Research Resource: Enhanced Genome-Wide Occupancy of Estrogen Receptor α by the Cochaperone p23 in Breast Cancer Cells

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p23 is a chaperone with multiple heat shock protein 90 dependent and independent cellular functions, including stabilizing unliganded steroid receptors and modulating receptor-DNA dynamics. p23 protein is also up-regulated in several cancers, notably breast cancer. We previously demonstrated that higher expression of p23 in the estrogen-dependent breast cancer line MCF-7 (MCF-7+p23) selectively increased estrogen receptor (ER) target gene transcription and ER recruitment to regulatory elements, promoted cell invasion, and predicted a poor prognosis in breast cancer patients. To probe the impact of p23 on ER binding throughout the human genome, we compared ER occupancy in MCF-7+p23 cells relative to MCF-7-control cells by using chromatin immunoprecipitation followed by ultrahigh-throughput DNA sequencing in the absence and presence of 17β-estradiol (E2) treatment. We found that increased expression of p23 resulted in a 230% increase in the number of E2-induced ER-binding sites throughout the genome compared with control cells and also increased ER binding under basal conditions. Motif analysis indicated that ER binds to a similar DNA sequence regardless of p23 status. We also observed that ER tends to bind closer to genes that were induced, rather than repressed by either E2 treatment or p23 overexpression. Interestingly, we also found that the increased invasion of MCF-7+p23 cells was not only p23 dependent but also ER dependent. Thus, a small increase in the expression of p23 amplifies ER-binding genome wide and, in combination with ER, elicits an invasive phenotype. This makes p23 an attractive target for combating tumor cell metastasis in breast cancer patients. (Molecular Endocrinology 26: 194–202, 2012)

NURSA Molecule Pages†: Nuclear Receptors: ER-α; Ligands: 17β-estradiol.

The chaperone p23 is traditionally thought of as impacting regulatory signaling cascades and steroid hormone ligand binding in the cytoplasm (1–3), yet recent studies have alluded to important effects of p23 on protein-DNA dynamics in the nucleus (4–6). However, the impact of p23 on transcription factor binding to DNA on a genome-wide scale has not been investigated.

We previously identified p23 as a modulator of estrogen receptor (ER)α-dependent transcriptional activity (7, 8). Gene expression profiling of MCF-7 cells with a small (<2-fold) increase in p23 protein expression relative to endogenous levels uncovered p23-dependent changes in the mRNA expression of a subset of genes under both basal and 17β-estradiol (E2)-treatment conditions, in-
including genes involved in metastasis and drug resistance (9). In addition, higher p23 expression converted MCF-7 cells from a noninvasive to invasive phenotype in a matrix gel invasion assay, and this phenotype correlated with findings in breast cancer patients where higher p23 expression was associated with increased lymph node metastases, elevated disease recurrence, and higher mortality (8, 9). Thus, p23 elicits changes in gene expression and cellular responses that promote a more aggressive cancer phenotype.

Although ER binding throughout the genome of MCF-7 cells has been examined (10–12), the effect of p23 expression on ER-binding genome wide has not been explored. Using ultrahigh-throughput sequencing of chromatin immunoprecipitated (ChIP) DNA fragments (ChIP-seq), we have determined the ER-bound sites as a function of p23 protein levels under basal conditions and in response to E2. We found that higher p23 expression resulted in a large increase in the number of sites bound by ER genome wide, and the proximity of this enhanced binding was associated more with E2-induced, rather than E2-repressed, genes. Surprisingly, the effect of p23 overexpression in promoting cellular invasion also required ER. Thus, higher p23 expression resulted in greater ER-binding genome wide and facilitates cell invasion that is ER dependent.

**Results**

**Genome-wide binding of ER as a function of p23**

We used ChIP-seq to identify the genomic locations bound by ER upon overexpression of p23 in the MCF-7 breast cancer cell line in response to E2. We isolated DNA from ER immunoprecipitated chromatin samples from MCF-7-control cells and MCF-7+p23 cells treated for 45 min with either 10 nM E2 or ethanol as a control. We sequenced the ChIP-isolated DNA with an Illumina Genome Analyzer IIx and aligned the sequence reads to the human genome by using the ELAND program (Illumina, San Diego, CA). We then used the model-based analysis of ChIP-seq (MACS) algorithm (13) with a P value cutoff of $10^{-10}$ to identify peaks in the aligned sequence data.

In addition to comparing genome-wide ER binding from MCF-7-control and MCF-7+p23 cells upon E2 treatment, we evaluated ER occupancy under low hormone (basal) conditions from two independent biological replicates. Only binding sites determined to be significant in both experiments are reported and represent high confidence ER-binding sites under the conditions of our assay. In MCF-7-control cells, we identified 3123 genomic positions that became occupied by ER upon E2 treatment. Significantly, in MCF-7+p23 cells, there were 9392 genomic sites occupied by ER. Of the sites bound by ER in MCF-7-control cells, 2873 (92%) overlapped with those from the MCF-7+p23 cells, whereas we identified 6519 additional ER-binding sites in MCF-7+p23 cells compared with control cells (Fig. 1A). This represents a 230% increase in the number of ER-binding sites in MCF-7 cells that have higher p23 expression. In addition, we observed 250 sites in the MCF-7-control cells that were not occupied by ER in the MCF-7+p23 cells (Fig. 1A), suggesting that p23 expression not only enhances ER binding to chromatin but also promotes, either directly or indirectly, ER removal from a small number of genomic sites (Supplemental Fig. 1, published on The Endocrine Society’s Journals Online web site at http://mend.endojournals.org).

Up-regulation of p23 also resulted in an increase in the number of regions bound by ER under basal conditions. Although there are no significant ER-binding sites identified in MCF-7-control cells in the absence of E2 treatment, we observed 392 regions bound by ER in MCF-7+p23 cells under basal conditions (Supplemental Fig. 2). There was a 42% overlap between ER binding in MCF-7+p23 in the absence of E2 and MCF-7-control cells in the presence of E2 (Supplemental Fig. 2), suggesting that increased p23, even in the absence of hormone treatment, is promoting ER binding to the same regions occupied upon E2 treatment. Interestingly, over half of the sites bound by ER under basal conditions did not overlap the ER-binding regions in the MCF-7-control cells upon E2 treatment but did significantly overlap (~80%) with the sites bound by ER in the MCF-7+p23 cells (Supplemental Fig. 3). Thus, increased p23 expression modulates ER binding to chromatin in both the absence and presence of E2.

We next wanted to determine whether the additional approximately 6000 ER-binding regions in response to E2 in the MCF-7+p23 cells are new regions bound by ER or correspond to sites weakly bound by ER in MCF-7-control cells that were below the threshold used to “call” ER binding. To test this, we lowered the threshold for ER binding in the MCF-7-control cells from a $P$ value of $10^{-10}$ to a $P$ value of $10^{-5}$. Using this less stringent parameter, we observed 6636 binding sites bound by ER compared with 3123 ER-binding sites obtained with a more stringent cutoff in MCF-7-control cells. Of the 6636 ER-binding sites, 4980 (80%) overlap with the sites bound by ER in MCF-7+p23 cells (Supplemental Fig. 4). Additional ER-binding sites are also observed in MCF-7+p23 cells compared with control cells, suggesting that p23 has multiple modes of regulating ER binding to chromatin. However, overall, these results suggest that a majority of the ER sites occupied in MCF-7+p23 cells are also bound, albeit much more weakly in MCF-7-control cells upon E2 treat-
ment. Consistent with this observation, we plotted the enrichment of ER-binding signal in MCF-7-control vs. MCF-7+p23 cells and observed that p23 overexpression caused an overall increase in ER-binding signal in response to E2 (Fig. 1B, top panel). In fact, more than 99.7% of ER-binding sites in the MCF-7-control cells displayed more ER binding in MCF-7+p23 cells (Fig. 1B, bottom panel). These results indicate that increased p23 expression results in a stronger binding response of ER to chromatin.

We also examined whether ER sites bound upon p23 overexpression would be associated with ER-mediated long-range interactions identified in an assay capable of interrogating three-dimensional chromosomal interactions, termed ChIA-PET (chromatin interaction analysis using paired end tag sequencing), in MCF-7 cells (14). We compared the locations of ER-binding sites from the published ChIA-PET database with the ER sites bound in MCF-7+p23 cells. There appears to be several binding sites involved in long-range interactions that are in common with the ER sites bound upon p23 overexpression (Supplemental Fig. 5), suggesting that p23 is contributing, either directly or indirectly, to DNA chromatin looping.

Next we compared our ER-interaction sites from MCF-7-control [3123 (P < 10^{-10}) and 6636 (P < 10^{-5})] with...
and MCF-7/p23 cells [9392 ($P < 10^{-10}$)] with genome-wide profiles determined in MCF-7 cells using either a ChIP-chip platform from Carroll et al. (15) [3665 ($P < 10^{-5}$)] or a ChIP-seq approach from Welboren et al. (16) [7713 sites ($P < 10^{-5}$)]. As shown in Table 1, when we compared ER-bound sites from our MCF-7-control cell dataset using a $P$ value cutoff of $10^{-10}$, we found a substantial 57% overlap with ChIP-seq targets as well as a 43% overlap with the target from the ChIP-chip study. Reducing the stringency of ER binding to a $P$ value cutoff of $10^{-5}$ in our MCF-7-control cells increased the total number of ER-interaction sites but reduced the overlap with ChIP-seq and ChIP-chip targets to 41 and 30%, respectively. This likely reflects the emergence of less specific ER-interaction sites when the threshold for ER binding is reduced. We also compared ER interactions from MCF-7+p23 cells to ChIP-seq and ChIP-chip datasets and found an overlap of 39 and 26%, respectively. This is similar to the overlap of ER-interaction sites found in MCF-7-control cells with a $P$ value cutoff of $10^{-5}$ and is consistent with the finding that p23 overexpression enhances ER binding to sites weakly occupied in MCF-7-control cells. Variations in cells, culture conditions, and sample handling likely account for some of the differences in the quantity and overlap of ER-binding sites between our study and the other studies. In fact, such differences are also apparent when comparing ER-binding sites between the Carroll et al. (15) and Welboren et al. (16) studies, in which ER-binding sites overlap by 42%. Thus, evaluating datasets from multiple ChIP studies has been highly informative by revealing what are likely to comprise the highest affinity ER-binding sites.

To determine whether ER binding to its response elements [estrogen response element (ERE)] was affected by p23 overexpression, we performed in silico binding motif identification on the significant ER-binding sites determined by the MACS peak caller from: 1) MCF-7-control in the presence of E2 (3123 binding sites); 2) MCF-7/p23 in presence of E2 (9392 binding sites); and 3) MCF-7/p23 in absence of E2 (392 binding sites). The half-site AGGTCA sequence motifs identified in each set did not change as a function of p23 or E2 treatment, agreeing with earlier studies (Fig. 1C) (10, 17). Thus, increased p23 expression enables ER to bind to more ERE sites genome wide without changing the DNA sequence preference of ER.

ER binding to E2 activated and repressed genes as a function of p23

To investigate the role of ER binding in E2-mediated gene expression, we searched for ER-bound sites within...
10^7 bp upstream and downstream of the transcriptional start site (TSS) of E2-responsive genes from MCF-7-control and MCF-7+p23 cells using a previously published microarray dataset (9). For the E2-induced genes in MCF-7-control and MCF-7+p23 cells, the fraction of genes with ER bound within 10^5-10^7 bp of the TSS is significant but low (Fig. 2, A and B), which is consistent with previous reports (14, 15, 18, 19). As the distance from the TSS is extended, the fraction of E2-induced genes bound by ER increases for MCF-7-control and MCF-7+p23 cells, with a median distance from the TSS of 1.4 × 10^5 and 3.0 × 10^5 bp, respectively (Fig. 2, A and B, and Table 2).

However, the proximity of ER recruitment to the TSS differs for the E2-repressed genes compared with E2-induced genes. For example, in MCF-7-control cells, the fraction of E2-repressed genes with ER bound at 10^3 bp is insignificant, whereas a significant fraction of genes with ER bound is observed at 10^4 bp, and the fraction increases at 10^5 bp from the TSS (median distance of 4.02 × 10^5 from the TSS) (Fig. 2A). For the E2-repressed genes in MCF-7+p23 cells, there is a small but increasing fraction of genes bound by ER between 10^3 and 10^5 bp, and a substantial fraction of E2-repressed genes now displays ER binding at 10^5 bp upstream of the TSS (median distance of 6.23 × 10^4) (Fig. 2B and Table 2). We also observed a large difference in ER binding in the vicinity of genes affected by p23 overexpression (Fig. 2C). The median distance from the TSS of genes induced by p23 overexpression to ER-binding sites identified in MCF-7+p23 cells treated with E2 was 2.8 × 10^4, whereas the median distance to genes repressed by p23 overexpression was 1.2 × 10^5.

To determine whether this shift toward more proximal ER binding at the repressed genes by p23 is genuine, or a by-product of the increased ER binding upon p23 overexpression, we compared the fraction of genes bound by ER at E2-repressed genes vs. a random set of genes not regulated by E2. Although there was a small enrichment over random genes of ER binding at sites proximal to the TSS of repressed genes, it was still marginal compared with the amount of ER binding proximal and distal to induced genes (Fig. 2C). In fact, when plotted as a histogram, there is enrichment in ER binding in MCF-7+p23 vs. MCF-7-control cells at induced as compared with repressed genes (Fig. 2D).

Thus, genes induced by p23 have more ER-binding sites nearby than genes repressed by p23.

**Increased ER binding and expression of SERPINA1 by p23**

To validate our ChIP-seq experiments, we selected a representative gene, SERPINA1 [serpin peptidase inhibitor clade A (alpha-1 antiproteinase, antitrypsin), member 1], which exhibited a large increase in ER binding in the MCF-7+p23 cells compared with control cells. SERPINA1 is a protease inhibitor and has been shown to play important roles in promoting carcinogenesis. Although protease inhibitors are generally thought to be antimalignant, SERPINA1, by contrast, correlates with tumor progression in lung and pancreatic cancers and is up-regulated in liver metastasis from pancreatic tumors (20, 21).

In ChIP-seq experiments of MCF-7-control cells, SERPINA1 showed a small enrichment of sequence tags over a narrow range near a promoter proximal ERE in the absence of ligand, and this was slightly increased upon E2 treatment (Fig. 3A). By contrast, in MCF-7+p23 cells, there was a vast enrichment of tags over the ERE in the absence of E2 treatment and more upon E2 treatment (Fig. 3A). ChIP-quantitative real-time PCR (qPCR) validated the binding of ER to the SERPINA1 ERE upon p23 overexpression (Fig. 3B). This increased ER binding was also associated with a significant increase of histone H3 lysine 9 acetylation (Fig. 3C) and enhanced mRNA expression (Fig. 3D) in MCF-7+p23 cells compared with control cells especially under basal conditions. Thus, overexpression of p23 enhances ER binding and histone H3 lysine 9 acetylation to the SERPINA1 promoter, which in turn facilitates gene expression under both basal and E2-dependent conditions.

**p23 overexpression induces cell invasion via ER**

Our previous work demonstrated that p23 overexpression converts the noninvasive MCF-7 cells to an invasive phenotype under both basal and E2-treated conditions (8). This was substantiated in breast cancer patient samples, where high levels of p23 were associated with increased lymph node metastasis (9). To test the requirement for ER in the enhanced invasion upon p23 overexpression, we performed matrigel invasion assays in MCF-7-control and MCF-7+p23 cells treated with the pure ER antagonist ICI 182,780 (ICI) (Fulvestrant), which extinguishes ER action by promoting ER degradation (22, 23). MCF-7+p23 cells displayed increased invasion through matrigel under basal conditions (Fig. 4A). Importantly, this p23-dependent invasion was largely blocked by ICI treatment (Fig. 4A). Thus, the invasive phenotype of MCF-7+p23 cells requires both ER and p23.

### TABLE 2. Median distance between ER-binding sites and transcription start site of affected genes (base pairs)

<table>
<thead>
<tr>
<th></th>
<th>MCF-7-control E2-dependent</th>
<th>MCF-7-control E2-dependent</th>
<th>MCF-7-p23 E2-dependent</th>
</tr>
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<tbody>
<tr>
<td>Induced</td>
<td>1.42 × 10^6</td>
<td>3.03 × 10^4</td>
<td>2.27 × 10^5</td>
</tr>
<tr>
<td>Repressed</td>
<td>4.02 × 10^6</td>
<td>6.23 × 10^4</td>
<td>1.18 × 10^5</td>
</tr>
<tr>
<td>Random</td>
<td>4.74 × 10^5</td>
<td>1.24 × 10^5</td>
<td>1.24 × 10^5</td>
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Given that p23 overexpression promotes cell invasion under basal as well as E2-stimulated conditions, we examined the gene sets associated with ER binding within 5 × 10^4 bp of a gene’s TSS in both the absence and presence of E2. Our previous analysis of gene expression profiles of MCF-7-control and MCF-7+p23 cells revealed that p23 protein levels modulate the expression of genes misregulated in advanced breast cancers, including those involved in transcriptional regulation, migration and invasion, and metabolism (9). Consistent with our previous transcription profiling analysis, in the presence of E2, we found enrichment of genes in the gene ontology class representing “regulation of cell communication” \( (P < 3.09 \times 10^{-13}) \). These genes include glial cell-derived neurotrophic factor (GDNF), a ligand for the rearranged during transfection (RET) oncogene; Kirsten rat sarcoma viral oncogene homolog (KRAS), a member of the small GTPase family implicated in various malignancies; and fibroblast growth factor receptor 2 (FGFR2), a receptor for mitogenic signaling molecules. Without hormone treatment for genes with ER bound within 50 kb, we observed enrichment in “plasma membrane proteins” \( (P = 1.9 \times 10^{-7}) \). These included matrix metallopeptidase 13 (MMP13), a matrix metalloproteinase family member involved in the breakdown of extracellular matrix and tumor initiation; chemokine receptor 7 (CCR7), a chemokine receptor important in cell migration; and IL-6 receptor, a cytokine receptor that regulates cell growth and differentiation and implicated in carcinogenesis. Thus, genes bound by ER with E2 treatment are enriched for regulators of cellular communication, whereas genes bound by ER without hormone treatment are associated with cell adhesion and migration. This suggests that p23 differentially modulates ER binding to select genes in absence and presence of E2 that influence distinct pathways of cell movement and cell communication, respectively.

**Discussion**

We have examined the impact of higher p23 expression on ER-binding genome wide in MCF-7 cells. This revealed a large increase in the number of sites bound by ER throughout the genome under both basal and E2-induced conditions. This was not a result of alterations in ER protein, because we have previously shown that MCF-7-control and MCF-7+p23 cells have similar levels of ER protein expression (8).
We also observed p23-dependent eviction of ER at a small number (250) of sites such that less ER binding was evident at the targets in MCF-7+p23 compared with MCF-7-control cells (Fig. 1A and Supplemental Fig. 4). Thus, increasing p23 levels modulates ER occupancy at genomic sites.

Our findings also indicate that p23 is making ERE in the genome more accessible to ER, rather than altering ER DNA binding specificity. In addition, it does not appear that this enhanced ER binding with higher p23 is a result of variations in the neighboring transcription factor-binding sites (so called pioneer factors), because forkhead box protein A1 (FOXA1) is the most prevalent binding motif adjacent to the ER-binding sites irrespective of p23 status (data not shown).

p23 overexpression enhanced ER binding to the SERPINA1 promoter, increased histone H3 lysine 9 acetylation to this same region, and increased SERPINA1 mRNA expression under basal and E2-stimulated conditions (Fig. 3). This suggests that if enough ER is driven onto DNA, even in the absence of added E2, it is capable of eliciting a transcriptional response at certain target genes. This is reminiscent of what is observed when the intracellular concentration of ER is raised in MCF-7 cells, which results in ER-dependent gene activation under basal conditions (24). Thus, it appears that higher p23 levels modulate the specific activity of ER for chromatin.

So how is p23 affecting ER binding to chromatin? Given the recent elegant study from John et al. (25) demonstrating the propensity for GR to bind to open chromatin, we hypothesize that higher p23 expression in MCF-7 cells is influencing chromatin architecture, perhaps inducing a more open state, which would allow ER to bind more efficiently to DNA.

In fact, a recent study from the Freeman laboratory has demonstrated that p23 plays important roles in transcription independent of heat shock protein 90, including a synthetic lethal interaction in yeast between p23 and the histone acetyl transferase general control of amino acid synthesis protein 5 (GCN5) (6). In addition, we find that higher p23 expression also resulted in greater p23 levels in the nucleus of MCF-7 cells to control gene expression (9). Although Freeman and Yamamoto (26) and Stavreva et al. (27) have been able to localize p23 to glucocorticoid response elements in cells by ChIP and by immunofluorescence, respectively, we have been unable to ChIP p23 to specific genomic regions in MCF-7 cells. However, we have demonstrated that p23 binds chromatin in MCF-7 cells by isolating a nuclear fraction that contains DNA and proteins tightly bound to chromatin, treating with micrococcal nuclease to release chromatin-bound proteins (28) and blotting for p23 (Supplemental Fig. 6). Thus, we speculate that the gene-specific effects of higher p23 expression occur in response to p23 control of general control of acid synthesis protein 5 (GCN5) activity, which through changes in histone acetylation alter chromatin accessibility and ER DNA binding. Consistent with this is the enhanced histone H3 lysine 9 acetylation observed at p23-enhanced ER-target genes, such as SERPINA1 (Fig. 3C).

Multiple laboratories have previously examined ER binding in MCF-7 cells (10–12, 16). As with any genome-wide study, there are differences in the number of ER-bound regions and the extent of overlap. For example, the overlap in ER-binding sites between the Carroll et al. (10) and Welboren et al. (16) studies was 42%. The apparent discrepancy in the number of ER-bound regions between our study and that of Welboren et al. (16) is in part a result of different P values employed in calling the ER-binding sites using the MACS program. When we compare bound ER using the same algorithm (MACS) and P value ($1 \times 10^{-10}$), we find fewer ER-bound sites (~3000 compared with ~7000 for Welboren et al. (16)) and an overlap of 57%. However, if we use the same P value as Welboren et al. (16) ($1 \times 10^{-5}$), a similar number of ER sites bound (~7000) is observed between studies. Of those sites, 41% overlapped, which represent the highest affinity ER-binding sites. The observed nonoverlapping ER-interaction sites are likely the result of experimental variation, including MCF-7 cell line heterogeneity, culture conditions, the antibody used to ChIP ER, and the length of E2 treatment. Moreover, our findings that higher p23 expression modulates ER chromatin bind-
ing, we would also maintain that subtle differences in p23 expression, even within the same MCF-7 cell type and under the same set of experimental conditions, could influence the amount and location of ER-interaction sites genome wide. Thus, the collection of ER-binding sites derived from a given ChIP-seq study is a function of the methodological and statistical parameters employed, as well as the expression of p23.

Our data showed that the majority of down-regulation occurs independently of proximal ER-DNA interactions. A study on genome-wide binding of GR by Reddy et al. (29) first described this proximity difference in receptor binding between activated vs. repressed genes and suggests that receptor-mediated repression involves long-range cis interactions.

Another important finding from this study is that the invasive phenotype of MCF-7+p23 cells under basal conditions was also ER dependent. Importantly, this ties ER binding in MCF-7+p23 cells to invasion and metastasis, even under low E2 conditions. These findings also have important clinical ramifications in those breast cancer patients whose tumors express ER and higher p23 levels and might benefit from Fulvestrant when estrogen levels are low. Thus, understanding how breast cancers from spreading, even in the postmenopausal setting, where estrogen levels are low. This understanding of p23 protein levels low could also be an important chemoprevention strategy in breast cancer and delay early stage breast cancers from spreading, even in the postmenopausal setting, where estrogen levels are low. Thus, understanding how p23 protein expression is regulated in breast cancer and identifying ways to maintain low p23 protein expression are important future areas for translational research.

Materials and Methods

Cell lines

MCF-7-control and p23-overexpressing MCF-7 cells (MCF-7+p23) were cultured and hormone starved as previously described (8, 9).

ChIP and analysis

After induction with 10 nM E2 for 45 min, cells were fixed in 1% formaldehyde for 10 min at room temperature. Glycine was then added to a concentration of 125 mm. Cross-linked cells were washed with cold PBS and scraped into Farnham lysis buffer [5 mM PIPES (pH 8.0), 85 mM KCl, and 0.5% Nonidet P-40]. Nuclei were collected by centrifugation for 5 min at 1000 × g. ChIP was performed as previously described (29) using the ERα (HC-20) antibody (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). ChIP interrogating histone H3 K9 acetylation were performed with acetyl-histone H3-K9/14 antibody (Millipore, Inc., Bedford, MA) or equivalent amount of rabbit IgG (I-8140, Sigma, St. Louis, MO) antibody.

For ChIP-seq experiments, DNA purified after ChIP went through Illumina library construction and was sequenced on a Genome Analyzer IxI. Thirty-six base pair sequence reads were aligned to the hg19/GRCh37 assembly of the human genome using ELAND (Illumina). The program MACS (13) was used to find significant binding sites for each experiment with the P value cutoffs described in Results. To determine E2-induced-binding sites in MCF-7+p23 cells, we used the MCF-7+p23 sample treated with ethyl alcohol (EtOH) as the control. To determine E2-induced-binding sites in MCF-7 cells, we used the MCF-7 sample treated with EtOH as the control. To determine ER-binding sites in MCF-7+p23 cells under basal conditions, we used the MCF-7 sample treated with EtOH as the control. Each ChIP was performed in duplicate, and only binding sites that were identified as significant in each replicate were considered.

Motif finding was performed by searching for sequence patterns within 50 base pairs on each side of the summit of each binding site identified by MACS using the program BioProspector (30). To calculate enrichment scores for each binding site, the average number of sequence alignments that mapped to each binding site per 1 million aligned sequence reads was calculated. All comparisons with genes were done to RefSeq coordinates (31).

Directed ChIP-qPCR to validate ER recruitment and histone 3 K9/14 acetylation at the SERPINA1 ERE were preformed as described in Simpson et al. (9). ChIP Primers flanking the SERPINA1 ERE Forward: 5’-ACAAGTCACCCCTCTCCCTTGTGAG-3’ and SERPINA1 ERE Reverse, 5’-CCAGAAACCTGCCAGTTATGG-3’.

RNA isolation and qPCR

Total RNA from MCF-7-control and MCF-7+p23 cells was extracted with TRIzol (Invitrogen, Carlsbad, CA) as described by the manufacturer. cDNA was synthesized from 2 μg of RNA using the First-Strand cDNA Synthesis kit for Real-Time PCR (USB, Santa Clara, CA) and random primer hexamers following the manufacturer’s instructions. cDNA were amplified with the SYBR Green Taq Ready Mix (USB) using MyiQ Single-Color Real-Time PCR Detection System from Bio-Rad (Hercules, CA). The primers used for qPCR for SERPINA1: forward, 5’-GGAAATGAACCTCCACCACG-3’ and reverse, 5’-AGTTGACCACGGAGCCTCT-3’.

Cell invasion assay

Invasion assays were performed by using BD BioCoat growth factor reduced matrigel invasion chamber from BD Biosciences (San Diego, CA) according to the manufacturer’s instructions. Fibronectin was used as a chemoattractant at a final concentration of 20 μg/ml. After addition of the cell suspension to the matrigel inserts, the invasion chambers were incubated at 37 C for 48 h. The noninvading cells were then removed by using moistened cotton swabs. The invading cells on the lower surface of the membrane were fixed and stained by using the Richard-Allan Scientific three-step stain kit (Thermo Fisher Scientific, Auburn, AL). The membranes were then removed from the insert by using a scalpel and placed on a microscope slide with a drop of immersion oil. The cells were counted under a microscope.

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