos siRNA</td>
<td>Multicellular organismal development</td>
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<td></td>
<td>DNA replication</td>
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<td>Mitosis</td>
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<tr>
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Silencing of c-Fos downregulates an E2F1-dependent proproliferative gene network

To assess the influence of c-Fos on estrogen-induced proliferation gene networks, we performed an in silico analysis of the promoter regions of the differentially expressed genes. We assessed whether predicted transcription factor binding sites (TRANSFAC motifs) were overrepresented ±2kb around the transcription start site in the set of regulated genes compared with their genome-wide frequency (Supplementary Table 2, available at Carcinogenesis Online). The results showed that E2F1 motifs were overrepresented in regulatory regions of E2-induced genes that were attenuated by c-Fos silencing (P = 5 × 10^-4), strongly implicating E2F1 in their regulation. Furthermore, E2F1 motifs were also overrepresented in regulatory regions of genes that were c-Fos induced (downregulated upon silencing of c-Fos) independent of E2 (P = 1 × 10^-11).

Using literature data of E2F1 downstream genes with proven participation in the cell cycle, apoptosis, proliferation and differentiation, we found that these represented a significantly enriched subnetwork within the set of E2-regulated genes affected by c-Fos silencing (P = 4.6 × 10^-6). This network is illustrated in Figure 2A (top panel), and proposes that c-Fos and ERα coregulate E2F1 expression. E2F1-regulated genes were overrepresented also among genes regulated by c-Fos silencing in the absence of E2 (P = 1.7 × 10^-13). This gene network is illustrated in Figure 2A (lower panel). We hypothesize that E2F1 itself is regulated by c-Fos and, thereby, affects the regulation of numerous downstream genes. To further understand this cascade, we asserted the proportion of the E2F1-regulated genes that are proven direct targets of E2F1. Incorporation of the E2F1 cistrome defined in MCF7 cells (25) revealed that 68% of the E2F1 downstream genes are confirmed direct targets, as indicated in Figure 2A. E2F1 is a known ERα-regulated gene, but further comparison with the ERα cistrome data in MCF7 cells (19) showed that 21% of the E2F1-regulated genes have also adjacent ERα binding sites (Figure 2A). The genes that contain both E2F1 and ERα binding sites might be potential targets of both E2F1 and ERα, and represent an additional potential level of modulation of the E2F1 pathway by ERα. Genes regulated by estrogen that contain only E2F1 but no ERα binding sites, may be secondary targets of ERα. We propose that modulation of the E2F1 pathway by c-Fos occurs and represents a novel mechanism by which c-Fos enhances breast cancer cell proliferation.

c-Fos directly mediates the E2-induced activation of the E2F1 promoter

E2F1, a master regulator of cell cycle progression and, subsequently, its downstream target genes are critical for hormone regulation of the proliferative program of ERα-positive breast cancer cells. Our microarray results indicate that c-Fos silencing can attenuate E2-induced E2F1 expression, and even have a repressive effect on E2F1 expression in the absence of E2 treatment. These effects were further confirmed by real-time PCR, demonstrating that estrogen stimulation induced an increase in E2F1 mRNA after 6 and 24h, that this effect was significantly reduced by c-Fos silencing and that c-Fos alone can regulate E2F1 expression (Figure 2B).

To examine the mechanism by which c-Fos is involved in the E2-mediated control of E2F1 transcription, we assayed the binding of c-Fos to the E2F1 promoter. The E2F1 promoter region contains one predicted consensus half-estrogen-responsive element site (~310bp from transcription start site) and one predicted AP-1 site (~210bp). We observed the recruitment of ERα and c-Fos to the E2F1 promoter, a recruitment that was E2-dependent. Neither ERα nor c-Fos was recruited to the E2F1 exon 3, which serves as a negative control (Figure 2C). Collectively, these results provide a direct link of AP-1 to cell cycle regulation through the transcriptional activation of E2F1 and E2F1 target genes.

RNAi depletion of PKIB, a direct target of ERα and AP-1, suppresses breast cancer cell growth

To try to dissect the molecular details of the estrogen-AP-1 cross talk in breast cancer proliferation we aimed to further characterize a novel target that (i) was subject to cross talk between E2 and AP-1 signaling, (ii) has binding sites for both ERα and AP-1 in potentially regulatory regions and (iii) is known from the literature to be involved in cellular proliferation. PKIB is in the present study identified as an E2-induced gene with the regulation affected by c-Fos. Our group has recently pursued genome-wide identification of AP-1 binding regions in MCF-7 cells by the ChIP-on-chip assay (unpublished data). Comparison with the genome-wide ERα-binding sites (20) revealed an ERα-AP-1 shared binding region located in intron 1 of the PKIB gene (ENST00000258014). Moreover, the proliferative role of PKIB has been demonstrated in prostate cancer cells. We therefore focused on the PKIB gene to address the role of cooperativity between ERα and AP-1 in gene regulation.

Real-time PCR confirmed that E2 induced PKIB expression, and this effect was significantly reduced by knockdown of c-Fos (Figure 3A). We next examined the effect of short-time treatment with TPA, a potent protein kinase C pathway inducer that activates the
Fig. 2. Silencing of c-Fos regulates E2F1-dependent gene transcription. (A) Putative and confirmed E2F1 target genes regulated upon c-Fos silencing and E2 treatment. Genes above dashed line have confirmed E2F1-binding site and orange figures represent genes with confirmed ERα-binding sites (19,25). Lines with arrow-head (↓) means stimulation of downstream gene transcription, whereas lines with blunt line (⊥) means inhibition of downstream gene transcription. Figure shapes represent functional classes as follows: oval shape—general protein; rhomb—ligand; sickle—kinase; O-vertex—transcription factor; stick-vertex—receptor. (B) Effect of c-Fos silencing on E2F1 expression. Seventy-two hours after siRNA transfection, cells were treated with 10 nmol/l of E2 or vehicle for 6 and 24 h. Columns, mean; bars, SD (n = 3). *P < 0.05 relative to si_control. (C) Recruitment of ERα and c-Fos to the promoter and exon 3 regions of E2F1. Hormone-depleted MCF-7 cells were treated with vehicle or E2 for 45 min and then fixed with formaldehyde. ChIP assays were performed with anti-ERα, anti-c-Fos and normal rabbit IgG antibodies. Data presented are normalized to input DNA and expressed as fold enrichment of ERα or c-Fos relative to IgG. Columns, mean; bars, SD (n = 3). *P < 0.05 compared with IgG controls.
AP-1 transcription factor, and E2 on PKIB expression. MCF-7 cells were treated with TPA or E2 for 3 h. As shown in Figure 3B, both TPA and E2 were found to induce PKIB expression, indicating that PKIB is a direct target of AP-1 and ERα.

We next performed ChIP experiments to confirm the recruitment of ERα and AP-1 to the PKIB intron 1, as revealed by the ChIP-on-chip data. As shown in Figure 3C, both ERα and c-Fos were recruited to this region, and E2 enhanced this recruitment. These results demonstrate that ERα and c-Fos converge on the same regulatory regions potentially to induce PKIB gene expression.

A previous study has shown that PKIB is overexpressed in prostate cancers and promotes prostate cancer cell growth (26). To determine
in positive regulation of cell proliferation were upregulated by E2-positive breast cancer cells. Specifically, 11 genes involved in mitogenesis were found to be significantly upregulated by E2-ERα, whereas 12 genes involved in negative regulation of cell proliferation were downregulated by E2 and dependent on c-Fos. Furthermore, we identified 22 genes involved in negative regulation of cell proliferation that were specifically downregulated by E2-ERα-dependent and -independent manners. Estrogen has previously been shown to regulate the E2F1 pathway (17). In the present study, we show, for the first time, that c-Fos is involved in estrogen induction of E2F1 expression through a mechanism potentially involving ERα and AP-1 recruitment to the E2F1 promoter. We found that E2F1 expression was reduced upon c-Fos depletion, which is in line with the results of Shen et al. (39) where they found that overexpression of a dominant negative form of c-Jun, a partner of c-Fos, led to reduced expression of E2F1. Furthermore, we identified many E2F1 transcriptional targets that were regulated by both an ERα/c-Fos-dependent mechanism and by c-Fos alone. These genes have been shown to participate in regulation of cell cycle, apoptosis, proliferation and differentiation in various studies. Several of the downstream targets of E2F1 were found to have a binding site for ERα (Figure 2), suggesting that ERα is involved not only in the regulation of E2F1 itself but also in the modulation of its downstream function. The proportion of E2F1 target genes that also have a binding site for AP-1 is very low (41). Thus, the molecular mechanisms regulating E2F1 levels could be explored for breast cancer treatment. In patients with breast tumors, low E2F1 levels were found to be associated with favorable outcome (41). Thus, the molecular mechanisms regulating E2F1 levels and/or activity could be explored for breast cancer treatment. In line with this, small molecules that interrupt E2F1 function have been suggested as strategies for breast cancer treatment (42). We hope that a detailed understanding of the ERα/AP-1/E2F1 network in breast cancer cells and how it relates to proliferation will facilitate the identification of approaches to target this network and ultimately inhibit breast cancer proliferation.

**Discussion**

Recent studies mapping ER-binding events all show that AP-1 motifs are enriched within the regions bound by ER (19,20,23), suggesting an extensive interaction network between ER and AP-1 on a genome-wide scale. Such a mechanism of transcriptional modulation is not unique to ER and has been observed for other nuclear receptors. For instance, recent cistrome studies reveal AP-1 as a major partner for glucocorticoid receptor–chromatin interactions in murine mammary epithelial cells (37) and for liver X receptor–chromatin interactions in keratinocytes (38). In this study we explore the functional interaction between AP-1 and ERα at the global level. We show that for 37% of estrogen-regulated genes, the regulation was attenuated by silencing of c-Fos, a component of the AP-1 transcription factor, implying that c-Fos is a major contributor to ERα-mediated gene regulation. However, as no published data on the AP-1 cistrome in MCF7 cells is available, the genome-wide co-occupancy of ER and AP-1 on chromatin remains unknown.

Analysis of GO annotation allows identification of overrepresented families of genes in expression profiles, indicative of significant roles in specific molecular or biological processes. Our results show that the set of E2-regulated genes influenced by c-Fos contained a significant overrepresentation of genes involved in regulation of cell proliferation. Thus, c-Fos may be an important factor in the mitogenic effect of E2 on ERα-positive breast cancer cells. Specifically, 11 genes involved in positive regulation of cell proliferation were upregulated by E2 dependent on c-Fos (E2F1, SKP2, CDC25A, BCL2, AREG, CDC14A, PDZK1, ITGA2), whereas 12 genes involved in negative regulation of cell proliferation were downregulated by E2 and dependent on c-Fos (INHBA, IGFBP3, TGFβ3, TGFβ2, BTG2, CDK2NB2, AXIN2, GRM4, PMF22, SPEG, IL28RA, MXD4). Additionally, for c-Fos induced genes, those within the cell cycle, DNA replication, cell division and regulation of transcription were overrepresented functional groups. These results implicate a critical role of c-Fos in the regulation of cell proliferation. Indeed, the importance of the AP-1 factor in regulating breast cancer cell growth has been demonstrated by us and others (10,11,23).

Our results demonstrate that a subnetwork of E2F1-regulated genes was affected at the gene expression level by c-Fos in both E2-ERα-dependent and -independent manners. Estrogen has previously been shown to regulate the E2F1 pathway (17). In the present study, we show, for the first time, that c-Fos is involved in estrogen induction of E2F1 expression through a mechanism potentially involving ERα and AP-1 recruitment to the E2F1 promoter. We found that E2F1 expression was reduced upon c-Fos depletion, which is in line with the results of Shen et al. (39) where they found that overexpression of a dominant negative form of c-Jun, a partner of c-Fos, led to reduced expression of E2F1. Furthermore, we identified many E2F1 transcriptional targets that were regulated by both an ERα/c-Fos-dependent mechanism and by c-Fos alone. These genes have been shown to participate in regulation of cell cycle, apoptosis, proliferation and differentiation in various studies. Several of the downstream targets of E2F1 were found to have a binding site for ERα (Figure 2), suggesting that ERα is involved not only in the regulation of E2F1 itself but also in the modulation of its downstream function. The proportion of E2F1 target genes that also have a binding site for AP-1 should be a focus of future studies.

The E2F1 transcription factor is well known for being involved in the cyclin/cyclin-dependent kinase/retninoblastoma pathway and for regulating the expression of numerous genes involved in DNA synthesis and cell cycle progression (40). In MCF-7 cells, E2F1 is required for estrogen-stimulated cell proliferation (17). In patients with breast tumors, low E2F1 levels were found to be associated with favorable outcome (41). Thus, the molecular mechanisms regulating E2F1 levels and/or activity could be explored for breast cancer treatment. In line with this, small molecules that interrupt E2F1 function have been suggested as strategies for breast cancer treatment (42). We hope that a detailed understanding of the ERα/AP-1/E2F1 network in breast cancer cells and how it relates to proliferation will facilitate the identification of approaches to target this network and ultimately inhibit breast cancer proliferation.

**Fig. 5.** PKIB is overexpressed in ERα-positive tumors compared with ERα-negative tumors in two independent studies on human breast cancers (30,33). Data and statistics were obtained from www.oncomine.org. Number of samples is indicated in brackets. Whiskers, 10th and 90th percentile; box boundaries, 75th and 25th percentile; line within box, median. Dots above and below the boxes show sample maximum and minimum values.
In this study, we identified PKIB as a novel ERα/AP-1 target molecule. We found that ERαs and c-Fos interact with the same region of the PKIB gene. There are three CAMP-dependent protein kinase A (PKA) inhibitor (PKI) members reported (α, β and γ) so far. PKIA (PKI-α) is a totally specific inhibitor of PKA and its binding to and inhibitory effects on PKA have been extensively characterized (43). On the other hand, the function of PKIB, which shares only a 40% amino acid identity with PKIA, has not been fully characterized. In prostate cancer, it has been reported that PKIB promotes cell proliferation through increasing the kinase activity of PKA-C and Akt phosphorylation (26). Through siRNA-targeted downregulation in combination with a proliferation assay, we have identified a proliferative role for PKIB in breast cancer cells. Our results therefore extend the proliferative role of PKIB previously demonstrated in prostate cancer. The results from the Oncomine database indicated that PKIB expression levels were higher in ERα-positive breast cancer tissues than in ERα-negative breast cancer tissues. These data are compatible with a role of PKIB in the proliferative end point of ERα/AP-1 cross talk in breast cancer cells.

There is abundant evidence that AP-1 plays a critical role in breast cancer. The elucidation of the role of AP-1 in the transcriptional response modulated by ERα furthers our understanding of how critical estrogen-stimulated targets such as E2F1 and PKIB are regulated. Finally, the identification of the critical targets by AP-1 and ERα cooperation provides potential new targets for therapeutic intervention. Future studies will focus on the correlation of AP-1 and ER global DNA binding regions with estrogen and AP-1 gene regulatory networks.

Supplementary material
Supplementary Tables 1–2 and Figure 1 can be found at http://carcin.oxfordjournals.org/

Funding
Swedish Cancer Society (Cancerfonden) (ref. no. 110391); Karolinska Institutet, Center for Biosciences (to K.D.W.).

Acknowledgements
We are grateful to the Bioinformatic and Expression Analysis core facility at the Karolinska Institute (BEA, www.bea.ki.se) for performing the Agilent assays.

Conflict of Interest Statement: None declared.

References
Association of AP-1 and ERα


Received May 03, 2012; revised June 21, 2012; accepted June 28, 2012