Extranuclear expression of hormone receptors in primary breast cancer


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Background: Hormone receptor (HR)-positive breast cancer cells grow through estrogen receptor (ER)-signaling pathways that mediate both genomic and nongenomic actions, which cross-talk with growth factors associated with resistance to tamoxifen. The aim of this study was to explore the cross-talk between extranuclear expression of ER and progesterone receptor (PR) and growth factor signaling pathways in primary breast cancer.

Patients and methods: The extranuclear expression of ER and PR was examined in 219 primary breast cancers by immunohistochemical staining. Specimens showing such expression were further examined for the expression of pAkt and aromatase. Staining reactions were scored on the basis of intensity and distribution in the tumors.

Results: Extranuclear expression of ER or PR was observed in 21 cases (9.5%), which included four cases for ER and 20 cases for PR. Among these patients, HER-2, pAkt, and aromatase-positivity were observed in 14 cases (66.6%), 13 cases (61.9%), and 14 cases (66.6%), respectively. On the basis of nuclear HR expression, 11 of these cases were categorized as ER-positive/PR-negative, while two were ER-negative/PR-positive. Of these 13 cases, increased pAkt staining was found in 11 cases (84.6%). In particular, among the 11 ER-positive/PR-negative cases, elevated pAkt and aromatase were found in 10 (90.9%; \( P < 0.01 \)) and nine cases (81.8%), respectively.

Conclusions: PR is expressed extranuclearly more frequently than ER in primary breast cancer, and extranuclear HRs cross-talk with the Akt/HER-2-signaling pathways and activation of aromatase. These observations may explain the more beneficial effects of aromatase inhibitors than tamoxifen for ER-positive/PR-negative patients.

Key words: hormone receptor, extranuclear expression, Akt, HER-2, breast cancer

Introduction

Proliferation of breast cancer cells is divided into hormone-dependent and -independent tumor growth; the former is regulated by the expression of hormone receptors (HRs) such as estrogen (ER) and progesterone receptors (PR) [1, 2]. Estrogens such as estradiol are a crucial growth factor for HR-positive breast cancer cells. Growth stimulation is mediated by the genomic action of nuclear ERs: estradiol binds to these receptors causing them to dimerize and translocate to ER-responsive elements of target genes [3]. Coactivators such as amplified in breast cancer-1 (AIB1) [4] and p300/CBP-associated factor (PCAF) [5] then bind to the ER-DNA complex, resulting in the activation of various target genes such as c-Myc [6], VEGF [7], Bcl-2 [8], IGFR-1 [9], IRS1 [10], TGF-\( \alpha \) [11], and cyclin D1 [12]. The expression of these target genes is coregulated by corepressors such as nuclear receptor corepressor (NCoR) [13] and histone deacetylase 1 (HDAC1) [14] which compete with coactivators for binding, and mediate the inhibitory effects of estrogen receptor antagonists such as tamoxifen. In addition to this so-called classical pathway, ER can also activate gene expression through a nonclassical pathway, in which ER interacts with Jun-Fos heterodimers and causes coactivators to bind to AP-1 sites of target genes [15].

In contrast, extranuclear ER expressed in the cell membrane and cytoplasm modulates the proliferation of breast cancer cells nongenomically via crosstalk with components of growth factor-signaling pathways such as PI3K/Akt and MAPK [3, 16]. Tumors with extranuclear expression of ER or PR are included in the population of ER-negative or PR-negative cases, which are more resistant to tamoxifen than ER/PR-double positive patients [17, 18]. Nevertheless, either ER-negative or PR-negative patients are more sensitive to aromatase inhibitors than tamoxifen [19–22]. In fact, recent results of the pivotal ATAC trial of the therapeutic efficacy of aromatase inhibitor (AI) in postmenopausal breast cancer showed that the survival benefit of anastrozole is greater than that of tamoxifen, in particular for ER-positive/PR-negative patients [21, 22]. In addition, the...
IMPACT trial of neoadjuvant therapy for postmenopausal breast cancer showed that HER-2-positive patients were more responsive to aromatase inhibitor than tamoxifen [23]. Further, some previous experimental models suggested that resistance to tamoxifen is produced by cross-talk with growth factors, and in these situations, tamoxifen exerts an agonistic effect through the activation function-1 (AF-1) activity of ER [17]. In contrast to the ATAC trial, a subgroup analysis in the recent findings of the BIG 1–98 trial showed that the beneficial effect of letrozole was seen in patients with HR-positive tumors irrespective of their PR status [24]. Although the predominant effect of AIs for ER-positive/PR-negative patients remains controversial, the benefit of AIs to either ER- or PR-negative patients, in particular for ER-positive/PR-negative patients, and the lesser efficacy of tamoxifen may be explained by cross-talk between HRs and growth factor-signaling pathways in these patients. Nevertheless, extranuclear expression of ER and PR, which is critical for crosstalk with growth factors, has not been reported thus far in clinical samples of primary breast cancer.

Based on this background, the aim of the present study was to explore the cross-talk between extranuclear expressions of ER and PR and growth factor signaling pathways in primary breast cancer, which may provide a rationale for selecting anti-estrogens in either ER or PR-positive patients.

patients and methods

patients
Two hundred nineteen primary breast cancers, which were resected in the Department of Surgical Oncology, Research Institute for Radiation Biology and Medicine, Hiroshima University (Hiroshima, Japan) between October, 2001 and July 2004 were studied. The clinical stage and pathological characteristics were described according to the general rules for the clinical and pathological recording of breast cancer established by the Japanese Breast Cancer Society [25].

immunohistochemical staining and assessment
Immunohistochemical staining for ER, PR, and HER-2 was performed according to previously reported methods [26, 27]. All specimens were fixed in 10% formalin, and routinely processed for embedding in paraffin. Tissue sections were deparaffinized in xylene and rehydrated through 100%, 95% and 90% ethanol. To overcome cross-linking due to formalin fixation, the tissue sections were placed in an epitope retrieval solution fixed in 10% formalin, and routinely processed for embedding in paraffin.

Tissue sections were deparaffinized in xylene and rehydrated through 100%, 95% and 90% ethanol. To overcome cross-linking due to formalin fixation, the tissue sections were placed in an epitope retrieval solution composed of 10 mM citrate buffer (pH 6) and heat treated in a water bath at 95°C for 40 min followed by a 20 min cool-down period at room temperature (RT). The tissue sections were immunostained for ER and PR using an automated staining system (DAKO Autostainer, DakoCytomation A/S, Glostrup, Denmark). In brief, all sections were reacted with 0.3% hydrogen peroxide solution containing 15 mM sodium azide for 10 min at RT to block endogenous peroxidase activity, and then thoroughly rinsed in a washing buffer (50 mM Tris–HCl buffer containing a detergent, pH 7.6). The sections were incubated with anti-ER and anti-PR mAbs (ER1D5 and PR10A9, respectively, Immunotech, Marseille, France) at RT for 30 min, and then washed three times with the washing buffer. Bound antibodies were detected by 30 min incubation with dextran polymer reagent conjugated with peroxidase and secondary antibody (DAKO EnVision+, DakoCytomation A/S). The tissues were then washed three times with the washing buffer. Color development was achieved with 3,3′-diaminobenzidine (DAKO DAB+ Liquid System, DakoCytomation A/S) for 10 min. The tissues were counterstained with hematoxylin at RT for 2 min. If more than 10% of tumor cells showed nuclear staining for ER or PR, the specimen was considered positive for that receptor. The staining intensity of ER and PR was classified into four categories: 0, negative; 1, weak; 2, moderate; 3, strong. In contrast to nuclear staining of HRs, the extranuclear staining pattern of ER and PR in the membrane and cytoplasm was observed in three types: diffuse staining in the cytoplasm, D; punctate staining in the cytoplasm, P; membrane staining, M.

For assessment of HER-2, the Hercep TestTM (DAKO, Kyoto, Japan) was used accordingly to the manufacturer’s protocol. In brief, after being deparaffinized the sections were incubated in a water bath for 40 min at 95°C in a 10 mmol/l citrate buffer (pH 6.0). The slides were incubated for 30 min with the primary rabbit polyclonal antibody which was supplied prediluted in the kit. The immunoreaction was visualized by incubating slides at RT with the DAKO Visualization Reagent (DAKO A/S) (dextran polymer conjugated with horseradish peroxidase and goat-antirabbit immunoglobulin) for 30 min, followed by the chromogen 3,3′-diaminobenzidine tetrahydrochloride for 10 min. Negative controls consisted of substituting normal rabbit serum for the primary antibody. Assessment of Hercep TestTM was performed according to the following criteria: score 0, no staining at all, or membrane staining was observed in less than 10% of the tumor cells; 1, a faint/barely perceptible membrane staining was detected in more than 10% of the tumor cells; 2 and 3, a weak to moderate staining or a strong staining of the entire membrane was observed in more than 10% of the tumor cells. Only scores 2 and 3 were assessed as overexpression. For assessing the expression of pAkt and aromatase, the tissues were deparaffinized, and immunohistochemical staining was performed according to the manufacturer’s protocol (Santa Cruz Biotechnology, Inc., California). The slides were incubated with primary antibody for pAkt [p-Akt1/2/3 (Thr 308), rabbit polyclonal, Santa Cruz Biotechnology, Inc., California] and for aromatase (cytochrome P450 aromatase, H4 clone, mouse monoclonal, Serotec, Oxford), respectively. They were then incubated with the dextran polymer reagent conjugated with peroxidase and secondary antibody, and color development was achieved with 3,3′-diaminobenzidine. The staining grades were classified as follows: score 0, negative; 0.5, partial and weak; 1, weak; 2, moderate. Given that tumor cells in some specimens showed did not stain uniformly, the nuclear expression of pAkt was assessed by total scores derived from the intensity of staining and percentage of tumor cells. The score is the product of the intensity and percentage. In detail, if in the membrane and cytoplasm was observed in three types: diffuse staining in the cytoplasm, D; punctate staining in the cytoplasm, P; membrane staining, M.

statistical analysis
The data were analyzed using StatView (5.0) for Windows. The relationship between the expression of ER/PR, and HER-2, pAkt, and aromatase was tested by the chi-squared test of independence. P-values less than 0.05 were considered to be statistically significant.

results
distribution of expression of ER and PR
Among 219 breast cancer patients, distribution of the nuclear expression of ER and PR are summarized in Table 1.
ER/PR-double positive tumors were observed in 136 cases (62.1%), whereas ER/PR-double negative tumors were observed in 45 cases (20.5%). Of these 45 cases, extranuclear expression of ER and/or PR was found in 8 cases. ER-positive/PR-negative tumors were observed in 27 cases (12.3%), whereas ER-negative/PR-positive tumors were observed in 11 cases (5.0%). Eleven of the 27 ER-positive/PR-negative cases and two of the 11 ER-negative/PR-positive cases showed extranuclear expression of ER and PR. Thus, the overall frequency of extranuclear expression was 13 of 38 cases (34.2%) in which tumors were negative for either ER or PR, and eight of 45 cases (17.8%) which were negative for both receptors.

**Characteristics of patients with extranuclear expression of ER and PR**

Extranuclear expression of ER or PR was found in 21 cases (9.5%), which included four cases for ER and 20 cases for PR. Only two cases showed both nuclear and extranuclear expression of ER and PR. The clinical and pathological characteristics of these 21 patients are summarized in Table 2. Most of these patients were postmenopausal (17 cases). One of the four cases with extranuclear ER expression also showed nuclear expression of ER. Similarly, one of 20 the cases with extranuclear PR expression showed nuclear expression of PR. These findings seem to indicate that coexistence of nuclear and extranuclear expression of HRs is rare. It is noteworthy that, although the frequency of the extranuclear expression of HRs

**Table 1. Distribution of expression of ER and PR in 219 patients with breast cancer**

<table>
<thead>
<tr>
<th>Hormone receptor</th>
<th>Progesterone receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>Positive</td>
</tr>
<tr>
<td>Estrogen receptor</td>
<td>Positive</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
</tr>
</tbody>
</table>

( ), extranuclear expression of ER and/or PR.

**Table 2. Characteristics and expression of HER-2, pAkt, and aromatase in 21 breast cancer patients with extranuclear hormone receptors**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/menopause</th>
<th>Histological type</th>
<th>pT/n</th>
<th>Nuclear grade</th>
<th>ER (Nuclear/extranuclear staining)</th>
<th>PR (Nuclear/extranuclear staining)</th>
<th>HER-2 Assessment/score</th>
<th>pAkt</th>
<th>Aromatase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>31/pre IDC (Sol-tub)</td>
<td>1b/−</td>
<td>3</td>
<td>0</td>
<td>0/1D</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>43/pre IDC (Sol-tub)</td>
<td>2/1</td>
<td>3</td>
<td>0</td>
<td>0/1D</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>52/post IDC (Sci)</td>
<td>4b/2</td>
<td>2</td>
<td>1</td>
<td>0/2P</td>
<td>0</td>
<td>1.3</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>53/post Paget</td>
<td>−/0</td>
<td>1</td>
<td>0</td>
<td>0/2M</td>
<td>3</td>
<td>0.8</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>41/pre IDC (Sol-tub)</td>
<td>2/3</td>
<td>3</td>
<td>1</td>
<td>0/2P</td>
<td>3</td>
<td>0.7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>56/post Paget</td>
<td>−/0</td>
<td>1</td>
<td>0/1D in situ</td>
<td>2/2D</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>61/post IDC (Sol-tub)</td>
<td>1b/0</td>
<td>2</td>
<td>0</td>
<td>0/2D</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>68/post IDC (Sci)</td>
<td>2/2</td>
<td>2</td>
<td>2</td>
<td>0/2D</td>
<td>2</td>
<td>0.8</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>58/post IDC (Sci)</td>
<td>3/2</td>
<td>3</td>
<td>0/1D</td>
<td>1</td>
<td>3</td>
<td>0.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>54/post IDC (Sci)</td>
<td>2/0</td>
<td>2</td>
<td>1</td>
<td>0/2P</td>
<td>3</td>
<td>1.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>52/post Muc</td>
<td>1b/0</td>
<td>1</td>
<td>3</td>
<td>0/2MD</td>
<td>0</td>
<td>0.9</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>48/pre IDC (Pap-tub)</td>
<td>1b/0</td>
<td>2</td>
<td>1</td>
<td>0/2D</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>66/post IDC (Sol-tub)</td>
<td>2/1</td>
<td>3</td>
<td>0</td>
<td>0/2P</td>
<td>1</td>
<td>0.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>70/post IDC (Pap-tub)</td>
<td>1c/0</td>
<td>1</td>
<td>3</td>
<td>0/2M</td>
<td>0</td>
<td>1.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>56/post IDC (Sol-tub)</td>
<td>2/0</td>
<td>1</td>
<td>0</td>
<td>0/2M</td>
<td>2</td>
<td>0</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>70/post IDC (Sol-tub)</td>
<td>1c/1</td>
<td>3</td>
<td>2/1D</td>
<td>0/2MD</td>
<td>2</td>
<td>0.8</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>55/post IDC (Sci)</td>
<td>1c/0</td>
<td>2</td>
<td>2</td>
<td>0/2M</td>
<td>2</td>
<td>0.8</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>79/post IDC (Sci)</td>
<td>1b/0</td>
<td>1</td>
<td>3</td>
<td>0/2PM</td>
<td>2</td>
<td>0.2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>70/post IDC (Sci)</td>
<td>1c/1</td>
<td>3</td>
<td>0</td>
<td>0/1D</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>58/post Med</td>
<td>1b/0</td>
<td>3</td>
<td>0/2D</td>
<td>0/2D</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>56/post IDC (Sci)</td>
<td>1c/0</td>
<td>1</td>
<td>3</td>
<td>0/2D</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

The total (%) of extranuclear expression of ER and/or PR was 13 of 38 cases (34.2%) in which tumors were negative for either ER or PR, and eight of 45 cases (17.8%) which were negative for both receptors.

A pronounced expression of ER and PR was observed in 21 cases (9.5%), which included four cases for ER and 20 cases for PR. Only two cases showed both nuclear and extranuclear expression of ER and PR. The clinical and pathological characteristics of these 21 patients are summarized in Table 2. Most of these patients were postmenopausal (17 cases). One of the four cases with extranuclear ER expression also showed nuclear expression of ER. Similarly, one of 20 the cases with extranuclear PR expression showed nuclear expression of PR. These findings seem to indicate that coexistence of nuclear and extranuclear expression of HRs is rare. It is noteworthy that, although the frequency of the extranuclear expression of HRs

The presented number is the score of staining intensity for nuclear expression of ER and PR, and extranuclear expression of ER and PR. In the extranuclear expression of ER and PR, the distribution was classified with membrane staining (M), diffuse staining in cytoplasm (D), and punctate staining in cytoplasm (P).

The expression of pAkt is calculated by the total score derived from percentage of staining and the intensity in tumor cells.

The total number means extranuclear expression of ER and PR, respectively in the first two columns. The number and percentage of HER-2, pAkt, and aromatase are presented in the last three columns.

The No. of ER+/PR− or ER−/PR+ (%) means the number of presence or absence of nuclear expression of ER and PR in the first two columns. The number and percentage of HER-2, pAkt, and aromatase in these populations are presented in the last three columns.
was around 10% among tumors assessed as negative for ER or PR but not both, ER-positive/PR-negative or ER-negative/PR-positive tumors accounted for 13 (61.9%) of the 21 cases of extranuclear expression (more frequently of PR than of ER). No particular relationship was found between extranuclear expression and the factors described in the table, including histologic type, tumor size, lymph node involvement, and nuclear grade.

typical extranuclear expression of PR, and expression of pAkt and aromatase

Examples of typical extranuclear expression, as assessed by the methods and criteria described above, are shown in Figures 1 and 2. Patient no. 10, a case of invasive ductal carcinoma, showed no nuclear expression of PR, but punctate staining of PR was observed in the cytoplasm (Figure 1b). The level of pAkt was assessed by the intensity and distribution of staining in the tumor cells; this specimen was scored as 1.6 (Figure 1c). Aromatase expression was assessed as weakly positive (score 1, Figure 1d). Patient no. 4 (Paget tumor) showed membrane staining of PR in the absence of the nuclear expression of ER (Figure 2b). The expressions of pAkt and aromatase were assessed as scores of 0.8 and 1, respectively (Figures 2c and 2d).

HER-2, pAkt, and aromatase in patients with extranuclear expression of ER and PR

In the 21 patients showing extranuclear HR expression, we assessed the levels of growth factor-signaling pathway components such as HER-2 and Akt that could crosstalk with the extranuclear receptors. Overexpression of HER-2 was observed in 14 cases (66.6%), while an increased level of pAkt was observed in 13 cases (61.9%). However, among the 13 ER-positive/PR-negative or ER-negative/PR-positive cases, elevation of pAkt was observed in 11 cases (84.6%) at a higher rate. Furthermore, pAkt was elevated in 10 of the 11 ER-positive/PR-negative cases (90.9%). In contrast, the HER-2 overexpression was observed in nine of the 13 single-receptor-negative cases (69.2%) and seven of the 11 ER-positive/PR-negative cases (63.6%). Since HER-2 overexpressing and ER-positive/PR-negative tumors are more responsive to aromatase inhibitor than tamoxifen, the expression of aromatase in the 21 patients with extranuclear expression of ER/PR was assessed. Increased aromatase expression was observed in 14 cases (66.6%), and in nine of the 13 ER-positive/PR-negative or ER-negative/PR-positive cases (69.2%). However, of the 11 ER-positive/PR-negative cases, increased aromatase expression was seen at the higher rate of 81.8% (nine cases). Of the eight cases which were negative for nuclear ER and PR and positive for extranuclear expression, the following results were noted: HER-2 overexpression was observed in five cases (62.5%); increased level of pAkt was observed in two cases (25%); and increased expression of aromatase was observed in five cases (62.5%) (Table 2). The ER-positive/PR-negative and ER-negative/PR-positive or negative groups were compared for the presence or absence of an increase in HER-2, pAkt, and aromatase because extranuclear expression of HRs was not observed in ER-positive/PR-positive tumors. Increased level of pAkt was
significantly associated with the expression of ER-positive/PR-negative tumors (chi-square test, \(P < 0.01\)) (Table 3).

**discussion**

In the present study, we have shown that extranuclear expression of ER/PR occurs frequently in ER-positive/PR-negative and ER-negative/PR-positive tumors, and furthermore those with extranuclear expression of PR contribute substantially to the population of ER-positive/PR-negative tumors. Indeed, around 40% of the ER-positive/PR-negative cases show extranuclear expression of PR, which can cross-talk with the PI3K/Akt-signaling pathway whose activation by HER-2/neu overexpression contributes to the growth of some breast cancers. Further, it is notable that an increased level of pAkt is associated with increased expression of aromatase. These findings may explain why aromatase inhibitors are more effective than tamoxifen in ER-positive/PR-negative tumors, including those overexpressing HER-2.

We also found that elevation of pAkt is significantly associated with ER-positive/PR-negative status, suggesting that the cross-talk with PI3K/Akt-signaling pathway may play a critical role in the growth of these tumors.

An important issue with potential clinical implications brought out in the present study may be relevant to some aromatase inhibitor studies in which the subgroup of ER-positive/PR-negative tumors responds better to an AI than to tamoxifen. A possible explanation of this is that aromatase transcription is activated through COX-2 activation [28], which is mediated by the Akt/HER-2-signaling pathway. Although the activation of Akt is regulated in part by HER-2, HER-2 is also regulated by Akt with an interaction for tumor survival [29]. The activation of HER-2 and Akt is able to activate COX-2, which produces prostaglandin E2 (PGE2) [30], resulting in activation of aromatase transcription in tumor and stromal cells. Activation of HER-2 and Akt also induce several cytokines such as IL-10 and IL-6, and angiogenic factors, leading to tumor progression (Figure 3).

**Table 3.** Relationship between expression of hormone receptors and HER-2, pAkt, and aromatase in 21 breast cancer patients with extranuclear hormone receptors

<table>
<thead>
<tr>
<th>Other factors</th>
<th>HER-2 Positive</th>
<th>HER-2 Negative</th>
<th>pAkt Positive</th>
<th>pAkt Negative</th>
<th>Aromatase Positive</th>
<th>Aromatase Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR status(^a)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER-positive/PR-negative (n=11)</td>
<td>7</td>
<td>4</td>
<td>10</td>
<td>1</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>ER-negative/PR-positive or negative (n=10)</td>
<td>7</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>(P)-value</td>
<td>(P=0.56)</td>
<td>(P &lt; 0.01)</td>
<td>(P=0.14)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Hormone receptor (HR) status means nuclear expression of ER and PR.
Thus, in these situations, it is conceivable that aromatase inhibitors are more effective than tamoxifen in inhibiting the Akt/HER-2-signaling pathway.

Another reason for the superiority of aromatase inhibitors to tamoxifen in ER-positive/PR-negative tumors is the agonistic action of tamoxifen through AF-1 in the ER. In general, tamoxifen competes with estrogen for binding to ER at the AF-2 site, resulting in the activation of co-repressors such as NCOr and HDAC. However, where cross-talk occurs between the signaling pathways of nuclear ER and growth factor receptors such as HER-2, the ER can be activated in a ligand-independent fashion, rendering tamoxifen’s blockage of estrogen binding less effective. Consistent with this idea, a recent study on the effect of tamoxifen in HER-2-transfected MCF-7 cells indicated that tamoxifen recruited coactivator complexes (ER, AIB1, CBP, p300) to the ER-regulated pS2 gene promoter in MCF-7/HER-2–18 cells and corepressor complexes in MCF-7 cells [17]. This explanation is also supported by several previous studies showing that the forced overexpression of growth factor receptors such as EGFR and HER-2 resulted in resistance to tamoxifen [29, 33]. In clinical studies as well, less responsiveness to tamoxifen has been reported in patients with overexpression of EGFR and HER-2 [34, 35]. Therefore, ER-positive/PR-negative breast tumors might best be treated by completely blocking ER action via estrogen withdrawal with aromatase inhibitors, targeted ER degradation, or by combined therapy targeting both ER and growth factor signaling pathways.

The hormone receptors (ER and PR) are the critical predictors of responsiveness in the treatment of estrogen-dependent breast cancer. Down-regulation of ER or PR decreases the sensitivity to SERMs such as tamoxifen, which is associated with inferior survival compared with ER/PR-double positive patients [34]. In the results of the pivotal ATAC study comparing the therapeutic efficacy of tamoxifen, the aromatase inhibitor anastrozole, and their combined application in postmenopausal breast cancer, time to recurrence was longer for anastrozole- than for tamoxifen-treated patients in both the ER-positive/PR-positive and ER-positive/PR-negative subgroups, but further subset analysis showed that the benefit was substantially greater in the PR-negative subgroup [22]. Indeed, the superior effect of anastrozole over tamoxifen on disease-free survival and incidence of recurrence noted in the ER-positive/PR-negative group was significantly greater than in any of the other subgroups.

Of interest, the survival benefit for the combined treatment with tamoxifen and anastrozole was inferior to either tamoxifen or anastrozole alone. The reason could be that in situations of cross-talk with growth factors, the agonistic effect of tamoxifen can interfere with the effect of estrogen deprivation by anastrozole, thereby decreasing the effect of anastrozole in the combination treatment. The survival benefit of aromatase inhibitors such as exemestene and letrozole following tamoxifen for HR-positive postmenopausal breast cancer has been reported in other studies to be superior to that of tamoxifen alone [36, 37]. The reason for the superiority of the sequential treatment with tamoxifen and aromatase inhibitor may be explained by the greater effectiveness of aromatase inhibitors on either ER-negative or PR-negative tumors, which manifest growth factor cross-talk. Despite the hypothesis that AIs may be more beneficial for the patients with ER-positive/PR-negative tumors, the recent findings of BIG 1–98 trial showed that all patients with ER-positive tumors had a similar reduction in the risk of a disease-free-survival event associated with letrozole irrespective their PR status [24]. It is possible that the above hypothesis was not supported by the BIG 1–98 trial because of the relatively short follow-up and multiple subgroup analyses in the study. Nevertheless, the
differential benefit noted in patients with PR-negative tumors may be due to such tumors having cross-talk with HER-2/Akt- and other growth factor-signaling pathways, in which some are expressed with extranuclear expression of ER and PR. This cross-talk may result in tamoxifen resistance by promoting agonist properties of tamoxifen.

Finally, it is likely that the extranuclear expression of ER/PR is a factor in the cross-talk with growth factor-signaling pathways such as Akt and HER-2. Also, such an association can explain the biological behavior of ER-positive/PR-negative and ER-negative/PR-positive patients. Possibly other clinical manifestations of the cross-talk between HRs and growth factors could exist. Given that the extranuclear expression of ER and PR is associated not only with increased activity of the PI3K/Akt-signaling pathway but also with increased expression of aromatase, aromatase inhibitors may be more beneficial than tamoxifen for some patient populations in ER-positive/PR-negative and ER-negative/PR-positive tumors. The results from the present study may provide an explanation for several previous experimental and clinical studies showing that ER-positive/PR-negative and ER-negative/PR-positive patients are less responsive to SERMs such as tamoxifen, and more responsive to aromatase inhibitors.

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references
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