The role of AIB1 and PAX2 in primary breast cancer: validation of AIB1 as a negative prognostic factor

S. Alkner1,2, PO. Bendahl1, D. Grabau3, P. Malmström1,2, M. Femö1, L. Rydén4,5
& On behalf of the South Swedish Breast Cancer Group

1Department of Oncology, Clinical Sciences, Lund University, Lund; Departments of 2Clinical Oncology; 3Pathology, Skåne University Hospital Lund, Lund; 4Department of Surgery, Clinical Sciences, Lund University, Lund; 5Department of Clinical Surgery, Skåne University Hospital Lund, Lund, Sweden

Background: The steroid-receptor coactivator amplified in breast cancer one (AIB1) is implicated to be a prognostic factor, although the results are not unanimous. Recently its effect was suggested to be modified by paired box 2 gene product (PAX2).

Patients and methods: Using immunohistochemistry (IHC) AIB1 and PAX2 were investigated in two cohorts of early breast cancer, including systemically untreated premenopausal lymph-node-negative women and pre- and postmenopausal women receiving tamoxifen.

Results: AIB1 scores were available for 490 patients and PAX2 scores were available for 463 patients. High AIB1 was a negative prognostic factor for distant disease-free survival (DDFS, \( P = 0.02 \)) and overall survival (OS, \( P < 0.001 \)) in systemically untreated women, while no prognostic effect was seen in the tamoxifen-treated cohort, indicating AIB1 to be a predictor of tamoxifen response. In systemically untreated patients, PAX2 was not a prognostic factor, nor did it modify the effect of AIB1. However, in ER-positive patients receiving tamoxifen, PAX2 appeared to be a positive prognostic factor in premenopausal patients, while a negative factor in postmenopausal. The interaction between the menopausal status and PAX2 was significant (\( P = 0.01 \)).

Conclusions: In an independent cohort of low-risk premenopausal patients, we validate AIB1 as a negative prognostic factor, indicating AIB1 to be an interesting target for new anti-cancer therapies. The effect of PAX2 warrants further studies.

Key words: AIB1, breast cancer, clinical trial, PAX2, prognosis, tamoxifen

introduction

A great challenge in breast cancer care today is to customize adjuvant treatment to the patients’ individual needs. We, therefore, need to learn more about the factors that determine the risk of relapse and the effect of systemic treatment. One recently suggested prognostic and treatment-predictive factor is the steroid-receptor coactivator amplified in breast cancer one (AIB1). Using a controlled trial of premenopausal patients randomly assigned to tamoxifen versus control, we have previously found AIB1 to be a negative prognostic factor [1].
In the present study, we validate these results in an independent cohort of low-risk lymph-node-negative premenopausal women.

In our previous study, we found patients with a high AIB1 to respond well to tamoxifen, improving prognosis to the same levels as in systemically untreated patients with low AIB1. Although these results are in line with some previous trials [2, 3], AIB1 has also been implied to be involved in tamoxifen resistance [4–6]. Reasons for the discrepancies between studies are unclear. However, since our previous study includes only premenopausal patients, we here explore the effect of AIB1 in relation to the menopausal status.

In a recent article, Hurtado et al. suggest the relationship between AIB1 and paired box 2 gene product (PAX2) to influence HER2, prognosis and tamoxifen response [7]. PAX2 plays an important role during embryogenesis, while overexpression might be of importance in cancer development [8–10]. However, clinical studies of the prognostic effect of PAX2 on breast cancer are very sparse [7, 11], and none have investigated PAX2 in a well-defined breast cancer cohort. Here, we investigate the relationship between AIB1 and PAX2, their effect on prognosis with or without tamoxifen treatment, and whether this is modified by menopausal status.

**materials and methods**

**patients**

**cohort 1—no tamoxifen**

The initial cohort consisted of 237 premenopausal, lymph-node-negative women included in a prospective study (1991–1994) of the prognostic value of S-phase fraction [12]. Detailed treatment information has been described before [12]. A flow chart is given in Figure 1A. Seven patients received adjuvant tamoxifen, one oophorectomy and 21 chemotherapy. The eight patients receiving endocrine treatment were excluded from further analysis. For 14 cases, the paraffin blocks could not be retrieved, in the remaining 215 tumours PAX2 scores were evaluable in 208 and AIB1 scores in 205. Non-evaluable cases were due to the loss of the tissue microarray (TMA) core, the TMA containing only cancer in situ or ≤10 cancer cells. Two hundred and five patients had both AIB1- and PAX2 scores. The median duration of follow-up for distant disease-free survival (DDFS) was 10.8 years for patients alive and free from metastases. The median duration of follow-up for distant disease-free survival (DDFS) was 6.0 years and for OS it was 21.4 years.

Both the studies were approved by the ethics committee of Lund University Hospital.

**tissue microarray and immunohistochemical staining**

TMAs were constructed using a tissue array machine according to the manufacturer’s instructions (Beecher Instruments, Sun Prairie, WI). Two cores were taken from each tumour. A mouse monoclonal antibody was used for AIB1 detection (Cat No. #611105 BD Bioscience, CA), as previously described [5]. For PAX2, the sections (3–4 μm) were transferred to glass slides, and baked for 2 h at 60°C. Deparaffinizing and antigen retrieval were carried out in PT-Link (Dako, Sweden) with high pH-buffer. Staining was carried out in an automatic immunohistochemistry (IHC) staining machine (Autostainer Plus Dako, Sweden) according to the standard procedures. PAX2 (Cat No. #2549–1 Epitomics Inc. CA; Diluted 1:500) primary antibody was applied for 60 min at room temperature and staining was detected using a Dako EnVision kit K8010. Validation of the AIB1 antibody has been reported before [1]. The antibody used for PAX2 was validated by western blot, and is approved for IHC and western blot [16]. The TMAs included positive controls (kidney), as well as internal negative controls represented by TMA cores not stained.

**AIB1 and PAX2 staining evaluation**

IHC staining was examined using a light microscope by two independent viewers blinded for clinical and tumour characteristics (SA and DG). For cohort 2, AIB1 scores were already available from a previous study [5]. Since scoring had been done in the same way as in the present study, and by the same pathologist (DG), the available scores were used in the present study. Staining for both AIB1 and PAX2 was seen in the cell nuclei (supplementary Figure S1, available at Annals of Oncology online). Each sample was given semi-quantitative scores from 0 to 3 for both the percentage of stained nuclei (proportion score) and the intensity of positive cells (intensity score). For AIB1, proportion score 0 represents no stained cell nuclei, score 1: 1–10%; score 2: 11–50% and score 3: 51–100%. Staining intensity 0 represents negative staining, 1 weak, 2 moderate and 3 intense staining.

Scoring for PAX2 was done in a similar way. However, to make it as similar as possible to previous studies [7, 8, 10, 11, 17], the proportion scores were instead determined as score 0, no stained cell nuclei; score 1, 1–10%; score 2, 11–80% and score 3, 81–100%. The staining intensity was determined as above.

The proportion and intensity scores were added to a total score ranging from 0 to 6. Cases, for which the scores differed more than one step between viewers (8%), were reexamined to reach consensus. In tumours with only one step difference, the mean score was used. In case of discrepant staining between the two cores from the same tumour, the core with the highest score was used. No consensus for the choice of a cut-off value for PAX2 was found in the literature. However, previous studies have found 40–60% of breast cancers to express high PAX2 [7, 8, 10, 11]. A cut-point of ≥5 resulted in 43 and 56% PAX2-positive patients in the respective cohort, and was chosen for further analysis. In accordance with two previous studies by our group, a total score of ≥5 was considered as high AIB1 [1, 5].
other tumour characteristics

The estrogen (ER) and progesterone receptors (PgR) were analysed with IHC or cytosol-based biochemical assays [12, 15], and HER2 with IHC and fluorescent or chromogenic in situ hybridization (FISH/CISH). All patients with amplified tumours according to FISH/CISH and patients with an IHC score of 3+, where FISH could not be evaluated, were considered HER2 positive [18, 19]. The Ki67 proliferation index was evaluated with the antibody MIB-1 [18], and considered high if >20% of the cells were stained. Nottingham histological grade (NHG) was re-evaluated according to the method by Elston and Ellis [12, 20].

statistical analysis

The statistical software package Stata 11.1 (StataCorp. 2009. College Station, TX) was used for statistical calculations. DDFS and OS were primary and secondary end points. DDFS includes distant metastasis and breast cancer deaths as primary event. OS includes death from any cause.
All analyses were done with the intention-to-treat rule. Correlation between AIB1, PAX2 and other prognostic factors was evaluated by \( t \)-test, \( \chi^2 \)-test or, where appropriate, \( \chi^2 \)-test for trend. The log-rank test was used to evaluate hypotheses of equal survival. For variables with more than two categories, the log-rank test for trend was used. The hazard ratios (HR) were estimated with Cox regression, univariate to compare survival in subgroups and multivariate to adjust the effects of AIB1 and PAX2 for other prognostic factors. To assess whether the effect of a factor differed in different subgroups, a Cox model with a term for interaction was used. Assumptions of proportional hazards were checked using Schoenfeld’s test \([21]\). All \( P \) values correspond to two-sided tests and the values <0.05 were considered significant.

**results**

**correlation between AIB1, PAX2 and other prognostic factors**

We first investigated the correlations between AIB1, PAX2 and other factors in two well-defined cohorts of early breast cancer patients: Cohort 1 included premenopausal lymph-node-negative women not receiving tamoxifen, whereas cohort 2 consisted of pre- and postmenopausal women receiving tamoxifen. In cohort 1, 42% of patients were AIB1 positive and 43% PAX2 positive. In cohort 2, the corresponding numbers were 47 and 56%, respectively.

A high expression of AIB1 was significantly associated with a high NHG (cohort 1: \( P = 0.008 \), cohort 2: \( P < 0.001 \)), a high expression of Ki67 (cohort 1: \( P = 0.04 \), cohort 2: \( P = 0.02 \)) and ER negativity in cohort 2 (\( P = 0.03 \)) (Table 1). AIB1 was also correlated with HER2 positivity in cohort 2 (\( P < 0.001 \)).

PAX2 was associated with ER negativity (cohort 1: \( P = 0.02 \), cohort 2: \( P = 0.01 \)), and high expression of Ki67 in cohort 2 (\( P = 0.02 \)). AIB1 and PAX2 also strongly correlated with each other (cohort 1: \( P < 0.001 \), cohort 2: \( P = 0.004 \)).

**AIB1 as a prognostic factor**

In cohort 1, there were 48 events for the end point DDFS, and 59 events for OS, in patients with either an AIB1- or a PAX2-positive tumour.

### Table 1. Relationship between AIB1, PAX2 and other prognostic factors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cohort 1 (no tamoxifen) percent in high AIB1/PAX2 (% high/N total)</th>
<th>Cohort 2 (tamoxifen) percent in high AIB1/PAX2 (% high/N total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Node status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>42% (86/205)</td>
<td>48% (47/98)</td>
</tr>
<tr>
<td>N+</td>
<td>0</td>
<td>46% (92/199)</td>
</tr>
<tr>
<td>Tumour size (mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 20 )</td>
<td>45% (69/154)</td>
<td>41% (30/73)</td>
</tr>
<tr>
<td>( &gt; 20 )</td>
<td>33% (17/51)</td>
<td>49% (109/224)</td>
</tr>
<tr>
<td>NHG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>29% (19/66)</td>
<td>18% (2/11)</td>
</tr>
<tr>
<td>2</td>
<td>44% (31/70)</td>
<td>40% (51/129)</td>
</tr>
<tr>
<td>3</td>
<td>52% (34/66)</td>
<td>62% (39/63)</td>
</tr>
<tr>
<td>Missing (2/3)</td>
<td></td>
<td>47/94</td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>41% (30/73)</td>
<td>57% (48/84)</td>
</tr>
<tr>
<td>Positive</td>
<td>42% (56/132)</td>
<td>43% (87/201)</td>
</tr>
<tr>
<td>Missing (0/0)</td>
<td></td>
<td>4/12</td>
</tr>
<tr>
<td>Ki67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 20% )</td>
<td>39% (48/124)</td>
<td>39% (52/134)</td>
</tr>
<tr>
<td>( &gt; 20% )</td>
<td>55% (33/60)</td>
<td>56% (40/72)</td>
</tr>
<tr>
<td>Missing (5/21)</td>
<td></td>
<td>47/91</td>
</tr>
<tr>
<td>HER2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>41% (70/170)</td>
<td>41% (101/244)</td>
</tr>
<tr>
<td>Positive</td>
<td>59% (13/22)</td>
<td>72% (38/53)</td>
</tr>
<tr>
<td>Missing (3/13)</td>
<td></td>
<td>0/0</td>
</tr>
<tr>
<td>PAX2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>31% (36/115)</td>
<td>33% (26/79)</td>
</tr>
<tr>
<td>Positive</td>
<td>56% (50/90)</td>
<td>54% (61/113)</td>
</tr>
<tr>
<td>Missing (0/0)</td>
<td></td>
<td>52/105</td>
</tr>
<tr>
<td>AIB1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>34% (40/119)</td>
<td>34% (40/119)</td>
</tr>
<tr>
<td>Positive</td>
<td>58% (50/86)</td>
<td>58% (50/86)</td>
</tr>
<tr>
<td>Missing (0/3)</td>
<td></td>
<td>0/3</td>
</tr>
</tbody>
</table>

\( \chi^2 \)-test for 2 × 2 tables or, for NHG with three categories, \( \chi^2 \)-test for trend. Tumours with missing values were not included in analyses.

AIB1, amplified in breast cancer 1; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; N, number; N0, no lymph-node-metastases; N+, lymph-node-metastases; NHG, Nottingham histological grade; PAX2, paired box 2 gene product.
score (208 patients). All analyses presented below were repeated excluding patients receiving chemotherapy, with similar results observed.

A significant trend for a worse prognosis was seen with increasing AIB1 scores (0–6) (log-rank test for trend: OS $P = 0.005$, DDFS $P = 0.07$). We here confirmed that $\geq 5$ was a reasonable AIB1 cut-off value. Under the hypothesis of no trend, a turning point from fewer to more events than expected was seen for an AIB1 score of $\geq 5$. Using $\geq 5$ as a cut-off value for AIB1 high versus low, a high AIB1 was a significant negative prognostic factor. (Table 2). Subgroup analysis showed the prognostic effect to be strongest, and only significant in ER-positive patients (Table 2) (Figure 2). In multivariate analysis (adjusted for age, tumour size, NHG and HER2), significance remained for OS but not for DDFS. Moreover, there was a trend towards AIB1 being a negative prognostic factor also in ER-negative patients (Table 2) (Figure 2).

Since previous publications suggest a relationship between AIB1 and HER2 [4, 7, 22], we further explored this. Although tumours positive for both AIB1 and HER2 seem to have a worse prognosis than tumours positive for one or neither of the factors, AIB1 did not significantly modify the prognostic effect of HER2 (data not shown). However, only 22 (11%) patients in this low-risk group were HER2 positive, making it hard to draw any conclusions in regard to this.

AIB1 in tamoxifen-treated patients

In cohort 2, there were 106 events for the end point DDFS and 248 for the end point OS in patients with either an AIB1- or a PAX2 score (369 patients). Data regarding AIB1 for all patients in this cohort (pre- and postmenopausal together) have been published before [5]. In the following analysis, we wished to investigate whether the prognostic effect of AIB1 on tamoxifen-treated patients (cohort 2) differed according to the menopausal status. AIB1 did not remain a significant negative prognostic factor in either ER-positive pre- or postmenopausal tamoxifen-treated patients (Table 3) (Figure 2). Additional graphs of DDFS in relation to ER- and menopausal status are provided in supplementary Figure S2, available at Annals of Oncology online.

PAX2 as a prognostic factor

PAX2 had no prognostic impact in cohort 1 (Table 2) (Figure 3), neither with a log-rank test for trend for scores 0–6, or as a dichotomized variable.

Also, PAX2 did not affect prognosis when analysing the tamoxifen-treated cohort (cohort 2) as a whole (Table 3) (Figure 3). However, in the subgroup analysis of cohort 2, PAX2 appeared to be a negative prognostic factor in postmenopausal patients, and a positive prognostic factor in premenopausal patients (Table 3) (Figure 3). This effect was predominantly seen in ER-positive patients, although there was a trend also in ER-negative patients in multivariate analysis (supplementary Figure S3, available at Annals of Oncology online). Using a Cox model with PAX2 (high versus low), menopausal status (pre- versus postmenopausal) and an interaction term, we found a significant interaction between the menopausal status and the prognostic effect of PAX2 in ER-positive patients (OS HR 3.3, 95% CI 1.2–9.5, $P = 0.02$, DDFS HR 7.7, 95% CI 1.6–36, $P = 0.01$). Significance remained when the interaction analysis was adjusted for age, tumour size, node status, NHG and HER2.

When examining if PAX2 modified the prognosis in patients with high versus low AIB1 expression, no difference in the prognostic effect of AIB1 was seen in relation to PAX2 in any of the cohorts (data not shown).

Discussion

Using a controlled trial of premenopausal women randomly assigned to tamoxifen versus control, we have previously found AIB1 to be a negative prognostic factor [1]. Although others have shown similar results [3, 6, 22–24], conclusions from studies are not unanimous [4], and AIB1’s prognostic effect is still being discussed. Here, we confirm AIB1 as a negative prognostic factor in an independent cohort of lymph-node-

![Table 2. Cox regression analysis for DDFS and OS in cohort 1, lymph-node-negative premenopausal patients without tamoxifen](image)

<table>
<thead>
<tr>
<th>AIB1* (N 205)</th>
<th>HR</th>
<th>95% CI</th>
<th>$P$ value</th>
<th>DDFS multivariateb</th>
<th>HR</th>
<th>95% CI</th>
<th>$P$ value</th>
<th>OS univariate</th>
<th>HR</th>
<th>95% CI</th>
<th>$P$ value</th>
<th>OS multivariateb</th>
<th>HR</th>
<th>95% CI</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>2.0</td>
<td>1.1–3.6</td>
<td>0.02</td>
<td>1.5</td>
<td>0.79–2.8</td>
<td>0.2</td>
<td>2.8</td>
<td>1.6–4.8</td>
<td>&lt;0.001</td>
<td>2.3</td>
<td>1.3–4.2</td>
<td>0.005</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>2.6</td>
<td>1.2–5.9</td>
<td>0.02</td>
<td>2.1</td>
<td>0.87–4.9</td>
<td>0.1</td>
<td>3.8</td>
<td>1.8–7.9</td>
<td>&lt;0.001</td>
<td>3.6</td>
<td>1.6–7.8</td>
<td>0.001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER−</td>
<td>1.6</td>
<td>0.70–3.7</td>
<td>0.3</td>
<td>1.3</td>
<td>0.46–3.9</td>
<td>0.7</td>
<td>1.9</td>
<td>0.87–4.3</td>
<td>0.1</td>
<td>1.5</td>
<td>0.55–3.8</td>
<td>0.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAX2* (N 208)</td>
<td>1.0</td>
<td>0.57–1.8</td>
<td>1.0</td>
<td>0.94</td>
<td>0.50–1.8</td>
<td>0.8</td>
<td>1.3</td>
<td>0.76–2.1</td>
<td>0.4</td>
<td>1.2</td>
<td>0.68–2.1</td>
<td>0.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+</td>
<td>0.85</td>
<td>0.38–1.9</td>
<td>0.7</td>
<td>1.1</td>
<td>0.47–2.5</td>
<td>0.8</td>
<td>1.2</td>
<td>0.63–2.4</td>
<td>0.6</td>
<td>1.3</td>
<td>0.66–2.6</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER−</td>
<td>1.0</td>
<td>0.43–2.3</td>
<td>1.0</td>
<td>0.89</td>
<td>0.31–2.5</td>
<td>0.8</td>
<td>1.2</td>
<td>0.52–2.6</td>
<td>0.7</td>
<td>0.99</td>
<td>0.35–2.8</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a AIB1 and PAX2 high versus low.

b Adjusted for age (years), tumour size ($\leq 20 \text{ mm}$), NHG (1–3), ER status (positive versus negative) and HER2 (positive versus negative). Analyses divided by ER status are not adjusted for ER status again.

AIB1, amplified in breast cancer 1; BCSS, breast cancer-specific survival; CI, confidence interval; DDFS, distant disease-free survival; ER, estrogen receptor; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; N, number; NHG, Nottingham histological grade; OS, overall survival; PAX2, paired box 2 gene product.
negative premenopausal patients not receiving tamoxifen. The finding that AIB1’s prognostic information is extended to a low-risk group of patients supports its role as an independent prognostic factor.

A deregulation of AIB has been suggested in tamoxifen resistance [4, 25], though the results from preclinical as well as clinical studies are ambiguous. Some find high AIB1 to be associated with a worse outcome in tamoxifen-treated patients.
while others find no such correlation [22, 26], or a good response to endocrine treatment [2]. In our previous study, we found ER-positive patients with a high AIB1 to respond well to tamoxifen, improving prognosis to the same level as in patients with low AIB1 [1]. Since this trial included only premenopausal patients, one hypothesis to explain the discrepancies between studies could be that AIB1’s effect on tamoxifen response differs in relation to menopausal status. Apart from acting as a coactivator to the ER, AIB1 can interact with other signalling pathways, inducing hormone-independent proliferation [27–30]. It is also involved in cell cycle regulation, apoptosis and distant metastasis [31–34]. One theory could hence be that AIB1 in the presence of estrogen acts as a coactivator to the ER, but in the postmenopausal setting, with low estrogen levels, through other pathways.

The results regarding AIB1 for all patients included in the tamoxifen-treated cohort 2, pre- and postmenopausal together, have been published before [5]. More recurrences were seen with a high AIB1 during the first 2 years, but not with a longer follow-up. When dividing the patients according to the menopausal status, we found no difference in prognosis in relation to AIB1 in ER-positive pre- or postmenopausal tamoxifen-treated women. The prognostic effect of AIB1 seems to be restricted in tamoxifen-treated patients compared with the effect in untreated patients. This finding could support the results from our previous study, indicating tamoxifen to improve progno

**Figure 3.** DDFS in relation to PAX2 alone and in combination with AIB1. (A) Cohort 1, premenopausal women not receiving tamoxifen. (B) PAX and AIB1 in cohort 1. (C) Cohort 2, PAX2 in pre- versus postmenopausal ER-positive patients. (D) Cohort 2, ER-positive patients receiving tamoxifen, PAX2 and AIB1. Abbreviations: AIB1, amplified in breast cancer 1; AIB1—low AIB1 (score <5), AIB1 + high AIB1 (score ≥5); ER, estrogen receptor; DDFS, distant disease-free survival; PAX2, paired box 2 gene product; PAX2—low PAX2 (score <5), PAX2 + high PAX2 (score ≥5); Premenop, premenopausal; Postmenop, postmenopausal; PrePAX—premenopausal low PAX2, PrePAX + premenopausal high PAX2, PostPAX—postmenopausal low PAX2, PostPAX + postmenopausal high PAX2.
No association was found between PAX2 and HER2. In human breast cancer cell lines, Hurtado et al. [7] suggested the balance between PAX2 and AIB1 to influence tamoxifen resistance via regulation of ERBB2. In luminal breast cancer cell lines PAX2 also seems to be of importance for estrogen-induced decrease of HER2 and cell invasion [37]. Clinical studies of PAX2 are few and lack menopausal data. In 109 tamoxifen-treated patients, Hurtado et al. [7] found PAX2 to be a positive prognostic factor, and within the PAX2-positive subgroup those who were also AIB1 positive had a worse prognosis. PAX2 has also been associated with a lower recurrence risk in an unselected cohort of 74 breast cancer patients [11]. To our knowledge, this is the first time PAX2 and its relation to AIB1 is studied in a cohort of patients with or without tamoxifen. For the whole cohort, PAX2 did not bring prognostic information in tamoxifen-treated or untreated patients on its own. Nor did it modify the prognostic effect of AIB1. Interestingly, however, an interaction was seen between the menopausal status and PAX2 in tamoxifen-treated ER-positive patients. One explanation could be levels of circulating estrogen, affecting which proliferation pathways the tumour mainly depends on. A recent article of interest shows activation of PAX2 by estradiol to be specific for breast cancer cell lines of the luminal type, and indicates the activation and effect of PAX2 to differ in relation to the tumour’s dependence on the ER [37]. Theoretically, the data presented here can support that premenopausal women with high circulating estrogen levels might activate PAX2 resulting in a beneficial outcome, in contrast to estrogen poor postmenopausal women. However, our study contains few premenopausal tamoxifen-treated patients with PAX2 scores (57 patients). Hence, this result needs to be interpreted with some caution.

Although previous studies of PAX2 in breast cancer also have used IHC [7, 8, 10, 11], techniques and cut-off levels differ. However, in line with ours, previous studies have found PAX2 positivity in 40–62% of breast cancers. Also, investigating PAX2 scores from 0 to 6 showed similar results as with a cut-off of ≥5. Recently, the levels of phosphorylated PAX2 were indicated to be more important than total protein levels [37]. However, previous clinical studies finding PAX2 to influence prognosis have not separated phosphorylated from non-phosphorylated PAX2 [7, 11]. Another potential bias is that although follow-up is longer for OS, this end point includes non-breast cancer deaths. In cohort 1 (premenopausal), we expect by far the most deaths to be due to breast cancer, though in cohort 2 including older women, several might have died of other causes. We have investigated both DDFS and OS, and achieved similar results.

In conclusion, we here confirm AIB1 to be a negative prognostic factor in an independent cohort of lymph-node-negative premenopausal patients. AIB1 is thus an interesting possible target for new anti-cancer therapies. An interaction between the menopausal status and the prognostic effect of PAX2 was found in ER-positive tamoxifen-treated patients supporting experimental studies that the functional role of PAX2 is dependent on estrogen levels, but this observation needs further investigation.

Acknowledgements

We are indebted to the South Swedish Breast Cancer Group for participating in the controlled, clinical trials and for providing samples. We also want to thank Kristina Lövgren for help with the IHC staining and Looket Dihge for help with evaluation of the AIB1 scores in cohort 2.

Funding

This work was supported by funds from the Swedish Cancer Society [grant number 100391, 11 0464, 11 0076]; the Swedish Research Council [grant number 2001-18840-83692-86]; Gunnar, Arvid, and Elisabeth Nilsson Foundation [grant number 2010-05-26]; Mrs Berta Kamprad Foundation [grant number BKS 7/2011]; the University Hospital of Lund Research Foundation [grant number 2011-05-02]; the Swedish Society for Medical Research and Governmental Funding of Clinical Research within Nation Health Service [grant number ALF/FUA 10623, ALFSKANE-57071] and Skåne county council’s research and development foundation.

Disclosure

The authors have declared no conflicts of interest.

References


