Cyclin E expression in breast cancer: predicting germline BRCA1 mutations, prognosis and response to treatment

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Received 8 September 2004; revised 17 December 2004; accepted 22 December 2004

Background: Elevated levels of the cell cycle protein cyclin E, and low levels of its inhibitor, p27Kip1, have been associated with a poor prognosis following breast cancer. Some studies have found that germline mutations in the breast cancer susceptibility gene, BRCA1, are also associated with an inferior survival rate. The relationship between cyclin E/p27 Kip1 levels, BRCA1 status and outcome has not been studied in detail.

Patients and methods: We analyzed a historical cohort of 288 Ashkenazi Jewish women who were diagnosed with breast cancer between 1980 and 1995 and were previously tested for BRCA1/2 mutations. Protein levels of cyclin E and p27 Kip1 were assessed by immunohistochemistry. Breast cancer-specific survival (BCSS) was the main outcome measured.

Results: The median follow-up was 8 years. Thirty tumors carried germline BRCA1 mutations. These tumors were more likely to have high cyclin E protein levels [odds ratio (OR) 9.5; P <0.001] and low p27 Kip1 protein levels (OR 2.8; P = 0.03) than tumors from patients without BRCA1/2 mutations. High cyclin E expression level was the strongest predictor of BRCA1 germline mutations (multivariate OR 4.7; P = 0.004). On univariate analysis, high cyclin E protein levels [relative risk (RR) 2.6; P <0.001] and low p27 Kip1 protein levels (RR 2.3; P = 0.006) were significant prognostic factors for a poorer BCSS. In Cox multivariate models, high cyclin E levels remained an independent indicator of poor outcome only in the subgroup of patients who did not receive chemotherapy (P = 0.002).

Conclusions: In this ethnically restricted cohort, a high level of cyclin E is a characteristic of BRCA1-related breast cancer, and is a marker of poor prognosis following breast cancer, particularly in the absence of adjuvant chemotherapy.

Key words: BRCA1, breast cancer, chemotherapy, cyclin E, KIP1, prognosis

Introduction

Breast cancer is a major problem in public health. Its incidence is increasing in many countries. Despite much research, the precise spatiotemporal sequences of genetic alterations underlying this heterogeneous disease remain obscure. Definite progress has been achieved in the management of breast cancer patients, but despite a plethora of studies, identification of clinically useful, readily available prognostic or predictive markers is still a major challenge. Management of patients is currently based on easily identifiable clinical and pathological characteristics, which only partially reflect disease heterogeneity. Standard predictive factors include age, tumor size, histological type, axillary node involvement, histological or nuclear grade (Elston’s method), and steroid receptor expression [1]. The vast majority of patients with a localized disease will receive adjuvant treatment, despite the fact that only a small proportion will benefit from this treatment, particularly in the node-negative subgroup. Without adjuvant treatment, ~ 30% of node negative breast cancer patients will die after a period
Germline mutations in the tumor suppressor genes BRCA1 and BRCA2 predispose individuals to early onset breast and ovarian cancers [2]. The biological activities of the BRCA1 gene product are not completely understood; nevertheless, roles in DNA damage repair, cell-cycle control and transcriptional regulation have been identified. From a clinical standpoint, BRCA1-related tumors demonstrate distinct features in histopathological [3], immunohistochemical (IHC) [4], cytogenetic [5] and gene expression profile [6, 7] studies, when compared with either non-familial breast cancer cases or BRCA2-related breast cancer. Other studies have suggested an inferior survival rate for women developing a BRCA1-related breast cancer [8–11].

Cyclins bind to and activate cyclin-dependent kinases to form serine/threonine kinase complexes that regulate eukaryotic cell cycle. The cyclin E/CCNE1 gene is located on chromosome 19q. In normal dividing cells, cyclin E, in collaboration with cyclins A and D, regulates the transition through the G1 phase to enter the S phase by activating CDK2, and high levels of cyclin E protein accelerates this transition [12]. In a mouse model, conditional expression of cyclin E in mammary tissue resulted in the induction of mammary gland hyperplasia and carcinomas [13]. In several studies, high levels of cyclin E expression have been associated in multivariate analyses with a worse outcome after breast cancer [14, 15].

The cyclin-dependent kinase inhibitor 1B (CDKN1B) gene is located on chromosome 12p. The major function of the gene product, known as p27Kip1, is to inhibit cyclin E–CDK2 complexes and thus control cell proliferation [16]. In some breast cancer survival studies, low levels of p27Kip1, as detected by IHC, have been associated with a poor prognosis in univariate and multivariate analyses [16], but other studies did not confirm these results [17, 18].

Several previous studies have shown that cyclin D1 levels are lower in BRCA1-related breast cancer than in cancers occurring in either BRCA2 carriers or non-carriers [6]. Recently, it has been suggested that the perturbation of the cyclin D1 and cyclin E pathways lead to separate and alternative breast cancer subtypes [19]. Using a subset of the cases presented here, we previously showed that BRCA1/2-related breast cancer was associated with low levels of p27Kip1 [10]. Furthermore, using the same dataset as that presented here, we have recently shown that the ‘basal-like’ phenotype of breast is characterized by high levels of expression of cyclin E, expression of p53, the presence of glomerular microvascular proliferation and low levels of p27Kip1 [20]. The latter three factors have previously been shown by our group and others to be associated with BRCA1-related breast cancer [10, 21, 22]. Here, we wished to extend our analysis of the two functionally interacting cell cycle proteins, cyclin E and p27Kip1, both of which had a strong prognostic influence in the previous analysis, and to study their relationship with BRCA1-related breast cancer. These two areas were not covered in our previous publication [20].

We compared the clinicopathological features of BRCA1-related and BRCA1/2-unrelated breast cancer, evaluated cyclin E and p27Kip1 expression levels as predictor of the BRCA1 status, and then performed survival analyses including standard prognostic variables such as tumor size, nuclear grade and axillary node status. The main end point studied was breast cancer-specific survival (BCSS). In addition, we studied the effect of adjuvant treatment on outcome in those whose tumors featured differing levels of cyclin E/p27Kip1 proteins.

Patients and methods

Patients and clinicopathological review

The study design was an ethnically restricted, single hospital-based, retrospective cohort study. The anonymized study design was approved by the relevant Institutional Review Boards. Of 309 consecutive cases of Ashkenazi Jewish women aged ≤65 years diagnosed with a first primary, non-metastatic, invasive breast cancer between 1 January 1980 and 1 November 1995 at the Sir Mortimer B. Davis–Jewish General Hospital (Montreal, QC, Canada), 17 (5.5%) were excluded because of inability to locate path blocks, because a component of carcinoma in situ was only present on the available path blocks or because DNA could not be adequately amplified after repeated attempts, leaving 292 cases. Four patients were excluded as there was no follow-up, or they had no information on disease or vital status at the time of last follow-up. Breast cancer blocks were identified from each of these women, and clinicopathological and follow-up information were obtained by chart review. All specimens were reviewed by one pathologist (L.R.B.) for histological type, nuclear grade, lymph node status and estrogen receptor (ER) IHC assessment. The results of the cyclin E and p27Kip1 IHC tests were read by P.P. and L.R.K., respectively. Specimens were coded and DNA was extracted from the paraffin wax-embedded blocks using standard techniques.

BRCA1 and BRCA2 mutation status

Mutation analysis was carried out as described previously [8, 10], looking specifically for the recurrent mutations in the Ashkenazi Jewish population (BRCA1: 185delAG, 5382insC; BRCA2: 6174delT). Haplotype analysis was also used to confirm 5382insC mutation. The BRCA2 mutation 6174delT was sought using single-strand conformation analysis, by a mutation-specific PCR-RFLP endonuclease digestion assay and by direct sequencing. We also used a size assay as a second assay for all three mutations. BRCA2 mutation carriers (n=10) were excluded from further analysis because it is not established that they can be combined with BRCA1 carriers in survival analyses, and they constituted a group too small to be analyzed alone.

IHC analyses

All interpretation of IHC assays were made without knowledge of clinical outcome or any other clinical variables. ER protein levels were detected using a standard streptavidin–biotin–peroxidase complex IHC technique on paraffin-embedded tissue. Positivity was recorded when >10% of tumor cell nuclei showed immunoreactivity.

Cyclin E protein levels by IHC were determined using affinity-purified polyclonal cyclin E antibody as described previously [14]. Comparison of the polyclonal antibody with HE12 cyclin E monoclonal antibody (Oncogene Research Products, Cambridge, MA, USA) by western blot showed similar patterns of staining, including identification of multiple bands of smaller molecular weight products with both antibodies (data not shown).
Scoring of cyclin E IHC was a subjective interpretation of staining intensity and the percentage of tumor cells positive, as described previously [14]. p27Kip1 expression was also evaluated as described previously [10]. Briefly, streptavidin–biotin–peroxidase complex IHC technique on paraffin-embedded tissue with an anti-p27 monoclonal antibody (Transduction Laboratories, Lexington, KY, USA) was used. Low levels of protein were recorded when ≤50% of tumor cell nuclei were stained, as previously indicated [10].

**Statistical analysis**

Clinical, pathological and molecular data were collected in a mutually blinded fashion. Patient characteristics were compared using non-parametric Wilcoxon’s test and Fisher’s exact test. Trends in increasing odds ratios (ORs) were assessed by the Cochran–Armitage test. Exact confidence intervals (CI) were calculated for the proportions of BRCA1/2 mutation carriers, and tumors with high levels of cyclin E and low levels of p27Kip1 present in the cohort. A logistic regression model was developed to predict BRCA1 status where the following parameters were considered: age at diagnosis, tumor size, nuclear grade, axillary lymph nodes status, ER status, and cyclin E and p27Kip1 expression levels. This model was adjusted for missing values wherever possible.

Survival rates were calculated from the date of primary surgery until death from breast cancer for BCSS, which was the main outcome measure. Ten-year survival curves were estimated using the Kaplan–Meier method and significance was assessed using the log-rank test. To estimate the relative risk (RR) of death from breast cancer, Cox’s proportional hazards model was used where the following prognostic factors were examined: tumor size, axillary lymph node status, nuclear grade, age at diagnosis, ER status, cyclin E, p27Kip1, and BRCA1 mutation status; multivariate models were adjusted for missing variables wherever possible.

**Table 1. Patient characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All subjects (n = 278) [n (%)]</th>
<th>BRCA1/2 mutation non-carriers (n = 248) [n (%)]</th>
<th>BRCA1 mutation carriers (n = 30) [n (%)]</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at diagnosis, years (278)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>53.4</td>
<td>53.8</td>
<td>46.7</td>
<td>0.004</td>
</tr>
<tr>
<td>Range</td>
<td>26.5–64.9</td>
<td>26.5–64.9</td>
<td>31.6–62.1</td>
<td></td>
</tr>
<tr>
<td>Tumor size, cm (266)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>1.6</td>
<td>1.6</td>
<td>2.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Range</td>
<td>0.10–14</td>
<td>0.10–14</td>
<td>0.15–5.0</td>
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<tr>
<td>Nuclear grade (276)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>73 (26)</td>
<td>72 (29)</td>
<td>1 (3)</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>112 (41)</td>
<td>104 (42)</td>
<td>8 (27)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>91 (33)</td>
<td>70 (29)</td>
<td>21 (70)</td>
<td>&lt;0.001</td>
</tr>
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<td>Estrogen receptor IHC (272)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>100 (37)</td>
<td>77 (32)</td>
<td>23 (77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>172 (63)</td>
<td>165 (68)</td>
<td>7 (23)</td>
<td></td>
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<tr>
<td>Axillary lymph nodes (254)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>141 (56)</td>
<td>122 (54)</td>
<td>19 (66)</td>
<td>0.3</td>
</tr>
<tr>
<td>Positive</td>
<td>113 (44)</td>
<td>103 (46)</td>
<td>10 (34)</td>
<td></td>
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<tr>
<td>Cyclin E IHC (253)</td>
<td></td>
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</tr>
<tr>
<td>Negative</td>
<td>186 (74)</td>
<td>178 (79)</td>
<td>8 (29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive</td>
<td>67 (26)</td>
<td>47 (21)</td>
<td>20 (71)</td>
<td></td>
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<tr>
<td>p27Kip1 IHC (248)</td>
<td></td>
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<td></td>
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<tr>
<td>Negative</td>
<td>153 (62)</td>
<td>129 (59)</td>
<td>24 (80)</td>
<td>0.03</td>
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<tr>
<td>Positive</td>
<td>95 (38)</td>
<td>89 (41)</td>
<td>6 (20)</td>
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<tr>
<td>Surgery (278)</td>
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<td></td>
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<tr>
<td>Lumpectomy</td>
<td>218 (78)</td>
<td>193 (78)</td>
<td>25 (83)</td>
<td>0.6</td>
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<tr>
<td>Mastectomy</td>
<td>60 (22)</td>
<td>55 (22)</td>
<td>5 (17)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant chemotherapy (272)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>143 (53)</td>
<td>130 (54)</td>
<td>13 (43)</td>
<td>0.3</td>
</tr>
<tr>
<td>Yes</td>
<td>129 (47)</td>
<td>112 (46)</td>
<td>17 (57)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant hormonotherapy (260)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>131 (50)</td>
<td>112 (48)</td>
<td>19 (68)</td>
<td>0.07</td>
</tr>
<tr>
<td>Yes</td>
<td>129 (50)</td>
<td>120 (52)</td>
<td>9 (32)</td>
<td></td>
</tr>
</tbody>
</table>

IHC, immunohistochemistry.
All data were censored at 10 years time, significance was assessed at the 5% level, 95% CI were constructed and all statistical tests were two-sided. A competing risk analysis was not performed.

Results

Patients characteristics

Of the 288 patients tested, 10 (3.5%; 95% CI 1.7% to 6.3%) were identified as BRCA2 6174delT germline mutation carriers and were excluded from the analysis. We identified 30 patients (10.8%; 95% CI 6.1% to 12.4%) with BRCA1 mutations (20 with 185delAG and 10 with 5382insC) in the remaining 278 women. Eighty-five distant recurrence and 78 deaths (67 breast-related deaths, five other cancer deaths, six non-cancer deaths, including one death attributable to chemotherapy) were recorded at 10 years follow-up (median 8 years; range 0.1–20.7; median among non-breast cancer-related deaths 9.2 years). Patients and disease characteristics by mutation status are presented in Table 1. We identified 67 patients (26.5%; 95% CI 21.2% to 32.4%) with high cyclin E protein levels and 153 patients (61.7%; 95% CI 55.3% to 67.8%) with low protein levels of p27Kip1. Patients with BRCA1 mutation-related tumors were more likely to be diagnosed at a younger age ($P=0.004$), to have higher grade tumors ($P<0.001$ for trend), to have ER-negative tumors ($P<0.001$) and to have high cyclin E protein levels (OR 9.5; 95% CI 3.9–22.8; $P<0.001$) and low p27Kip1 protein levels (OR 2.8; 95% CI 1.1–7; $P=0.03$) than patients without BRCA1/2 mutations. Patients with high cyclin E protein levels had tumors with a higher nuclear grade ($P<0.001$ for trend), that were more commonly ER-negative ($P<0.001$) and that demonstrated lower p27Kip1 levels ($P=0.05$). Patients with low p27Kip1 protein levels had tumors that were ≥2 cm ($P=0.02$), had a higher nuclear grade ($P<0.001$ for trend) and were more commonly ER-negative ($P<0.001$).

Predictive markers of BRCA1 status (Table 2)

In an univariate analysis, younger age (OR 3; $P=0.006$), nuclear grade 3 (OR 21.6; $P=0.003$), absence of ER expression (OR 7; $P<0.001$), high cyclin E protein levels (OR 9.5; $P<0.001$) and low p27Kip1 protein levels (OR 2.8; $P=0.03$) were all predictive markers of BRCA1 germline mutations. In the multivariate analysis, expression of high levels of cyclin E was the single independent significant predictive marker (OR 4.7; $P=0.004$) of BRCA1 mutations.

Breast cancer-specific survival

Survival curves for patients dichotomized according to the protein levels of cyclin E and p27Kip1 in their breast tumors are shown in Figures 1 and 2. On univariate analysis (Table 3), high cyclin E protein level (RR 2.6; $P<0.001$) and low p27Kip1 protein level (RR 2.3; $P=0.006$) were significant prognostic factors for a poorer BCSS, as well as younger age at diagnosis, larger tumor size, higher nuclear grade, positive lymph node status and absence of ER expression; a positive BRCA1 mutation carrier status was of borderline statistical significance ($P=0.052$). In the Cox multivariate model, tumor size (RR 1.9; $P=0.04$), involvement of axillary lymph nodes

![Figure 1. Breast cancer-specific survival according to the cyclin E immunohistochemical status (n = 253).](image-url)
remained statistically significant, but high cyclin E protein levels and low p27Kip1 protein levels did not.

When BCSS was examined in the lymph node-negative subgroup (n=140), younger age at diagnosis (RR 3.9; P=0.003), nuclear grade 3 (RR 4.3; P=0.01), absence of ER expression (RR 5.9; P<0.001), high protein levels of cyclin E (RR 5.4; P<0.001), low p27Kip1 protein level (RR 4.9; P=0.01) and a positive BRCA1 mutation status (RR 5.6; P<0.001) were all significantly associated with higher mortality on Cox univariate analysis. In the multivariate model, only younger age at diagnosis (RR 3.6; P=0.01) and low p27Kip1 protein levels (RR 4; P=0.04) retained significance. On univariate analysis in the 112 patients with axillary node positive tumors, larger tumor size (RR 3.7; P<0.001), nuclear grade 2 and 3 (RR 8.9, P=0.03; and RR 16.9, P=0.006, respectively), absence of ER expression (RR 2.4; P=0.006) and high cyclin E levels (RR 2; P=0.04) were all statistically significant predictors of a poorer BCSS. In a multivariate model, only nuclear grade 3 (RR 10.5; P=0.03) retained statistical significance, and larger tumor size was of borderline significance (RR 2.5; P=0.0501).

When the BCSS analysis was performed according to the type of adjuvant treatment, high cyclin E protein level was an independent prognostic factor (RR 5.7; P=0.002) among 142 patients evaluable who did not receive adjuvant chemotherapy and among 128 patients with adjuvant hormonal therapy (RR 4.7; P=0.03). Cyclin E protein level was not an independent prognostic factor in other groups. In contrast, low levels of p27Kip1 were associated with a worse survival only in Cox univariate models and when the analyses were performed among the subgroup of patients (n=128) who received adjuvant chemotherapy (RR 2.9; P=0.02) and the subgroup of patients (n=130) who did not receive hormonotherapy (RR 2.2; P=0.03).

Combined effect of cyclin E and p27Kip1 protein levels

We compared BCSS among four groups of patients according to the combination of various levels of cyclin E and p27Kip1 protein level (Figure 3). An overall difference was found between the four curves (P<0.0001). Patients whose breast cancers featured low cyclin E and high p27Kip1 tumor protein levels had the highest BCSS, particularly when compared with patients with high cyclin E and low p27Kip1 protein levels (85% versus 46% respectively; P<0.0001, at 10 years follow-up).

When various combinations of expression were dichotomized according to BRCA1 mutation status, a worse survival was observed in both BRCA1 mutation carriers and non-carriers with high cyclin E and low p27Kip1 protein levels in the breast cancer. On dichotomizing the dataset into only two subgroups, those with high cyclin E and low p27Kip1 tumor protein levels and all other groups, the first group had a 10 year BCSS of 46%, whereas the survival in the latter group was 77% (P<0.001).
Discussion

We have shown here that cyclin E overexpression, as measured by IHC analysis of tumor protein, is a prognostic factor for breast cancer. In multivariate Cox analyses cyclin E was an independent prognostic factor only in those who did not receive adjuvant chemotherapy. High levels of cyclin E were strongly associated with BRCA1 germline mutation status. In this (Table 1) and previous studies using this dataset, we have shown that germline BRCA1 mutations were associated with nuclear grade 3 (versus grade 1; OR 21.6; \( P<0.0001 \)), ER-negative (OR 7; \( P<0.0001 \)) breast cancers, with the expression of nuclear p53 (OR 3.6; \( P=0.002 \)) [23], with low levels of p27\(^{\text{kip1}} \) expression (OR 2.8; \( P=0.03 \)) and with glomeruloid microvascular proliferation (OR 2.6; \( P=0.04 \)) [22]. Here, we found an OR of 9.5 and \( P<0.0001 \) for the association between BRCA1 mutations and high cyclin E levels. Thus the association between high cyclin E levels and BRCA1 mutations is as strong as the established relationship between the presence of a BRCA1 mutation and high-grade, ER-negative breast cancers. Furthermore, when these pathological characteristics were evaluated in a multivariate logistic regression model (Table 2), the strongest parameter that predicted BRCA1 germline mutations was the expression of high cyclin E levels in the malignant breast tissue.

A recent study showed that a high level of cyclin E as measured by western blotting was the strongest independent factor in predicting survival following breast cancer [15]. It was pointed out by several commentators that grade was not included in the final multivariable model, thus diluting the impact of their finding. The authors argued in a response letter that grade is not universally recognized as a prognostic factor [24]. This viewpoint is somewhat debatable. In the last international consensus established by the Early Breast Cancer Trials’ Collaborative Group, both histological and nuclear grade were considered as prognostic factors and thus are used to select patients who will receive adjuvant treatment, particularly in the subgroup of node-negative patients [1]. These recommendations were reinforced during the last meeting that took place in 2003 in St Gallen, Switzerland. Interestingly, when we excluded grade from the Cox multivariate analysis, high cyclin E expression levels (RR 1.9; 95% CI 1–3.3; \( P=0.04 \)), but not low p27\(^{\text{kip1}} \) levels, was an independent prognostic factor in the entire cohort.

When we evaluated BCSS according to the type of adjuvant treatment, high levels of cyclin E protein expression was the strongest independent prognostic factor (RR 5.7; \( P=0.002 \)) among the group of patients who did not receive adjuvant chemotherapy. When the analysis was adjusted for the type of chemotherapy received (anthracyclin-based versus other regimens), high cyclin E levels remained a prognostic factor, but of borderline significance in the multivariate analysis (RR 1.8; \( P=0.07 \)). As expected, high cyclin E expression was also an independent prognostic factor (RR 4.7; \( P=0.03 \)) among patients who received adjuvant hormonotherapy, reflecting the fact that most of these patients did not receive chemotherapy. This result suggests that chemotherapy may ameliorate the poor prognosis conferred by high levels of cyclin E protein. The role of differential protein levels of cyclin E and p27\(^{\text{kip1}} \) as potential predictive markers of response to chemother- or hormonotherapy is unclear [18, 25–27]. Available data are based on retrospective studies that employed non-standardized IHC methods, various adjuvant treatments and different stages of the disease, precluding any definitive conclusion. Nevertheless, this important issue deserves further well-designed prospective studies.

Simultaneous analysis of the expression of thousand of genes by DNA microarrays techniques have opened a new area in the search of markers that can predict the outcome of an individual patient. A recent study using this new high-throughput molecular technique has defined gene expression profiles associated with either a poor prognosis or a good prognosis after breast cancer [7]. In an extended study, the same research group recently showed that among patients associated with a genetically defined poor prognosis, 50% develop a distant progression of the disease and 45% died within 10 years. In the good prognosis group, 85% of patients stayed disease-free and 95% survived [28]. The genetic profile was the strongest independent prognostic indicator in this series. These molecular signatures consisted of a selection of a subset of 70 genes the expression of which defined the outcome. Notably, cyclin E2, a recently discovered protein with activity similar to cyclin E [29], was one of the genes identified in the 70-gene profile. In the van de Vijver et al. [28] study, DDFS of 151 patients with lymph node-negative disease was evaluated according to the prognosis signature. At 10 years, distant disease free survival (DDFS) for 60 patients (40% of node-negative patients) with tumor harboring a good prognosis profile was 87%, and 44% for 91 patients (60%) with a poor prognosis profile. These data can be compared with the survival results in association with cyclin E protein levels in our cohort. The DDFS was 85.6% at 10 years follow-up for 90 (70%) node-negative patients with low levels of cyclin E expression compared with 59.4% for 38 (30%)
patients with high levels of cyclin E expression ($P < 0.001$). Therefore, in our study, low levels of cyclin E staining identified 70% of all node-negative patients, amongst whom the DDFS was $>85$% at 10 years.

This study has a robust design in that, our historical cohort approach, results in an unbiased ascertainment. The restriction of our study population to the Ashkenazi limits the genetic heterogeneity that could obscure associations between putative disease markers and germline mutations. In particular, we are confident that the restriction of the BRCA1/BRCA2 mutation analysis to the three common Ashkenazi Jewish founder mutations has not resulted in many women being misclassified as non-carriers [30]. The number of individuals with BRCA1 mutations is small, so larger, confirmatory studies are required. However, the overall cohort is now fairly mature with a median follow-up of survivors of nearly 10 years; one of the longest periods of follow-up for a study evaluating cyclin E as a prognostic factor in breast cancer. The studied population is ethnically restricted. As such, the expansion of these results to other populations should be evaluated.

This study has confirmed that cyclin E is an important prognostic marker, particularly when evaluated along with p27Kip1 levels. It has been shown that overexpression of cyclin E can promote chromosomal instability [31]. The observations that: (i) BRCA1-related breast cancers tend to be highly aneuploid [5]; (ii) these cancers commonly overexpress cyclin E and underexpress p27Kip1; (iii) cyclin E overexpression and p27Kip1 underexpression are both associated with a poor outcome following breast cancer; and (iv) cyclin E overexpression mimicks the effect of germline BRCA1 mutations on response to adjuvant chemotherapy [23] suggests strongly that, directly or indirectly, cyclin E overexpression is a major determinant of the BRCA1 breast cancer phenotype. While the data remain preliminary, it will be important to determine, in a prospective study, whether adjuvant chemotherapy can significantly ameliorate the poor survival associated with high protein levels of cyclin E.

Acknowledgements

We thank Nancy Hamel and Ann-Josée Paradis for technical assistance. This work was supported by the Canadian Genetic Diseases Network, Fonds de la Recherche en Santé du Québec (FRSQ) Cancer Network-Breast and Ovarian Tumor Bank Axis and Susan G. Komen Foundation. P.O.C. was funded by the University Hospital of Geneva, Switzerland. J.R.G. was a recipient of the FRSQ/CAMO Fellowship. W.D.F. is funded by the FRSQ.

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