

# “Dwarf” Estrogen Receptor in Breast Cancer and Resistance to Tamoxifen

Amy M. Fowler, *Washington University School of Medicine, St. Louis, MO*  
Richard J. Santen, *University of Virginia Health System, Charlottesville, VA*  
D. Craig Allred, *Washington University School of Medicine, St. Louis, MO*

A major problem in breast cancer treatment and research today is why so many patients with estrogen receptor alpha (ER $\alpha$ )–positive tumors have de novo resistance to tamoxifen and why even more acquire resistance at a later time. An interesting study by Shi et al in this issue of *Journal of Clinical Oncology* suggests that one mechanism (and there are probably many) involves a truncated variant of ER $\alpha$ , which may predict and possibly mediate resistance.<sup>1</sup>

In previous studies, these investigators identified a novel 36-kDa variant of ER $\alpha$  using a homology search against a portion of the ER $\alpha$ 66 ligand binding domain, and cloned it from human placental cDNA.<sup>2</sup> Like ER $\alpha$ 66, this ER $\alpha$  variant is expressed from the *ESR1* gene, but it is transcribed from an alternative start site. Following transcription, it also undergoes alternative splicing at the 3' end, yielding a protein lacking the AF-1 and AF-2 *trans*-activation domains, as well as the last 138 amino acids of ER $\alpha$ 66, which are replaced with a unique 22–amino acid sequence. They developed an antibody specific to the unique C-terminus of ER $\alpha$ 36, which does not recognize ER $\alpha$ 66, and used it to show that ER $\alpha$ 36 is expressed in a significant proportion of ER $\alpha$ 66-positive and -negative breast cancer cell lines and human primary tumors.<sup>2</sup> Interestingly, ER $\alpha$ 36 is apparently not expressed in normal breast epithelial cells, or the MCF10A human mammary epithelial cell line.<sup>2,3</sup>

To study the function of this new variant, they stably transduced and overexpressed ER $\alpha$ 36 in human embryonic kidney cells (HEK293), which do not express the receptor.<sup>2</sup> They showed that ER $\alpha$ 36 lacks intrinsic transcriptional activity, consistent with its lack of both *trans*-activation domains. In cells cotransduced with ER $\alpha$ 66, they also demonstrated that the variant is a dominant inhibitor of ER $\alpha$ 66 transcriptional activity. An unexpected and particularly interesting finding was that ER $\alpha$ 36 localizes to the cytoplasm and surface membrane of cells, and that binding by both estrogen and tamoxifen activates the mitogen-activated protein kinase (MAPK) signaling pathway, stimulating cell growth. Based on these laboratory studies, they hypothesized that ER $\alpha$ 36 expression may be associated with tamoxifen resistance in patients with ER $\alpha$ 66-positive breast cancer. In this study,<sup>1</sup> Shi et al tested this hypothesis by measuring ER $\alpha$ 36 expression by immunohistochemistry (IHC) in archival tissue banks of several groups of patients with invasive breast cancer. The patients were treated with various combinations of tamoxifen and chemotherapy, and the associations with ER $\alpha$ 36 expression and disease-free survival (DFS) and disease-specific survival (DSS) were evaluated.

ER $\alpha$ 36 positive was artificially defined as moderate-to-strong IHC staining in 10% or more tumor cells.

The largest group of patients (n = 307) had ER $\alpha$ 66-positive tumors treated with tamoxifen with or without chemotherapy (median follow-up = 7.9 years), and showed significantly decreased benefit in ER $\alpha$ 36-positive compared with ER $\alpha$ 36-negative tumors in both univariate and multivariate (MVA) analyses of DFS and DSS (11% and 12%,  $P = .002$ , and hazard ratio [HR] 1.9 and 2.5,  $P \leq .002$ , respectively). A separate, independently evaluated group with ER $\alpha$ 66-positive tumors treated with tamoxifen alone (n = 156; median follow-up, 4.8 years) also showed significantly decreased benefit in ER $\alpha$ 36-positive versus ER $\alpha$ 36-negative tumors (eg, DFS HR, 5.47;  $P = .003$ ). In contrast, there was a hint of benefit in patients with ER $\alpha$ 66-positive/ER $\alpha$ 36-positive tumors (n = 129) treated with chemotherapy alone (DSS HR, 0.79;  $P = .58$  in MVA), and a stronger trend towards benefit in a group with ER $\alpha$ 66-negative/ER $\alpha$ 36-positive tumors (n = 149) treated in a similar manner (DSS HR, 0.52;  $P = .07$  in MVA). A smaller group of patients with ER $\alpha$ 66-negative/ER $\alpha$ 36-positive tumors (n = 73) treated with tamoxifen with or without chemotherapy showed a nonsignificant trend towards decreased benefit (DFS HR, 1.55;  $P = .35$  in MVA).

This study has strengths and limitations. Foremost among the strengths are the tantalizing results suggesting that ER $\alpha$ 66-positive/ER $\alpha$ 36-positive breast cancers are relatively resistant to adjuvant tamoxifen, which was demonstrated in two independent cohorts of patients of reasonable size and follow-up. Foremost among the limitations was the absence of an untreated group of patients for comparison, so it is difficult to determine if the apparent resistance to tamoxifen represents a truly significant negative interaction with the drug alone, or if there are additional prognostically detrimental biologic features associated with the variant receptor. Aggressive, fast-growing breast cancers often initially respond fairly well to the types of cytotoxic chemotherapies used in this study,<sup>4</sup> and there were weak trends towards benefit in ER $\alpha$ 36-positive compared with ER $\alpha$ 36-negative tumors treated with chemotherapy alone, regardless of ER $\alpha$ 66 status, suggesting that the variant may indeed be associated with aggressive behavior. The authors' previous *in vitro* studies of ER $\alpha$ 66-negative/ER $\alpha$ 36-positive breast cancer cell lines showing that estrogen activates the MAPK pathway and increases proliferation also support this notion.<sup>2</sup> Because of the retrospective and longitudinal study design, it is also difficult to separate de novo from acquired

tamoxifen resistance, which might be clarified by future studies in a neoadjuvant setting. This may be a relatively minor point, but the crude method of scoring and defining ER $\alpha$ 36 positivity by IHC used in this study may also be obscuring quantitative relationships between expression and response to tamoxifen.

An important question that was not addressed in this study is the relationship between ER $\alpha$ 36 expression and response to aromatase inhibitors (AIs), which are being increasingly used. Since AIs inhibit the synthesis of estrogen, rather than estrogen binding to ER $\alpha$ 66, it is reasonable to speculate that ER $\alpha$ 36 may not be associated with resistance to these drugs because MAPK-induced cell growth would not occur. If this is correct, then ER $\alpha$ 36 expression may even be partially responsible for the relative benefit of AIs over tamoxifen observed in previous clinical trials.<sup>5</sup> Hopefully, future studies will evaluate this. Because of significant adverse effects associated with AIs, tamoxifen is still being used with benefit in many patients, which will probably continue for some time.<sup>6</sup> Thus, it remains important to gain a better understanding of tamoxifen resistance, despite the increasing use of AIs as first-line hormonal therapy.

From a biologic perspective, this article reinforces growing evidence that truncated forms of hormone receptors may be important in regulating the transcriptional activities of hormone receptors as a class, which is novel and fascinating. Many of these “dwarf” receptors seem to exert a dominant-negative effect on transcription. For example, another dwarf receptor referred to as ER $\alpha$ 46 lacks the N-terminal A/B domain and suppresses ER $\alpha$ 66 AF-1 *trans*-activation.<sup>7</sup> There is a truncated form of ER $\beta$ , referred to as ER $\beta$ cx, or ER $\beta$ 2, which inhibits full-length ER $\alpha$ , but not ER $\beta$ .<sup>8</sup> Progesterone receptor (PgR)-C is an N-terminal truncated variant of PgR lacking the DNA binding domain, which inhibits the transcriptional activity of full-length PgR-B, contributing to the onset of labor.<sup>9</sup> Glucocorticoid receptor- $\alpha$  is inhibited by a truncated variant referred to as GR- $\beta$ ,<sup>10</sup> and there are more. Although ER $\alpha$ 36 shares the ability to suppress transcription of the full-length receptor, it differs from other known variants in its ability to activate MAPK-mediated signaling pathways through apparently independent extranuclear mechanisms, stimulating cell proliferation.

It will be important to determine the precise mechanism whereby ER $\alpha$ 36 activates MAPK activity in order to develop strategies to prevent the ensuing cell proliferation. The general model proposed by the authors is that ER $\alpha$ 36 localizes to the outer cell membrane and estradiol (E2) somehow activates the MAPK pathway through membrane-initiated signaling. Furthermore, ER $\alpha$ 36 is a promiscuous receptor in the sense that it can bind estradiol (E2), estretol (E4), tamoxifen, and fulvestrant, all of which activate MAPK and stimulate cell proliferation.<sup>2</sup> Since ER $\alpha$ 36 seems to lack an intrinsic *trans*-membrane domain, it is unclear exactly how it localizes to the surface membrane of cells. Small amounts of ER $\alpha$ 66 also localize to the surface membrane in certain situations through a mechanism involving palmitoylation at cysteine 447.<sup>11</sup> Perhaps ER $\alpha$ 36 uses a similar mechanism, or a related one involving myristoylation, as suggested by the authors, but the answer awaits future studies.

Studies during the past few years have demonstrated that transcriptional regulating functions of ER $\alpha$ 66 in the nucleus can be inhibited at the same time as its extranuclear (ie, membrane) functions are being activated.<sup>12-15</sup> The mechanisms and consequences of this divergent regulation have been controversial and difficult to study, primarily because it has been challenging to visualize and purify membrane

receptor, as well as to stimulate selectively membrane over nuclear receptor activity.<sup>12</sup> The recent development of dendrimeric estrogen conjugates that are unable to enter the nucleus has been helpful in understanding nuclear versus extranuclear ER $\alpha$ 66 signaling, but these compounds are still unable to distinguish cytoplasmic from surface membrane activation.<sup>16</sup> Perhaps ER $\alpha$ 36 may provide an additional tool to help understand this process, given that it represses nuclear signaling of ER $\alpha$ 66 while independently initiating estrogen-induced signaling at the cell membrane.

It is possible that the mechanism of ER $\alpha$ 36-mediated MAPK activation leading to cell proliferation is entirely independent of ER $\alpha$ 66 at the cell membrane. This is supported by recent studies using the MCF7 breast cancer cell line showing that dendrimeric estrogen conjugates preferentially induce ER $\alpha$ 66-mediated MAPK activation at the cell membrane, but does not stimulate proliferation.<sup>16</sup> However, there are reasonable alternative hypotheses. For example, perhaps ER $\alpha$ 36-mediated MAPK activation participates in phosphorylation of ER $\alpha$ 66 and its coactivator AIB1, which would facilitate nuclear transcription and might override the opposing ER $\alpha$ 36-mediated inhibition of nuclear ER $\alpha$ 66 transcriptional activities, leading to cell proliferation. Again, answers await future studies.

This study by Shi et al,<sup>1</sup> and their previous studies leading up to it, is an excellent example of well-conducted bench-to bedside translational research. The results suggest that ER $\alpha$ 66-positive/ER $\alpha$ 36-positive breast cancers may be relatively resistant to tamoxifen and treated more effectively with chemotherapy alone (or with other types of hormonal therapies such as AIs, if they are shown to remain effective in future studies). As the authors acknowledge, their results are preliminary but, because they seem promising, and because the issue is so important, they deserve additional validation. If confirmed to be truly predictive of tamoxifen resistance in a substantial proportion of patients with ER $\alpha$ 66-positive breast cancers, then they also deserve to be compared with other strategies under investigation addressing similar issues, such as OncotypeDX in the TailorRX trial,<sup>17</sup> and MammaPrint in the MINDACT (Microarray in Node-Negative Disease May Avoid Chemotherapy) trial.<sup>18</sup> A technically simple and economical test that accurately identifies tamoxifen resistance would be a welcome contribution.

#### AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

#### AUTHOR CONTRIBUTIONS

**Conception and design:** Amy M. Fowler, Richard J. Santen, D. Craig Allred

**Manuscript writing:** Amy M. Fowler, Richard J. Santen, D. Craig Allred

**Final approval of manuscript:** Richard J. Santen, D. Craig Allred

#### REFERENCES

- Shi L, Dong B, Li Z, et al: Expression of ER- $\alpha$ 36, a novel variation of estrogen receptor  $\alpha$ , and resistance to tamoxifen treatment in breast cancer. *J Clin Oncol* 2009 doi:10.1200/JCO.2008.17.2254
- Wang Z, Zhang X, Shen P, et al: A variant of estrogen receptor- $\alpha$  hER $\alpha$ 36: transduction of estrogen- and antiestrogen-dependent membrane-initiated mitogenic signaling. *Proc Natl Acad Sci U S A* 103:9063-9068, 2006
- Lee LM, Cao J, Deng H, et al: ER- $\alpha$ 36, a novel variant of ER- $\alpha$ , is expressed in ER-positive and -negative human breast carcinomas. *Anticancer Res* 28:479-483, 2008
- Razzak AR, Lin NU, Winer EP: Heterogeneity of breast cancer and implications of adjuvant chemotherapy. *Breast Cancer* 15:31-34, 2008
- Howell A, Cuzick J, Baum M, et al: Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* 365:60-62, 2005

6. Coates AS, Keshaviah A, Thurlimann B, et al: Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: Update of study BIG 1-98. *J Clin Oncol* 25:486-492, 2007
7. Flouriot G, Brand H, Denger S, et al: Identification of a new isoform of the human estrogen receptor- $\alpha$  (hER- $\alpha$ ) that is encoded by distinct transcripts and that is able to repress hER- $\alpha$  activation function 1. *Embo J* 19:4688-4700, 2000
8. Ogawa S, Inoue S, Watanabe T, et al: Molecular cloning and characterization of human estrogen receptor  $\beta$ c $\alpha$ : A potential inhibitor of estrogen action in human. *Nucleic Acids Res* 26:3505-3512, 1998
9. Condon JC, Hardy DB, Kovacic K, et al: Up-regulation of the progesterone receptor (PR)-C isoform in laboring myometrium by activation of nuclear factor- $\kappa$ B may contribute to the onset of labor through inhibition of PR function. *Mol Endocrinol* 20:764-775, 2006
10. Oakley RH, Jewell CM, Yudit MR, et al: The dominant negative activity of the human glucocorticoid receptor  $\beta$  isoform. Specificity and mechanisms of action. *J Biol Chem* 274:27857-27866, 1999
11. Acconcia F, Ascenzi P, Bocedi A, et al: Palmitoylation-dependent estrogen receptor  $\alpha$  membrane localization: Regulation by 17 $\beta$ -estradiol. *Mol Biol Cell* 16:231-237, 2005
12. Levin ER, Pietras RJ: Estrogen receptors outside the nucleus in breast cancer. *Breast Cancer Res Treat* 108:351-361, 2008
13. Boonyaratanakornkit V, Edwards DP: Receptor mechanisms mediating non-genomic actions of sex steroids. *Semin Reprod Med* 25:139-153, 2007
14. Moriarty K, Kim KH, Bender JR: Minireview: Estrogen receptor-mediated rapid signaling. *Endocrinology* 147:5557-5563, 2006
15. Song RX, Santen RJ: Membrane initiated estrogen signaling in breast cancer. *Biol Reprod* 75:9-16, 2006
16. Harrington WR, Kim SH, Funk CC, et al: Estrogen dendrimer conjugates that preferentially activate extranuclear, nongenomic versus genomic pathways of estrogen action. *Mol Endocrinol* 20:491-502, 2006
17. Zujewski JA, Kamin L: Trial assessing individualized options for treatment for breast cancer: The TAILORx trial. *Future Oncol* 4:603-610, 2008
18. Cardoso F, Van't Veer L, Rutgers E, et al: Clinical application of the 70-gene profile: The MINDACT trial. *J Clin Oncol* 26:729-735, 2008