An Estrogen Receptor Mutant with Strong Hormone-independent Activity from a Metastatic Breast Cancer

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Abstract

Thirty tumors from metastatic breast cancer patients were screened for mutations in the estrogen receptor (ER) gene using single-strand conformation polymorphism and sequence analysis. Three missense mutations, Ser47Thr, Lys531Glu, and Tyr537Asn, were identified in these lesions. To investigate these mutated ERs or altered transcriptional activation function, expression vectors containing wild-type (wt) and mutant ERs were constructed and cotransfected with different estrogen response element reporter gene constructs into HeLa cells and MDA-MB-231 human breast cancer cells. The first two ER mutants were similar to wt ER. However, the Tyr537Asn ER mutant possessed a potent, estradiol-independent transcriptional activity, as compared to wt ER. Moreover, the constitutive activity of the Tyr537Asn ER mutant was virtually unaffected by estradiol, tamoxifen, or the pure antiestrogen ICI 164,384. Tyr537 is located at the beginning of exon 8 in the COOH-terminal portion of the hormone-binding domain of the ER, to which dimerization and transcription activation functions have also been ascribed. It has been identified as a phosphorylation site implicated in hormone binding, dimerization, and hormone-dependent transcriptional activity. Our results suggest that the Tyr537Asn substitution induces conformational changes in the ER that might mimic hormone binding, not affecting the ability of the receptor to dimerize, but conferring a constitutive transactivation function to the receptor. If present in other metastatic breast tumors, this naturally occurring ER mutant may contribute to breast cancer progression and/or hormone resistance.

Introduction

The human ER\(^1\) belongs to the steroid/thyroid hormone receptor superfamily of ligand-activated transcription factors (1). Hormone binding is thought to induce conformational changes in the structure of the ER, homodimerization, and high-affinity binding of the hormone-ER dimer complex to well-defined palindromic DNA sequences, termed ER\(_E\)Es, usually located upstream of estrogen-responsive genes (2). Transcription is then induced by two separate AFs of the ER, the NH\(_2\)-terminally located and constitutive AF-1 region and the ligand-inducible AF-2 region located within the hormone-binding domain (3), both of which are probably dependent on further interaction with specific sets of ER-associated proteins (4). Receptor activity is also thought to be modulated by phosphorylation and dephosphorylation on multiple serine and tyrosine residues through more or less defined signaling pathways (5, 6).

It is well accepted that the presence of ER identifies those breast cancer patients with a lower risk of disease recurrence and better survival, who may also have a better response to endocrine interventions (7). The discovery of ER variants with altered function, e.g., transcriptionally active in the absence of estrogen (dominant-positive) or transcriptionally inactive but preventing the activity of wt ER (dominant-negative), may help elucidate the different responses of individual breast tumors to treatment (8). However, the ultimate clinical significance of the ER variants remains to be defined until their presence can be verified at the protein level in clinical studies. We and others have previously described the presence of several ER-splicing variants in human breast cancer cell lines and tumors (8–12). Single-bp changes within the ER, however, appear to be infrequent in primary breast tumors (13–15). To examine their occurrence in metastatic breast tumors, we used SSCP and sequence analysis to screen DNA from 30 tumor specimens for mutations in all eight coding exons of the ER gene. The transcriptional activity of the three missense mutations that we found was assessed by their transfection with different ER-reporter gene constructs into HeLa cells and human MDA-MB-231 breast cancer cells. One of these naturally occurring ER mutants, Tyr537Asn, is at a previously described phosphorylation site located in the COOH-terminal end of the hormone-binding domain. This alteration conferred a potent constitutive transcriptional activity that was independent of estradiol binding and was essentially unaffected by Tam or the pure antiestrogen ICI 164,384.

Materials and Methods

Tumor Samples. Thirty breast tumor samples from residual tumor material remaining after routine steroid receptor measurements were utilized for the study. Tumors samples were frozen within 1 h from surgical removal and kept at −80°C for the present investigation. Data for these tumors and patients were collected from pathological examination and follow-up of clinical records. These cases were all from metastatic sites distant from the original breast lesion. Genomic DNA was isolated from 50–100 mg of frozen tumor tissues using phenol-chloroform extraction (16) and kept at 4°C until use. The patient exhibiting the Tyr537Asn substitution presented with stage IV disease, and the sample used for the analysis was a bone metastasis that recurred after treatment with diethylstilbestrol hormonal therapy. The metastatic bone sample was ER negative and progesterone receptor negative by ligand binding analysis. No additional clinical follow-up is available on this patient.

PCR Amplification and SSCP Analyses. Twelve primers were designed for analysis of genomic DNA according to the exon/intron locations defined in Ponglikitmongkol et al. (17). All coding exons were examined. The primer sequences, the expected sizes of the PCR products, and their location within the ER are shown in Table 1. The forward primers were \(^5\) biotinylated for direct sequencing analysis.

Eighty ng of genomic DNA were used as template in PCR, which was performed in a 30-μl volume, including 10 mM Tris·HCl (pH 8.4), 50 mM KC\(_2\), 1.3–1.5 mM Mg\(_2\)Cl\(_2\), 200 μM of each deoxynucleotide triphosphate, 0.13 μM of each primer, and 0.75 units of Taq polymerase (Boehringer Mannheim).
The site within the plasmid to facilitate cloning of the ER mutations was:

Transformer site-directed mutagenesis kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions (Life Technologies, Inc., Gaithersburg, MD). Direct sequencing of the products of these ligation reactions showed the expected sizes and then stored at 4°C until used for direct sequence analysis. PCR products were first visualized on 7.8% polyacrylamide gels to verify the expected sizes and then stored at 4°C until used for direct sequence analysis. DMSO (5%) was also added for primer pair number 3 (Table 1) to facilitate amplification. Negative control reactions (H₂O instead of DNA) were included in every experiment. Amplification was carried out under the following conditions: 94°C for 3 min, followed by 32–42 repetitive cycles of 94°C for 1 min, 52–63°C for 45 s, 72°C for 1 min, and an extension step at 72°C for 5 min. PCR products were first visualized on 7.8% polyacrylamide gels to verify the expected sizes and then stored at 4°C until used for direct sequence analysis. 

PCR for SSCP analysis (18) was performed as above with the exception of including 5 μl of diluted (1:50 in dH₂O) [³²P]dCTP (10 μCi/μl, 3000 Ci/mmol; Amersham Corp.) and reducing the concentration of unlabeled dCTP to 100 μM. One μl of the PCR product was mixed with 9 μl of denaturing solution (95% deionized formamide, 10 mm NaOH, 0.25% xylene cyanol, and 0.25% bromphenol blue), denatured at 95°C for 3 min, and put on ice for 3 min. Four μl of the mixture were then run on a nondenaturing 0.5× mutation detection enhancement acrylamide gel (AT Biochem) without glycerol at 4°C and 40 W for 4–5 h or with 5% glycerol at room temperature and 15 W overnight. The gel was then transferred onto Whatman filter paper, dried under vacuum at 80°C for 2 h, and put on X-ray film at −70°C overnight or longer. Samples manifesting migration shifts in SSCP gels were analyzed further by DNA sequencing.

DNA Sequencing. DNA sequencing was performed both directly from PCR-amplified genomic DNA and from shifted bands cut from the SSCP gels. For direct sequence analysis, single-stranded DNA template was produced by binding the biotinylated strand from 20–30 μl of PCR product to Dynabead streptavidin solid supports (Dynal AS, Oslo, Norway). Sequence analysis was then performed, using the bound biotin labeled strand as template, according to the Sanger dideoxynucleotide chain termination (19) using 35S-labeled dCTP. In addition, 2 μl of the PCR product from samples displaying altered migration bands were cloned into the pCR-TO plasmid (Invitrogen), sequenced, and compared to the published sequence for ER (17, 20).

Expression Vector Construction. A wt ER expression vector was made by subcloning the BamH1 EcoRI fragment from the yeast expression vector YEPE 10 (6) into the pcDNA expression vector (Invitrogen, San Diego, CA). To study the function of the three identified ER mutants, we individually introduced these three substitutions into pcDNAI-wtER plasmid using the Transformer site-directed mutagenesis kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions. The following three mutagenic primers were used: Ser47Thr, 5'-CGAGAATCTCTCCAGGCTGGCGGCG-3'; Lys531Glu, 5'-CAGCATGAGCTCCGAGAAA-3'; and Tyr357Asn, 5'-GTGCCTCTCTCCTACCTG-3'. The selection primer employed to disrupt the unique site within the plasmid to facilitate cloning of the ER mutations was: 5'-GCAACGCAATGACTCTCCAGTGAAC-3'. The sequences of the three mutated ER expression plasmids were verified by sequence analysis.

ERE Reporter Construction. We have prepared CAT reporter constructs to the promoters of the four estrogen-inducible genes progesterone receptor (PgR), p23, lactoferrin, and cathepsin D. All the ERE-CAT vectors were derived by modification of the vector pT7-tk-CAT (21), provided generously by Dr. Benita Katzenellenbogen. This plasmid contains a multiple cloning site in the same orientation as the herpes simplex virus type 1 tk promoter (bases −150 to +56) controlling the expression of the CAT gene. This plasmid was linearized by BamHI digestion and dephosphorylated with calf intestine alkaline phosphatase (Promega, Madison, WI) to prevent recircularization. The ERE-CAT constructs were prepared by ligating this vector in the presence of a 100-fold molar excess of oligonucleotides containing a 13-bp ERE plus 9–11 bp of the requisite flanking gene sequence on either side. Oligonucleotides corresponding to the plus and minus strands of each ERE appended by a sequence generating a BamHI compatible overhang were kinased and annealed prior to addition to the ligation reaction. ERE oligonucleotide sequences (consensus sequences appear in bold and underlined and non-consensus sequences are non-bold; underlining designates two half-ERE sites) were derived from published promoter sequences of the human lactoferrin (GATCCAGGCTCCTCACAGACGCCG; Ref. 2), p52 (GATCCAGGCTCCTCACAGACGCCG; Ref. 23), and cathepsin D (GATCCAGGCTCCTCACAGACGCCG; Ref. 24) genes. Subsequent analysis of the products of these ligation reactions showed that the vectors contained three (p52) or four (cathepsin D and lactoferrin) inserted ERE sequences. A similar protocol was used to generate the plasmid pERE2-tk-CAT, which contains a tandem insertion of two vitellogenin A2 (consensus) EREs in the BamHI site of pT7-tk-CAT.

Cell Culture and Transient Transactivation Assays. HeLa cells were maintained in complete MEM supplemented with 10% fetal bovine serum (Sigma Chemical Co., St. Louis, MO). Cells were plated into six-well cluster dishes (Falcon, Franklin Lakes, NJ) at a density of 1.5 × 10⁵ cells/well and grown in the above medium. MDA-MB-231 breast cancer cells (2 × 10⁵) were plated and grown in the same medium supplemented with 2 mm isoglutamine and 6 mg/ml insulin and allowed to recover for 2 days at 37°C in 5% CO₂. Transient transfections were carried out in a total volume of 1 ml of OptiMEM reduced-serum medium with 8 μg of lipofectamine/well according to the manufacturer’s instructions (Life Technologies, Inc., Gaithersburg, MD). Transfections of individual wells were performed using 1 μg of ERE-CAT reporter vector. Cytomegalovirus-driven β-gal plasmid (100 ng) was added into each well to monitor transfection efficiency, and 20 ng of either the wt ER or ER-mutant plasmid was transfected into HeLa cells or MDA-MB-231, respectively. The reporter was also transfected into cells without ER as a control for background level of CAT activity. Following an 8-h incubation at 37°C, the transfection medium was removed, and 2 ml of phenol red-free MEM supplemented with charcoal-stripped fetal bovine serum was added and incubated at 37°C for 18–20 h. Cells were then treated with 10⁻¹¹ or 10⁻¹⁰ M estradiol, 10⁻⁷ M Tam, 10⁻⁹ M T3, 10⁻³ M ICI 16,384 (a kind gift from Dr. Allen Wakeman of Zeneca, Macclesfield, England), or an ethanol vehicle for 20–22 h at 37°C. All the transfections were performed in duplicate. The transfected cells were then rinsed once with PBS, and 150 μl of cell lysis buffer (Promega) were added. Cell extracts were spun for 5 min, and 20 μl of the supernatant were used in the β-gal assay. The remaining supernatant was heated to 65°C for 15 min to remove any endogenous acetylases or nonlabeled acetyl-CoA and centrifuged again. Various volumes of the extracts, calculated to contain equivalent amounts of β-gal activity, were incubated with substrate mix (1 ml of 1 M Tris (pH 7.8), 80 μl of 4C-labeled chloramphenicol, 800 μl of 4 mm acetyl-CoA, and 4.52 ml of dH₂O) at 37°C for 90 min (HeLa cells) or 120 min (MDA-MB-231 cells). The reaction was then terminated by adding 1 ml of cold ethylacetate vigorously mixed and centrifuged. The supernatant was dried under vacuum, and the residue was resuspended in 20 μl of ethyl acetate, spotted on precast TLC plates (Sigma Chemical Co.), chromatographed in 95% chloroform and 5% methanol at room temperature for 30–40 min, dried, and exposed to Biomax X-ray film (Kodak, Rochester, NY) overnight. All the assays were done in duplicate.

Results

ER Mutations Identified in Metastatic Breast Tumors. Thirty metastatic breast cancer samples were screened for mutations in the ER gene by SSCP analysis. SSCP shifts (evident by electrophoresis both at ambient and subambient temperatures) were identified in six of the metastatic tumors. The six positive samples were studied further.
both by direct sequencing analysis and by cloning of shifted bands followed by sequencing analysis. Three of the SSCP-shifted bands were found to constitute previously identified polymorphisms (TCT to TCC at nucleotide 30 in exon 1, TGC to TGT at nucleotide 720 in exon 3, and CGC to CGT at nucleotide 729 in exon 3), none of which results in an amino acid substitution (data not shown). The remaining three shifted SSCP bands (Fig. 1A, arrows) were found to constitute missense mutations: G140C (Ser47Thr) in exon 1, A1591G (Lys531Glu) in exon 8, and T1609A (Tyr537Asn) in exon 8 (Fig. 1B). Whereas the first two sequence changes exchange hydrophilic amino acids, the last mutation changes a hydrophobic tyrosine to a hydrophilic asparagine.

**Functional Studies.** The functional transactivational status of the three missense ER mutations isolated from the metastatic breast tumors was investigated using transient transactivation assays, measuring the transcriptional activity of the mutant ERs with ERE-reporter gene constructs. Although the majority of studies examining the effect of specific alterations in the ER on function have utilized consensus EREs, most commonly the vitellogenin A2 ERE, we felt that it would be important to test ER function on constructs that might be more relevant to breast cancer biology, e.g., those genes that are regulated endogenously by ER in breast cancer cells. We therefore prepared ERE-CAT reporters from the estrogen-regulated pS2, cathepsin D, and lactoferrin gene promoters. Each mutant ER was then cotransfected into HeLa cells (Fig. 2) with the different ERE reporter vectors and compared to wt ER activity. CAT activity was determined relative to the activity of the transfected reporter vector alone; activity was also corrected for transfection efficiency by cotransfection of a /3-gal vector.

The activities of Ser47Thr and Lys531Glu were not different from that of wt ER using any of the four different ERE constructs in either HeLa or MDA-MB-231 cells (data not shown). However, the Tyr537Asn mutant exhibited strong constitutive transactivation activity (15–20-fold over wt ER activity on the vitellogenin ERE) in the absence of hormone (Fig. 2, Vitellogenin, con). This elevated constitutive activity was also observed on the other ERE constructs (5-fold on the pS2 control [con], 8-fold on the cathepsin D control, and 17-fold on the lactoferrin ERE control). Estradiol was required for the induction of wt ER activity on all four of the ERE reporter constructs (maximum inductions were 11-, 2-, 3-, and 4-fold on the vitellogenin, pS2, cathepsin D, and lactoferrin reporters, respectively, using $10^{-9} \text{M}$ estradiol; Fig. 2). As expected, tamoxifen alone had no effect on basal activity of the wt ER and completely inhibited the stimulatory effect of estradiol (Fig. 2, E2+ Tam).

In contrast, the addition of estradiol had only minimal influence on the already high constitutive transcriptional activity of the Tyr537Asn mutant (Fig. 2; compare the control [con] with the estradiol-stimulated levels (E2-9 and E2-11)). Interestingly, tamoxifen appeared to slightly inhibit to varying degrees the basal activity of the Tyr537Asp mutant on three of the ERE promoters; for instance, 45% inhibition of basal activity was seen using the cathepsin D ERE reporter. The only notable difference among the four ERE reporters was in the insignificant tamoxifen inhibition of basal transcriptional activity on the lactoferrin promoter (Fig. 2, compare control with Tam levels). Similar results to those seen with tamoxifen were observed when these experiments were repeated using the pure antiestrogen ICI 164,384 in place of tamoxifen in these cells (data not shown).

Because we know that the transcriptional activity of the ER is highly dependent on the cell and the promoter context (25) in which the receptor is expressed, we also tested the transcriptional activity of the Tyr537Asn ER in MDA-MB-231 breast cancer cells. Similar results were seen in these cells, although the maximum inductions by wt ER (2–3-fold) over control in the absence of estradiol were all reduced due to the high basal activity of the tk-CAT reporter alone in these cells (compare the controls in Fig. 3 to the controls in Fig. 2). Again, the Tyr537Asn mutant displayed high constitutive activity on all four of the ERE reporters, ranging from 3- to 7-fold depending on the ERE reporter, and this activity was essentially unaffected by estrogen, tamoxifen (Fig. 3), and the ICI pure antiestrogen (data not shown).

**Discussion**

Although ER-splicing variants have been shown to be ubiquitous in human breast cancer (12), the number of naturally occurring missense mutations identified in primary breast cancers to date is extremely low. It has been estimated that missense mutations are present in only about 1% (2 of 188) of primary tumors (15). In agreement with this, we have not detected any missense ER alterations in 60 primary breast cancers that we have examined using SSCP analysis. Karnik et al. (14) have also recently examined five primary and metastatic breast tumor pairs for ER sequence alterations, again using SSCP analysis. They found that one of the five metastatic lesions, but not the primary tumor from the same patient, contained a single nucleotide deletion (1294delT) in the coding region of the ER. This deletion generates a frameshift in the hormone-binding domain of the receptor and is predicted to give rise to a premature translation termination with an ER protein maintaining an intact DNA-binding domain but with a defective ligand-binding domain. Functional analysis of this ER mutant, however, has not yet been reported. The fact that this ER mutation was present in the metastatic lesion but not in the corresponding primary tumor suggests that some ER mutations may be associated with tumor progression.

In the present study of 30 cases of metastatic breast cancer, we detected three missense ER mutations. Thus, ER mutations in meta-

4 Q.-X. Zhang, A. Borg, and S. A. W. Fuqua, unpublished data.
ER MUTANT WITH CONSTITUTIVE TRANSCRIPTIONAL ACTIVITY

Fig. 2. The Tyr537Asn ER mutant displays strong hormone-independent transcriptional activity in HeLa cells. Transactivation assay comparing the Tyr537Asn ER mutant (●) with wt ER (●) in the absence and presence of estradiol (con, control group; E2-11, 10⁻¹¹ M; E2-9, 10⁻⁹ M), Tam (Tam, 10⁻¹⁰ M), and a combination of both (E2+Tam, 10⁻¹⁰ M estradiol and 10⁻¹⁰ M Tam). The results from four different ERE constructs are shown in separate panels. Data are shown as percentage of CAT conversion (corrected for β-gal activity) from duplicate wells; bars, SD.

Static breast tumors may be more frequent than in primary lesions [3 of 30 as compared to 2 of 248 (see above); P = 0.004 with Fisher’s exact test]. Unfortunately, the corresponding primary tumors from these three patients were not available for ER mutational analysis, and future studies will be directed at addressing whether ER mutations arise during metastatic spread of the tumor in certain patients. Two of the identified ER mutations (Ser47Thr and LysS31Glu) did not alter ER transcriptional activity in transient transfection assays. The third mutation, however, resulted in an ER protein with a very high constitutive transcriptional activity. This mutation (Tyr537Asn) is located at the beginning of exon 8 of the ER gene encoding the COOH-terminal portion of the hormone-binding domain, a region of the ER to which dimerization and AF-2 functions have been ascribed (2). This is also a region that is evolutionarily conserved between species but is divergent from other members of the steroid and thyroid hormone receptor superfamily (17).

The Tyr537Asn mutation eliminates a tyrosine residue that is a potential phosphorylation site within the ER. We found that the Tyr537Asn ER mutant manifested strong transactivation activity in both HeLa and breast cancer cells in the absence of hormone binding and that this activity was only marginally affected by estradiol, tamoxifen, or the pure steroidal antiestrogen ICI 164,384. Furthermore, this constitutive activity was similarly evident whether using an idealized ERE (vitellogenin) or endogenous ERES such as the cathepsin D, pS2, and lactoferrin gene promoters. Several potential mechanisms could explain the high constitutive activity of the Tyr537Asp ER mutation. One explanation, and one that we favor (Fig. 4), is that the Tyr537Asn substitution may produce a conformational change in the receptor that mimics hormone binding. We know that this residue lies within the hormone-binding domain of the ER; functional analysis of ER deletion mutants has suggested that the COOH-terminal boundary of both the estrogen and tamoxifen-binding domains are similar, lying between residues 522 and 538 (26, 27). The role of ligand binding in the formation of AF-2 is unknown but is believed to involve conformational changes in the receptor that generate a productive association between the AF-1 and AF-2 domains (28). In our model (Fig. 4), we envision that similar conformational changes may be induced by the Tyr537Asn substitution. As a result of this conformational change, the mutant ER might only weakly bind estrogen and tamoxifen, explaining their limited effects on mutant ER activity. Although the metastatic bone lesion from which we isolated the Tyr537Asn mutant was indeed ER negative by ligand-binding analysis, this result may not be conclusive, because low tumor cellularity is often associated with bone lesions. Unfortunately, additional tumor material was not available to address the question of hormone binding ability of this variant using immunohistochemical techniques.

There is evidence to suggest that phosphorylation at this site is required for efficient estrogen binding. Arnold et al. (29) determined that Tyr 537 is a physiological phosphorylation site in ER isolated from MCF-7 human breast cancer cells. Using site-directed mutagenesis to separately replace all five tyrosine residues within the hormone-binding domain of the ER with phenylalanine, Castoria et al. (30) demonstrated that phosphorylation of in vitro synthesized ER at Tyr537 confers efficient estrogen binding ability. Thus, phosphoryl-
ER MUTANT WITH CONSTITUTIVE TRANSCRIPTIONAL ACTIVITY

Fig. 3. The Tyr537Asn ER mutant displays strong hormone-independent transcriptional activity in MDA-MB-231 cells. Transactivation assay comparing the Tyr537Asn ER mutant (■) with wt ER (□) in the absence and presence of estradiol (E2—10⁻¹¹ M; E2—10⁻⁹ M), Tam (10⁻⁷ M), and a combination of both (E2+Tam, 10⁻⁹ M estradiol and 10⁻⁷ M Tam). The results from four different ERE constructs are shown in separate panels. Data are shown as percentage of CAT conversion (corrected for β-gal activity) from duplicate wells; bars, SD.

phosphotyrosine residues and SH-2-like domains, similar to the activation of the STAT family of transcription factors (32). These data would therefore suggest that the Tyr537Asn ER mutant would be incapable of dimerization due to the absence of a target for the SH-2 like domain of its dimerization partner. However, as ER dimerization is necessary for ERE binding and transcriptional activity, this hypothesis is clearly inconsistent with our demonstration of strong transcriptional activity with the Tyr537Asn mutant and implies that other regions of the hormone-binding domain, such as the adjacent leucine zipper motif (32), are involved in dimerization as well.

Tyr537 may represent a basal phosphorylation site of the human ER, which is under strict control by both specific tyrosine kinases and phosphatases. This was suggested by studies demonstrating that two members of the src family of tyrosine kinases were capable of Tyr537 phosphorylation, as well as by the finding that protein tyrosine phosphatase-1B and the SH-2 protein tyrosine phosphatase-1 dephosphorylated Tyr537 (6). Thus, the Tyr537Asn ER mutant may have escaped from phosphorylation-mediated transcriptional regulation that is present in vivo, as was seen in the HeLa and MDA-MB-231 cells used in the present transfection studies. As reflected in the model diagrammed in Fig. 4, the activity of the ER can be modulated by the phosphorylation of a number of residues through growth factor and oncogene signaling pathways. There are multiple sites of phosphorylation in the ER; several serine residues in the NH₂-terminal AF-1 domain are targeted through the Ras mitogen-activated protein kinase (5) and the protein kinase A or C signaling pathways (33). Recently, it was demonstrated that the HER-2 oncogene targets the ER leading to phosphorylation of the ER on tyrosine residues and ligand-inde-
dependent signaling through the receptor, resulting in ligand-independent signaling through the ER (34). Disruption or dysregulation of phosphorylation at specific sites within the ER may therefore be important in the clinical problem of hormone-independent tumor growth, as would be predicted for patients harboring the Tyr537Asn mutation detected in this study. It is of note that the patients from which this mutation was identified presented with advanced metastatic disease. Future studies will be directed at investigating the frequency of this specific alteration in patients with metastatic breast cancer to determine whether this constitutive mutation is common in patients with dissemination of their disease.

References

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