The Expression of ERβcx in Human Breast Cancer and the Relationship to Endocrine Therapy and Survival

Carlo Palmieri,1 Eric W.-F. Lam,1 Janine Mansi,2 Claire MacDonald,2 Sami Shousha,3 Peter Madden,4 Yoko Omoto,5 Andrew Sunters,1 Margaret Warner,5 Jan-Åke Gustafsson,5 and R. Charles Coombes4

1Department of Cancer Medicine, Cancer Cell Biology Group, Cancer Research UK Laboratories, Imperial College-London, Hammersmith Hospital, London, United Kingdom; 2Department of Medical Oncology, St. George’s Hospital, London, United Kingdom; 3Department of Histopathology, Imperial College Faculty of Medicine and Charing Cross Hospital, London, United Kingdom; 4Department of Social Science and Medicine, Faculty of Medicine, Imperial College School of Science, Technology and Medicine, London, United Kingdom; and 5Department of Medical Nutrition and Biosciences, Karolinska Institute, Huddinge, Sweden

ABSTRACT

Purpose: Estrogen receptor (ER) α-positive breast cancer is often treated with endocrine therapy using either antiestrogens or aromatase inhibitors. However, 30% of patients who receive endocrine therapy will derive no benefit from such treatments and may indeed suffer adverse effects. Currently, there are no ways to predict response to such treatments. ERβcx, a variant of ERβ, has a dominant-negative effect over ERα, and its expression thought to modulate response to endocrine treatment may represent a predictor of response to endocrine therapy.

Experimental Design: We investigated the expression of the ERβcx in 82 frozen breast samples (8 benign, 1 ductal carcinoma in situ, and 73 malignant) by Western blot analysis. The relationship between the expression of ERβcx variants with prognosis and outcome of endocrine therapy was examined.

Results: There was a statistically significant association between the presence of ERβcx and the response to endocrine therapy (Fisher’s exact test, P = 0.04). We also examined the influence of the ERβcx status of a tumor on time to progression and death. There was a relationship between the presence of ERβcx and survival, with patients whose tumors express ERβcx having a longer survival rate (P = 0.05).

INTRODUCTION

One of the main features of breast cancer is that in some tumors there is a dependence on estrogen for continued growth. Consequently, endocrine therapy is one of the most widely used treatments for breast cancer both in the adjuvant and metastatic setting. The adjuvant use of the antiestrogen tamoxifen is associated with a reduction in relapse and death from breast cancer, as well as protection against contralateral breast cancer (1). The most reliable predictors of response to hormonal therapy at present are estrogen receptor (ERα) and progesterone receptor (2–4). However, the predictive value of ERα is not perfect, because ~30% of metastatic ERα-positive tumors fail to respond to endocrine therapy (5). An improvement in the ability to predict the outcome of response to endocrine therapy would prevent patients from receiving inappropriate treatment and could also enhance the prognostic stratification of ERα-positive patients.

ERα and ERβ represent distinct gene products, and have 96% and 60% homology in the DNA-binding domains and ligand-binding domains, respectively (6, 7). Since the initial cloning of ERβ, several splice variants of this receptor have been identified in humans and other species (6–13); however, the role of ERβ in breast cancer is still unclear. Leygue et al. (14) found that those tumors coexpressing ERα and ERβ were node positive and tended to be of a higher grade, whereas Jarvinen et al. (15) proposed that ERβ was often associated with lower-grade tumors and negative axillary node status. A decreased expression of ERβ in proliferative preinvasive tumors has been reported (16), whereas Speirs et al. (17) found that ERβ was increased in expression in tamoxifen-resistant tumors. ERβ expression has also been found to be associated with elevated levels of the proliferation markers Ki67 and cyclin A, especially in recurrent cancers (18). A novel human ERβ variant, which is truncated at the COOH terminus, was identified in 1998 by the screening of a human testis cDNA library (19). It was named hERβcx by Ogawa et al. (19) and hERβ2 by Moore et al. (20), and will be referred to as ERβcx in the present study. The coding sequence of this variant was found to be identical to ERβ cDNA except that the COOH-terminal 61 amino acids of ERβ were replaced by a unique 26 amino acid sequence, resulting in a protein with 495 amino acids (M, 55,000). The 61 amino acids that are replaced in ERβcx are encoded by exon 8 of the ERβ gene, making up the AF-2 core, which is essential for ligand-dependent transcriptional activation. The ERβcx variant...
has been found to be expressed in ovary, testis, prostate, and thymus (19, 21–23). Transfection of ERβcx expression constructs into COS cells did not reveal either detectable binding of estrogen in whole cell extracts or activation of an estrogen response element–containing reporter plasmid in either the presence or absence of estrogen (19, 24). Consistent with the presence of both the DNA-binding and dimerization domains, mobility shift assays have shown that ERβcx binds to DNA containing an estrogen response element consensus sequence as a heterodimer with either ERα and ERβ1 (20). It was also found that ERβcx was unable to interact with TIF1α, a co-regulator that interacts with ligand activated hERα via the AF-2 domain (19). ERβcx preferentially forms heterodimers with ERα and ERβ; whereas ERα is inhibited by ERβcx, ERβ1 is unaffected, suggesting that ERβcx can act as a dominant-negative inhibitor of ERα. In support of this notion, Tremblay et al. (25) have shown that in mice ERα and ERβ lacking an intact AF-2 can impair transcriptional activity of the ERα-ERβ heterodimer.

The often contradictory results published regarding the role of ERβ in breast cancer could be related to the presence or absence of a number of splice variants, which exert dramatically different biological effects, in particular ERβcx. ERβ is known to be expressed in both normal and malignant breast tissue, and presence of ERβcx in breast cancer has been demonstrated by both PCR and Western blot of sucrose density gradients (26). In certain breast tumors, ERβ was detected but there was no 4S estradiol binding peak in sucrose density gradients, and subsequent PCR revealed the presence of ERβcx. This observation using human breast tissues supports the results of the in vitro experiments of Ogawa et al. (19) that ERβcx can dimerize with ERα and negatively modulate its ligand-binding activity.

Consequently, we sought to determine the expression patterns of ERβcx in normal and malignant breast tissue to correlate this expression with clinical outcome. Frozen breast samples (82) were analyzed for ERβcx expression by Western blotting, and in a complementary approach, the cell-type specificity was analyzed by immunohistochemistry. The relationship between the expression of ERβcx variants with prognosis and outcome of endocrine therapy was also examined.

### MATERIALS AND METHODS

**Breast Cancer Samples.** Frozen breast tissue samples (82) were collected from Charing Cross Hospital and St. George’s Hospital, and analyzed for their ERβcx content. Samples were composed of 8 benign, 1 ductal carcinoma in situ, 63 invasive ductal carcinoma, 5 medullary carcinoma, and 5 lobular carcinoma (Table 1). Information on patient age, menopausal status, pathological diagnosis, and differentiation grade were recorded, and the notes reviewed for response to endocrine treatment.

**Assessment of Response to Endocrine Therapy.** Clinical records of 23 patients who had assessable disease and had received neoadjuvant or palliative hormonal treatment were identified and evaluation of response to hormonal treatment determined. Response was evaluated using Response Evaluation Criteria in Solid Tumors guidelines (27). Complete response was defined as resolution of all detectable disease for 4 weeks; partial response was defined as ≥50% reduction in tumor size as determined by two measurements of tumor diameter or the product of two perpendicular diameters, at least 4 weeks apart. No change was defined as <50% tumor regression or <25% progression, and progressive disease as ≥25% increase in tumor measurements. Time to progression was defined as the time

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* Mean, (median).
* SD, (range).
* t test.
* Mann-Whitney.
from initial diagnosis to documented date of first relapse, and time to death was defined as the time from initial diagnosis to death.

Preparation of Cell Lysates from Frozen Tissue Samples. Frozen tissue samples were pulverized in a dismembrator (Braun Melsungen, Melsungen, Germany) for 45 s at 1800 rpm. Pulverized tissue was added to a buffer composed of 10 mM Tris-HCl (pH 7.5), 1.5 mM EDTA, and 5 mM sodium molybdate, using 1 ml/100 μg tissue. Cytosol was obtained by centrifugation of the homogenate at 204,000 × g for 1 h in a 70Ti (Sorvall) rotor at 4°C.

Immunohistochemistry for Erβcx. Immunohistochemistry was performed on paraffin sections of breast tissue obtained from the histopathology archive at Charing Cross Hospital using a new anti-Erβcx sheep polyclonal antibody that recognizes the Erβcx-specific sequence (MKMETLLPEATMEQ) at the COOH terminus of Erβcx but not other ER variants; hence, this antibody is specific for Erβcx. The sections were also stained with a rabbit polyclonal protein A purified polyclonal antibody that reacts with the NH2-terminal region (YAPQKSPWCEARSLEHT, amino acids 46–63) of Erβ (28), and this antibody recognized the majority of Erβ isoforms. Two-μm paraffin sections were dewaxed in xylene and rehydrated through graduated alcohol to water. Antigen retrieval was performed by microwaving sections in 5% urea for 15 min at 800 W. Endogenous peroxidase was blocked by incubation for 30 min with a solution of 0.3% hydrogen peroxide at room temperature and washed twice for 5 min in PBS. Tissue sections were incubated for 10 min at room temperature with normal rabbit serum diluted at 1:10 in PBS and excess was removed. Sheep anti-Erβcx sheep polyclonal antibody (1:800 dilution) in PBS was then applied to sections incubated overnight at 4°C. Negative controls consisted of the substitution of the primary antibody with PBS and with preabsorbed antibody. Sections were subsequently rinsed in PBS three times for 5 min before addition of the secondary antibody. Biotinylated antiship antibody (1:200 dilution) in PBS was applied for 60 min at room temperature and subsequently washed off with PBS. Slides were then incubated with Vectastain ABC Regent (Vector Lab) for 1 h at room temperature and then washed in PBS. Color was developed with diaminobenzidine tetrahydrochloride. Sections were counterstained, dehydrated through graduated alcohol to xylene, and mounted with pertex. Evaluation of immunohistochemistry was carried out according to the methods published by Harvey et al. (29) and Leake et al. (30).

Briefly, this involves using a simple, additive scoring system based on the proportion and intensity of staining, resulting in a score of 0–8. Any score >2 were considered positive. This is the same method of evaluation and cutoff score used by Saji et al. (28) in their evaluation of Erβcx staining. Staining for Erα was performed using the mouse monoclonal Erα (F-10; Santa Cruz Biotechnology) antibody described as before.

Western Blotting. For Western blotting, proteins were size fractionated on 8% SDS-PAGE gel and electroblotted on to polyvinylidene difluoride membrane. After transfer these blots were probed using a specific Erβcx antibody raised in sheep (28). Of the 82 samples, 8 were benign, 1 was a ductal carcinoma in situ, 63 were invasive ductal carcinomas, 5 were medullary carcinomas, and 5 were lobular carcinomas. The characteristics of the tumors are summarized in Table 1. In total, 37 (45%) were positive and 45 (55%) negative for Erβcx. Of the benign samples, 25% were Erβcx positive and 75% were Erβcx negative. For the malignant breast cancers, 48% were Erβcx positive and 52% Erβcx negative. Fig. 1 shows representative Western blots for Erβcx.

RESULTS

Expression of Erβcx in Human Breast Samples. Cell lysates were prepared from 82 frozen breast tissue samples, and proteins were size fractionated on 8% SDS-PAGE gel and electroblotted on to polyvinylidene difluoride membrane. After transfer these blots were probed using a specific Erβcx antibody described (diluted 1:500). Bound antibodies were detected using a horseradish peroxidase-conjugated donkey anti-sheep antibody and goat-antirabbit antibody (diluted 1:10,000; both from Dako). Antibody staining was detected using the enhanced chemiluminescence visualization system (ECL; Amersham Pharmacia Biotech) according to the manufacturer’s instructions. All of the immunoblots were strained with Ponceau S (Sigma) after electroblotting to ensure even-transfer and protein integrity. The “nuclear” protein integrity was additionally confirmed by staining with two pan-Erβ antibodies, which recognize the majority of all known Erβ isoforms (data not shown).

Every set of samples was electrophoresed with ~5 ng of recombinant Erβcx and Erβ1 (see Fig. 2A controls) as an expression level/exposure standard. Western blots were simply scored positive or negative based on the presence of the appropriate sized band for Erβcx. The data from Western blotting and immunohistochemical staining were then assessed to see if a correlation existed between these two methods.

Statistical Analysis. Associations between Erβcx status and a number of clinicopathological features (menopausal status, size, l-V invasion, nodal status, ER, and age) and response to endocrine therapy (complete or partial response, no change, and progressive disease) were investigated using χ tests for categorical variables (where applicable, Fisher’s exact tests were used) and both t and Mann Whitney tests for continuous data.

Kaplan-Meier plots were used to compare survival (death due to breast cancer) and relapse between the two groups with respect to Erβcx status, and differences were assessed using log rank tests. Estimates of relative survival were obtained using Cox proportional hazards models. Stata 7 program was used for all of the statistical analyses.

Immunohistochemical Staining for Erβcx in Breast Tissue. Given that frozen breast tissue was used for the Western blotting, which could be composed of both epithelial and stromal components, the results of the Western blots were validated by a small immunohistochemistry study. This revealed that of the 23 samples stained, >80% correlated with the Western results. In addition, the immunostaining revealed predominantly epithelial staining; however, some stromal staining was also present (Fig. 2).
Association between ERβcx Status and Histopathological Features. An analysis of whether any of the clinical or histopathological characteristics of the studied patients were associated with ERβcx status was conducted. This revealed no statistically significant associations between ERβcx and any of the clinicopathological features of the breast tumors. These results are summarized in Table 1.

Analysis of ERβcx Status, and Time to Progression and Death. To determine whether ERβcx status was predictive of time to disease progression or cancer death, the clinical notes of all of the women were reviewed to assess whether they had relapsed and/or died, and the time of these events from the initial diagnosis of breast cancer was recorded in months.

There were 15 relapses in the ERβcx-positive and 21 relapses in the ERβcx-negative group. Fig. 3 shows the Kaplan-Meier survival curves for relapse by ERβcx status. There was no statistically significant difference in relapse between the two groups (log rank test for equality of survivor functions $x^2 = 2.32, P = 0.13$ hazard ratio of 0.60 [95% confidence interval (CI), 0.31–1.17] from Cox regression). Seven patients in the ERβcx-positive and 13 in the ERβcx-negative group died because of breast cancer. There was a statistically significant difference in survival between the two groups (log rank test for equality of survivor functions, $x^2 = 3.86; P = 0.05$; hazard ratio, 0.41; 95% CI, 0.16–1.03), and the Kaplan-Meier plot is shown in Fig. 3.

We also investigated the relationship of ERα status with time to relapse and death. There were 23 relapses in the ERα-positive and 12 relapses in the ERα-negative group. There was no statistically significant difference in relapse between the two groups (log rank test for equality of survivor functions, $x^2 = 0.13; P = 0.72$ hazard ratio of 0.88; 95% CI, 0.44–1.78) from Cox regression. There were 11 deaths in the ERα-positive and 9 in the ERα-negative group. There was again no statistically significant difference in death between the two groups (log rank test for equality of survivor functions $x^2 = 1.59; P = 0.21$ hazard ratio of 0.57; 95% CI, 0.24–1.38) from Cox regression.

After adjusting for ERα status, the hazard ratio for ERβcx in relation to time to relapse changed slightly to 0.62 (95% CI, 0.31–1.21). In adjusting for ERα, there was a decrease from 73 to 69 in the number of patients with complete data. After adjusting for ERα, the hazard ratio for ERβcx in relation to death remained the same at 0.41 (95% CI, 0.16–1.03), despite a slight reduction in the number of cases with complete records from 73 to 69. In conclusion, these data suggested that ERα status alone or in association with ERβcx status does not offer better prediction of time to relapse or death than ERβcx status on its own.

Association between ERβcx Status and Response to Endocrine Treatment. The records of all patients were reviewed, and patients who had received either neoadjuvant or palliative endocrine therapy and who had measurable disease were identified. The response to endocrine therapy was assessed according to the Response Evaluation Criteria in Solid Tumors guidelines (27). In a total of 23 patients identified, 12 ERβcx were positive and 11 ERβcx negative. Of the 12 ERβcx-positive tumors, 6 had a complete or partial response, 5 had no change, and 1 had progressive disease, whereas among the 11 ERβcx negative patients, 4 had a complete or partial response, 1 had no change, and 6 had progressive disease (Table 2). Statistical analysis revealed that there was a statistically significant association between the ERβcx expression within breast tumors and the response to endocrine therapy (Fisher’s exact test, $P = 0.04$). The response to endocrine therapy was also reanalyzed based on ERα status (Table 2). The result showed that there was no evidence of a statistically significant association between ERα and response to endocrine therapy (Fisher’s exact test, $P = 0.16$). However, due to the small sample size concerning response to endocrine therapy and the fact that the majority of the samples were ERα positive (20 of 23; 87%), it was not possible to assess whether the association between ERβcx and response to endocrine therapy was confounded by ERα status.

**DISCUSSION**

Initial studies demonstrated that ERβ is expressed in breast cancer and plays a role in breast cancer cell proliferation, and resistance to endocrine therapy was proposed (18, 26). We have shown that the splice variant ERβcx was expressed in a limited number of breast cancer samples, but in these tumors there was an absence of estradiol binding to the 4S peak corresponding to the ERβ containing fractions in density gradients experiments (20, 31). Whereas the exact mechanism for ERβ function in modulating estrogen signaling is unclear, it is interesting to note that the ERβ splice form ERβcx is capable of heterodimerizing with ERα, and yet does not bind estradiol, suggesting a dominant-negative effect on ERα function (20, 31). These results led us to hypothesize that ERβcx expression in breast cancer could modulate the response to antiestrogens in cells coexpressing ERα, and, thus, influence clinical outcome.

In the current study we have demonstrated that ERβcx protein is expressed in 48% of breast cancer samples and 25% of the benign samples by Western blotting with a specific antibody to ERβcx. We also validated the expression of ERβcx using immunohistochemistry in a number of cases and showed that the immunohistochemical analysis generally correlated with the Western blot results. Statistical analysis revealed that there was no significant associations between the expression of ERβcx and any of the clinicopathological features. We then examined the relationship between ERβcx status, and the time to progression and death from breast cancer in this group of
Fig. 2  Analysis of estrogen receptor (ER$\beta$) and ER$\beta$cx expression by Western blotting and immunostaining in 10 representative breast tumors. A, representative Western blot analysis of ER$\beta$ and ER$\beta$cx expression in breast tumor samples from 10 patients. B, representative immunostaining of paraffin sections of breast tissue obtained from patients whose ER$\beta$ and ER$\beta$cx expression were investigated by Western blotting as in (A; ×400 magnification). Sections a, c, e, g, i, and j were stained with the ER$\beta$cx-specific antibody and b, d, f, h, k, and l were stained with the ER$\beta$-specific antibody. Sections a and b were from patient 1, c and d were from patient 3, e and f were from patient 7, and g and h from patient 9. Slides j and l were stained in the presence of ×100 molar excess of the immunogen over the antibody.
The Expression of ERβcx in Human Breast Cancer

There was also no evidence of an interaction between ERα and response to endocrine therapy (Fisher’s exact test, \( P = 0.16 \)). However, we were unable to test whether the association between ERβcx and response to endocrine therapy was confounded by ERα status, because the majority of the samples were ERα positive (20 of 23; 87%). The high percentage of ERα-positive samples probably reflects the rationale behind endocrine therapy, because antiestrogens are the treatment strategy adopted for ERα-positive patients.

This is the first investigation to assess the relationship of ERβcx status to response to endocrine therapy, time to progression, and death in breast cancer using frozen clinical samples. Omoto et al. (32) have shown previously by reverse transcription-PCR analysis that ERβcx and ERβ5 mRNAs are more highly expressed than ERβ1 transcripts, with ERβcx mRNA expression significantly higher in breast cancers than normal tissue. In this particular study, immunohistochemical staining of clinical samples was also carried out using three different ERβ antibodies and one ERβcx antibody. The authors detected an association of ERβ expression with low histopathological grade breast cancer, but did not perform any correlation analysis between ERβcx positivity and any clinicopathological factors. The difference between the present study, which found an association between survival and the presence of ERβcx, and that of Omoto et al. (32) could be due to a number of factors. Primarily, the COOH-terminal region of ERβ was assessed differently in the two studies. Our current study used an ERβcx COOH-terminal-specific antibody, whereas the negative sample group in the study of Omoto et al. (32) could also consist of samples with an alternatively truncated ERβ COOH terminus or other COOH-terminal variants not recognized by their ERβ1. Furthermore, in the study by Omoto et al. (32), a sample was taken to be ERβcx positive if 25% of nuclei were stained positive, potentially allowing for a great deal of heterogeneity within the positively scored epithelial group, and possibly a majority of the sample could be negative as a whole and yet the sample classified as positive. Finally, the follow up in the current study was more than twice as long as that by Omoto et al. (32), and this longer follow up would be more likely to reveal any effects of ERβcx on clinical outcome, if one existed.

Some clinical correlations with ERβcx expression have been observed previously. In a microarray analysis of 82 normal and malignant breast samples a cluster of 20 tumors with reduced expression of ERβcx was observed (33, 34). This group

![Kaplan-Meier survival estimates for relapse by ERβcx status (n=72)](image)

**Fig. 3** Statistical analysis of time to first relapse and death from breast cancer in relation to estrogen receptor (ER) βcx. A, statistical analysis of time to first relapse from breast cancer with reference to ERβcx by the Kaplan-Meier method. There was no significant difference between relapse in those positive and those negative for the ERβcx (log rank statistic = 2.32; \( P = 0.13 \)), hazard ratio from Cox regression 0.60 (0.31–1.17). B, statistical analysis of death from breast cancer in relation to ERβcx by the Kaplan-Meier method. The result showed a significant difference between survival in those positive and those negative for the ERβcx receptor (log rank statistic = 3.86; \( P = 0.05 \)), hazard ratio from Cox regression, 0.41 (0.16–1.03).

women. With reference to the time to progression there was no statistical difference based on the ERβcx status of the breast tumors. However, when survival was investigated there was evidence of a statistically significant relationship between ERβcx status and survival, with ERβcx-positive patients having a survival advantage, hazard ratio of 0.41, and 95% CI, 0.16–1.03. These results, whereas significant, should be interpreted cautiously due to the relative small number of cases. An additional analysis should be undertaking adjusting for other prognostic/histopathological features. Because ERα status has been shown previously to be useful for predicting overall patient survival as well as response to endocrine therapy in breast cancer, we also compared the ability of ERβcx and ERα status in predicting relapse, patient survival, and response to endocrine therapy. Our result showed that ERα status does not offer better prediction of time to relapse and survival than ERβcx status. There was also no evidence of an interaction between ERβcx and ERα for either death (likelihood ratio test, \( P = 0.66 \)) or time to relapse (likelihood ratio test, \( P = 0.16 \)). When the response to endocrine therapy was reanalyzed based on ERα status, the result again showed no evidence of a statistically significant association between ERα and response to endocrine therapy (Fisher’s exact test, \( P = 0.16 \)). However, we were unable to test whether the association between ERβcx and response to endocrine therapy was confounded by ERα status, because the majority of the samples were ERα positive (20 of 23; 87%). The high percentage of ERα-positive samples probably reflects the rationale behind endocrine therapy, because antiestrogens are the treatment strategy adopted for ERα-positive patients.

**Table 2** Endocrine therapy response

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<th>Response</th>
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*PR, partial response; CR, complete response; NC, no change; PD, progressive disease.
of tumors was found to have a high number of node-positive breast cancers, and was at increased risk of developing metastatic breast cancers, as well as distant metastasis at the time of diagnosis. In addition, Saji et al. (28), in the correlation of pathological characteristics of the breast cancers with ERβcx, found that vascular invasion correlated significantly with an ERβcx-negative phenotype. The studies of Ahr et al. (33, 34) and Saji et al. (28) also highlighted the fact that the loss of ERβcx may be associated with a phenotype that is generally: (a) more aggressive; (b) more likely to invade vasculature; (c) has a higher number of lymph nodes involved; (d) is more likely to have distant metastasis at the time of diagnosis; and (e) has an increased risk of developing metastatic disease. These reports generally support the notion that high levels of ERβcx expression correlate with better prognosis.

Results from the current study showed that the presence of ERβcx correlates with favorable response to endocrine therapy. In the ERβcx-positive tumors, 6 of 12 (50%) had either a partial or complete response to endocrine therapy, compared with 4 of 11 (36%) who were ERβcx negative. With regard to progressive disease, 1 of 12 (8%) in the ERβcx-positive group developed progressive disease, whereas on endocrine therapy, this compared with 6 of 11 (55%) who were ERβcx negative. This represents a statistically significant association between the presence of ERβcx and response to endocrine therapy (Fisher’s exact test, \( P = 0.04 \)). When patients were included in the analysis who had a stabilization of their disease and could, therefore, be interpreted as to have derived clinical benefit from endocrine therapy, there were 11 of 12 (92%) in the ERβcx-positive group and 5 of 11 (45%) in the ERβcx negative group. This finding of ERβcx being associated significantly with response to endocrine therapy is in contrast to the result of Saji et al. (28). There are a number of potential reasons for the discrepancy. Firstly, the populations studied and the techniques used differed. In this study the population had locally advanced or metastatic disease, and ERβcx expression was determined using Western blotting, whereas the population in the study by Saji et al. (28) was newly diagnosed and treated neoadjuvantly, and immunohistochemistry was used to measure ERβcx. Secondly, Western blotting was used to assess the ERβcx status in the frozen breast tissue in the current study, whereas Saji et al. (28) used immunohistochemistry. However, our Western blot data were also validated by parallel immunohistochemical analysis. It should also be noted that in both Saji et al. (28) and the current study, the number of patients assessed was small (18 and 23, respectively). Nevertheless, the current study has a longer follow-up, and the results are statistically significant.

There is still considerable controversy concerning the significance and function of ERβcx in breast cancer. Whereas this study has shown evidence of a direct and significant link between the expression of ERβcx and the response to endocrine therapy, the mechanism for this role is not clear. The dependence of tumors on estrogen, and, hence, their suitability for endocrine therapy, is known to be associated with the expression of functional ERαs. The rationale behind endocrine therapy for breast cancer is that ERα-positive breast tumors depend on estrogen for growth and survival. As a result, ER antagonists, such as tamoxifen and faslodex, or aromatase inhibitors (compounds that inhibit local synthesis of estrogens from circulating C19 steroids) were used to disrupt the estrogen-dependent signaling (35). Given that ERβcx can have a dominant-negative effect on estrogen-dependent signaling through forming inactive heterodimers with ERα (19, 20, 24), it is, therefore, possible that the expression of ERβcx could block estrogen-dependent signaling via ERα and potentially synergize with antiestrogens, such as tamoxifen, thus making endocrine therapy more effective. If this hypothesis is correct, expression of ERβcx could be beneficial for the prognosis of breast cancer patients. Nevertheless, the modulation of ERα-dependent estrogen signaling by ERβcx and ERβ, and, thus, the success of endocrine therapy, would appear to be more complicated than first thought, and may involve a complex interplay between different ERβ variants and ERα in the epithelium, as well as in the stroma. To investigate in greater detail and to define accurately and definitively the role and predictive value of ERβcx, a larger immunohistochemical study with greater power using archival paraffin material from patients who have been treated and followed up for a long period of time is currently underway.

REFERENCES


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