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Cyclin D1 expression is associated with poor prognostic features in estrogen receptor positive breast cancer

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Abstract Cyclins D1 and E play an important role in breast carcinogenesis. High cyclin E expression is common in hormone receptor negative and high grade aggressive breast cancer, whereas cyclin D1 in hormone receptor positive and low grade breast cancer. Experimental data has suggested that cyclin D1 and E mediate cell proliferation by different mechanisms in estrogen receptor (ER) positive and negative breast cancer. To test this hypotheses in large breast cancer material and to clarify the histopathological correlations of cyclin E and D1, especially the association with proliferation, we analyzed cyclin E and D1

immunohistochemical expression on breast tumour micro-arrays consisting of 1348 invasive breast cancers. High cyclin D1 expression was associated with high grade ($P < 0.0005$), high cyclin A ($P < 0.0005$) and Ki67 ($P < 0.0005$) expression among ER positive but with low grade ($P = 0.05$) and low Ki67 ($P = 0.01$) expression among ER negative breast cancers. Cyclin E and D1 expression correlated positively in ER positive ($P < 0.0005$) but had a negative correlation in ER negative tumours ($P = 0.004$). Cyclin E associated with high grade among all tumours ($P < 0.0005$). In conclusion, the findings of this study show that cyclin D1 has separate roles, and proliferation is driven by different mechanisms in ER positive and negative breast cancers.

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Introduction

Cell cycle regulators cyclin D1 and E mediate positive growth stimuli to cell cycle progression and cell proliferation [1]. They control the G1 to S phase transition, a critical checkpoint controlling cell entry into division. Cyclin D1 activates the cyclin-dependent kinase (CDK) 4/6 and cyclin E CDK2.

Amplification of the coding gene CCND1 and post-transcriptional deregulation lead to high cyclin D1 expression that occurs in about 50% of breast cancers [2]. High cyclin D1 expression is associated with non-aggressive features in breast cancer including estrogen receptor (ER) positivity [3–8]. Estrogen stimulates cyclin D1 expression and cyclin D1 is an important mediator of estrogen stimulation of cell cycle progression [9]. Cyclin

D1 does not seem to be an independent prognostic factor in breast cancer [3–5, 10, 11] but has been linked to tamoxifen resistance [12–14]. Cyclin D1 also has CDK-independent functions and can directly, without estrogen, activate ER [15].

In normal cells cyclin E expression is under strict regulation but in cancers regulatory processes are often disturbed and high cyclin E expression is common [1]. Cyclin E gene amplification is rare, and disturbances in posttranslational as well as proteolytic regulation underlie the high expression in cancer cells [16, 17]. After estrogen treatment the cyclin E-CDK2 complex is activated by induction of cyclin D1-CDK4/6. High cyclin E expression causes chromosomal instability [18] and is often associated with aggressive disease features such as hormone receptor negativity, high grade and large tumour size in breast cancer [17, 19–23]. High cyclin E expression and particularly its low molecular weight (LMW) derivatives have in most studies been an adverse prognostic factor [17, 19, 20–26]. High cyclin E expression has also predicted endocrine therapy failure [27–29]. One study in patients treated with adjuvant chemotherapy failed to show an association with poor prognosis [30].

A study on 113 primary breast cancers and breast cancer cell lines suggested that cyclin D1 and E induce proliferation by different mechanisms [31]. ER positive tumours with high cyclin D1 had an increased proliferation rate but in ER negative tumours with low cyclin D1 proliferation was even higher. These tumours showed high cyclin E expression. In ER positive breast cancer cyclin D1 seems to activate the cell cycle, whereas in ER negative tumours high proliferation seems to be due to a cyclin E overexpression-dependent mechanism that does not require cyclin D1.

In order to test this hypothesis we analyzed cyclin D1 and E expression in a series of 1,348 invasive breast cancers to explore the role of cyclin D1 and E expression in proliferation among ER positive and negative breast cancer and their association with other histopathological variables.

Material and methods

Patients

The study material consists of 1,348 invasive breast tumours. Of these 884 are tumours from unselected patients collected at the Department of Oncology, Helsinki University Central Hospital between 1997 and 1998 [32] and 2000 [33] (79% of all consecutive, newly diagnosed breast cancer cases during the collection periods). Of the unselected tumours 439 are tumours from sporadic and 445 from patients with one first degree or two or more first or

second degree relatives with breast or ovarian cancer. A total of 464 additional tumours from familial breast cancer patients were collected by a systematic screening at the Department of Oncology, Helsinki University Central Hospital or were ascertained through genetic counselling at the Department of Clinical Genetics [34]. Of all tumours from familial patients 453 are from patients with stronger family history (at least three first or second degree relatives with breast or ovarian cancer, including the proband), and 341 from patients with two affected first degree relatives (including the proband), 56 from BRCA1, and 59 from BRCA2 mutation carriers.

All patients' cancer diagnoses were confirmed through the Finnish Cancer Registry. Non-invasive cancers were excluded. Patient characteristics are shown in Table 1. Information on tumour histology, grade, size, nodal status, distant metastases, and ER and PR status were obtained from pathology reports [35]. A breast cancer pathologist (P.H.) re-reviewed all tumours for tumour histology and grade. Grading was performed according to Scarff–Bloom–Richardson modified by Elston and Ellis. HER2 protein expression on TMAs was analyzed by immunohistochemical staining and gene amplification with chromogenic in situ hybridization (CISH) [36, 37], and p53 protein expression by immunohistochemical expression as previously described [38]. The TMAs were stained with a mouse monoclonal cyclin A antibody to evaluate cyclin A protein expression [39], and with Ki67 antibody (Dako cytometry, Sweden) to evaluate Ki67 protein expression [40]. The tumour was considered Ki67 strong positive if 30% or more of the cancer cells stained, intermediate if 20–29% of the cancer cells stained, weak positive if 5–19% of the cancer cells stained, and tumours with <5% of the cancer cells staining were considered Ki67 negative. Cyclin A was analyzed as a continuous variable.

Information on adjuvant treatment and distant metastases during the follow-up was collected from the patient records. The information on death due to breast cancer or other reason came from the Finnish Cancer Registry. A total of 797 patients were accepted to survival analysis, including the unselected series and familial patients attending the study within 6 months after diagnosis. The median follow-up time was 93 months (2–516 months). Of all patients in the survival analysis 127 (16%) relapsed with distant metastases during the follow-up time, of whom 91 (11%) died from breast cancer.

Tissue micro array construction

Paraffin blocks of the primary tumours were collected. The blocks' haematoxylin and eosin sections were studied and from the most representative tumour area four cores

Table 1 Patient and tumour characteristics

| | |
|-------------------------|--------------|
| <i>Grade</i> | |
| 1 | 318 (23.6%) |
| 2 | 606 (45.0%) |
| 3 | 399 (29.6%) |
| not known | 25 (1.9%) |
| <i>T</i> | |
| 1 | 782 (58.0%) |
| 2 | 448 (33.2%) |
| 3 | 51 (3.8%) |
| 4 | 38 (2.8%) |
| NA | 29 (2.2%) |
| <i>N</i> | |
| Positive | 585 (43.4%) |
| Negative | 731 (54.2%) |
| NA | 32 (2.4%) |
| <i>M</i> | |
| Positive | 48 (3.6%) |
| Negative | 1244 (92.3%) |
| NA | 56 (4.2%) |
| <i>ER</i> | |
| Positive | 989 (73.4%) |
| Negative | 288 (21.4%) |
| NA | 71 (5.3%) |
| <i>PR</i> | |
| Positive | 828 (61.4%) |
| Negative | 448 (33.2%) |
| NA | 72 (5.3%) |
| <i>HER2</i> | |
| Positive | 155 (11.5%) |
| Negative | 1074 (79.7%) |
| NA | 119 (8.8%) |
| <i>p53</i> | |
| Positive | 253 (18.8%) |
| Negative | 989 (73.4%) |
| NA | 106 (7.9%) |
| <i>Ki67 expression</i> | |
| <5% | 318 (23.6%) |
| 5–19% | 553 (41.0%) |
| 20–29% | 220 (16.3%) |
| >29% | 214 (15.9%) |
| NA | 43 (3.2%) |
| <i>Tumour pathology</i> | |
| Ca ductale | 924 (68.5%) |
| Ca lobulare | 254 (18.8%) |
| Ca medullare | 20 (1.5%) |
| Ca mucinosum | 25 (1.9%) |
| Ca papillare | 4 (0.3%) |
| Ca tubulare | 46 (3.4%) |
| Others | 75 (5.6%) |

Table 1 continued

| | |
|----------------------------|-------------|
| <i>Familial background</i> | |
| BRCA1 | 56 (4.2%) |
| BRCA2 | 59 (4.4%) |
| Familial non-BRCA1/2 | 794 (58.9%) |
| Sporadic | 439 (32.6%) |

(diameter 0.6 micrometer) were punched and included in the tissue micro arrays (TMA) (two cores from BRCA1 and BRCA2 tumours). Subsequently 3–4 micrometer thick sections were cut from array blocks and transferred to glass slides [35].

Immunohistochemistry

After deparaffinization in xylene and hydration in graded alcohols all immunostainings were done in automated immunostainer (Ventana Medical Systems Inc., Tuscon, AZ, USA) using a diaminobenzidine (DAB) kit (Ventana) to ensure standardized performance. Cyclin E (BD Pharmingen) and cyclin D1 (Novocastra) antibodies were diluted 1:20 and antigen retrieval was done using the iView-kit.

Evaluation of immunoreactivity scores

TMA slides were analyzed by one, and partly by two investigators (Ki.A. and R-M.A). We have earlier shown that the reproducibility of cyclin expression scoring on breast cancer TMAs is good [39]. Cyclin E and D1 positive cells were counted in one high-power field (40× objective) in each of the four cores on TMA. Only unequivocal positive nuclear staining was accepted as a positive reaction. A minimum of 200 cells was counted in each tumour. The result was the percentage of all positive cells from the entire number of breast cancer cells counted from the four biopsies. Tumours with expression above mean expression (6.8% for cyclin E and 9.1% for cyclin D1) of all tumours were considered as high expression tumours and those below mean expression as low expression tumours.

Statistical analysis

Statistical analyses were made with SPSS for Windows v12.0.1 (SPSS Inc., Chicago, IL, USA). The correlation of cyclin D1 and E with other histological variables was analyzed by Spearman's rho (continuous variables) or Mann–Whitney test (dichotomic variables). The correlations

were analyzed with cyclin E and D1 as continuous variables. Survival was assessed with Cox regression analysis with cyclin E and D1 dichotomized at median values. Statistical significance was set at 0.05, and all *P*-values are two-sided.

Ethics

This study was performed with informed consent from the patients as well as permission from the Ethics Committee of the Helsinki University Central Hospital and from the Ministry of Social Affairs and Health in Finland.

Results

Cyclin D1 result was obtained from 1,187 (88.1%) and cyclin E from 1,180 (87.5%) tumours. In the remaining cases the biopsy did not contain enough tumour cells to be evaluated or the staining was unsuccessful. The median cyclin D1 count was 9.0% (range 0–81%) and cyclin E 6.5% (range 0–68%).

High cyclin D1 expression correlated with low tumour grade ($P = 0.01$), positive nodal status ($P = 0.02$), ER ($P < 0.0005$) and PR ($P < 0.0005$) positivity, and negative p53 immunohistochemistry ($P < 0.0005$) (Table 2A). Among ER negative tumours high cyclin D1 similarly associated with low grade ($P = 0.08$) and low Ki67 expression ($P = 0.01$) (Table 2C). Among ER positive tumours, in contrast, high cyclin D1 expression correlated with high grade ($P < 0.0005$) and high Ki67 ($P < 0.0005$) and cyclin A ($P < 0.0005$) expression (Table 2B). Of ER positive grade 3 tumours 67% had high cyclin D1 expression (dichotomized at mean value), while only 21% of ER negative grade 3 tumours showed high expression (Table 3). The correlation between cyclin D1 and grade as well as Ki67 expression were similar among HER2 positive and negative cases irrespective of ER status (data not shown).

When all tumours were analyzed together, cyclin D1 and E as continuous variables did not correlate ($P = 0.99$), but in ER positive cases there was a positive ($P < 0.0005$), and in ER negative cases a negative ($P = 0.004$) correlation (Table 2A–C). Figure 1 shows cyclin D1 plotted against cyclin E expression among ER positive and ER negative breast cancers.

Tumours with low cyclin D1 expression were more often of medullary histology (2.4%) than tumours with high cyclin D1 expression (0.5%, $P = 0.007$) ones. The frequencies of ductal and lobular histology were not related to cyclin D1 expression.

High cyclin E expression correlated with large tumour size ($P = 0.009$), high grade ($P < 0.0005$), ER ($P < 0.0005$) and PR ($P < 0.0005$) negativity, HER2 ($P < 0.0005$) and p53 ($P < 0.0005$) positivity, high Ki67 ($P < 0.0005$) and cyclin A ($P < 0.0005$) expression, and younger age at disease onset ($P < 0.0005$) (Table 4A). All cyclin E correlations except the correlation with cyclin D1 expression, as mentioned above, were similar among ER positive and negative tumours (Table 4B and C). Among tumours with high cyclin E more ductal (77.6%) and medullary (2.8%) tumours were found than among those with low expression (64.1%, $P < 0.0005$ and 0.3%, $P = 0.0008$, respectively). Lobular histology was seen more frequently in tumours with low cyclin E (23.7%) compared to tumours with high cyclin E (10.0%, $P < 0.0005$).

Cyclin D1 expression did not affect survival among all patients, or among ER positive and negative cases analyzed separately. Among ER positive cases not treated with adjuvant chemotherapy, however, high cyclin D1 showed a trend toward shorter metastasis free survival (RR 1.74, 95%CI 0.93–3.24, $P = 0.08$). High cyclin E expression correlated with poor overall survival (RR 1.62, 95%CI 1.05–2.52, $P = 0.03$) in univariate analysis but not in a multivariate model including tumour size, nodal status, grade, ER, PR, and HER2 status (RR 1.01, 95%CI 0.91–2.34, $P = 0.41$). The association with poor metastasis free survival was significant in univariate (RR 1.77, 95%CI 1.21–2.61, $P = 0.003$) as well as in multivariate analysis (RR 1.76, 95% CI 1.17–2.64, $P = 0.006$). Hormone receptor status, chemotherapy, or endocrine treatment did not affect cyclin E associations with survival.

Discussion

In this study we investigated the associations of cyclin D1 and E expression with other tumour histopathological features, addressing on hormone receptor status, tumour grade and proliferation, in an extensive breast cancer material of 1,348 invasive tumours. The most important finding of the study was that among ER positive tumours high cyclin D1 expression showed a highly significant correlation with high tumour grade and high Ki67, cyclin A and E expression but among ER negative tumours, on the contrary, with low grade and low Ki67 and cyclin E expression. High cyclin D1 also showed a trend towards poor metastasis free survival among ER positive chemotherapy-naïve tumours. The results show that cyclin D1 has different roles and proliferation is driven by different mechanisms in ER positive and negative breast cancer, supporting the important role of cyclin D1 in pathogenesis

Table 2 Correlation of cyclin D1 with other tumour characteristics (Spearman's Rho)

| Variable | Correlation coefficient | 95% CI | P-value |
|--|-------------------------|------------------|----------|
| <i>A. Among all tumours</i> | | | |
| T | -0.120 | -0.081 to 0.035 | 0.43 |
| N | 0.070 | 0.013 to 0.127 | 0.02* |
| M | 0.021 | -0.037 to 0.079 | 0.49* |
| Grade** | -0.074 | -0.131 to -0.017 | 0.01 |
| Estrogen receptor | 0.373 | 0.323 to 0.422 | <0.0005* |
| Progesterone receptor | 0.297 | 0.243 to 0.350 | <0.0005* |
| HER2 | 0.043 | -0.015 to 0.101 | 0.15* |
| Ki67** | -0.039 | -0.096 to 0.018 | 0.18* |
| Cyclin A** | 0.031 | -0.027 to 0.089 | 0.30 |
| p53 | -0.170 | -0.226 to -0.113 | <0.0005 |
| Age at onset | 0.011 | -0.046 to 0.068 | 0.69 |
| Cyclin E** | 0.000 | -0.057 to 0.057 | 0.99 |
| <i>B. Among estrogen receptor positive tumours</i> | | | |
| T | 0.040 | -0.027 to 0.107 | 0.24 |
| N | 0.062 | -0.006 to 0.127 | 0.07* |
| M | 0.036 | -0.032 to 0.103 | 0.30* |
| Grade** | 0.151 | 0.085 to 0.216 | <0.0005 |
| Progesterone receptor | 0.068 | 0.001 to 0.134 | 0.05* |
| HER2 | 0.073 | 0.005 to 0.140 | 0.04* |
| Ki67** | 0.156 | 0.089 to 0.221 | <0.0005 |
| Cyclin A** | 0.261 | 0.197 to 0.323 | <0.0005 |
| p53 | -0.027 | -0.095 to 0.041 | 0.43 |
| Age at onset | -0.042 | -0.109 to 0.025 | 0.22 |
| Cyclin E** | 0.231 | 0.166 to -0.294 | <0.0005 |
| <i>C. Among estrogen receptor negative tumours</i> | | | |
| T | -0.02 | -0.142 to 0.103 | 0.74 |
| N | 0.107 | -0.015 to 0.226 | 0.09* |
| M | 0.026 | -0.097 to 0.148 | 0.68* |
| Grade** | -0.109 | -0.228 to 0.014 | 0.08 |
| Progesterone receptor | 0.130 | 0.009 to 0.247 | 0.04* |
| HER2 | 0.192 | 0.067 to 0.311 | 0.003* |
| Ki67** | -0.155 | -0.273 to -0.033 | 0.01 |
| Cyclin A** | -0.002 | -0.125 to 0.121 | 0.97 |
| p53 | -0.076 | -0.198 to 0.048 | 0.23 |
| Age at onset | 0.036 | -0.086 to 0.157 | 0.56 |
| Cyclin E** | -0.178 | -0.294 to -0.056 | 0.004 |

* P-value from Mann–Whitney test

** Correlation is different among all tumours, or estrogen receptor positive and negative tumours analyzed separately

of hormone receptor positive breast cancer. Furthermore cyclin E was shown to clearly associate with aggressive breast cancer phenotype and it was found as an

independent marker of poor metastasis free survival. This study, to our knowledge, is the largest by far to study these aspects and first to show the differential association of cyclin D1 with proliferation and hormone receptor status in a large breast cancer material.

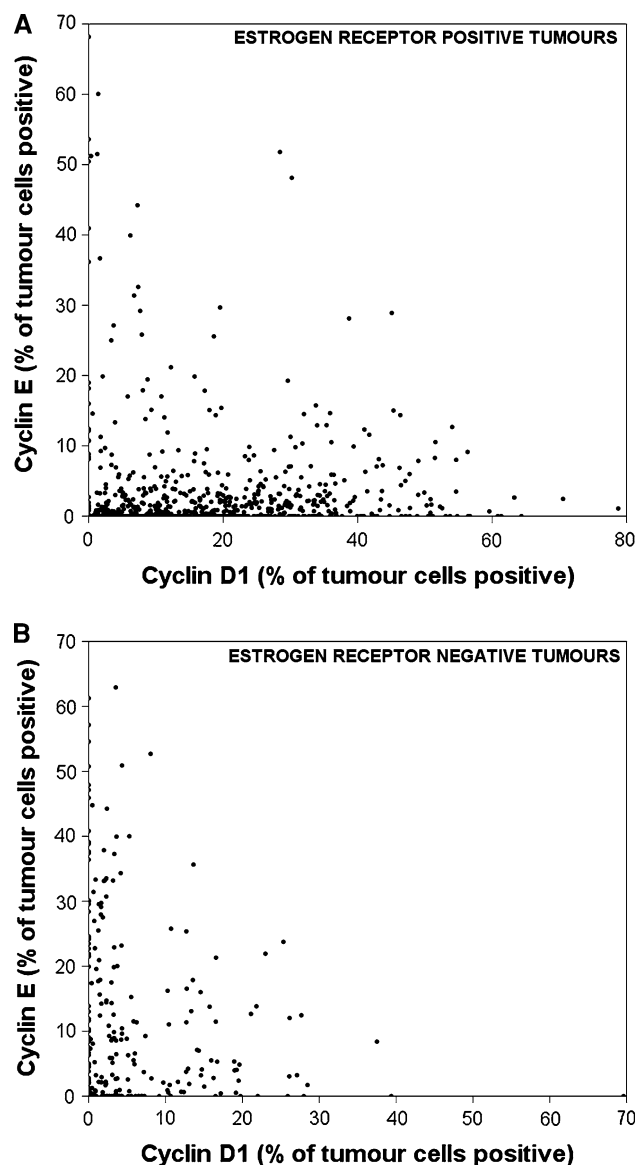
As regulators of critical cell cycle checkpoint G1/S, cyclins D1 and E have been implemented in breast carcinogenesis but their associations with other histopathological features and prognosis of breast cancer are still not fully understood. High cyclin D1 expression has been associated with less aggressive tumour characteristics including hormone receptor positivity. It has associated with low tumour grade [4, 6, 7, 14], although a few studies have failed to confirm this [3, 5, 11]. The role of cyclin D1 in breast cancer prognosis remains unclear; some studies show correlation to poor, some to good prognosis, and some have not been able to show any correlation. In our study cyclin D1 did not correlate with survival in the whole patient material, but among ER positive chemotherapy-naïve tumours showed a trend towards poor metastasis free survival. Cyclin D1 effects not only cell cycle regulation; it binds to, and activates the estrogen receptor, and also co-operates with c-myc in ER signalling [15, 41]. Thus cyclin D1 may have an oncogenic role particularly in hormone receptor positive breast cancer. It also has been suggested that cyclin D1 may mediate tamoxifen resistance [12–14].

Experimental data implied that cyclin D1 is needed to drive proliferation in ER positive breast cancer cells while in ER negative cells proliferation proceeds through other, cyclin D1 independent mechanisms [31]. Our results support this view, since we show in large breast cancer material that proliferation in ER positive tumours indeed seems to be mediated by cyclin D1, but in ER negative tumours to be driven by other, cyclin D1 independent mechanisms. This is in accordance with data implying that in ER negative breast cancer cell cycle regulation does not occur through cyclin D1 and ER [15] but proliferation may be activated through deregulation downstream from the pRB-node with cyclin E being overexpressed. It can be hypothesized that in ER positive breast cancer cells with pathologically active cell cycle the deregulated pathway causing cell cycle activation may be cyclin D1-pRB, and that in these cases cyclin E expression may be a physiological consequence of cell cycle activation. This hypothesis warrants further investigation of the cyclin D1-pRB pathway. If confirmed this pathway may be a source for developing new targeted biological therapies. Promising results in MCF-7 breast cancer cells imply that pharmacological shutting down of the cyclin D1/CDK4-complex inhibits cell proliferation and could be a useful strategy to limit tumour growth [42].

Table 3 Proliferation (Ki67) and tumour grade in tumours with high and low cyclin D1 expression (dichotomized at mean expression 9.1%)

| | Among estrogen receptor positive tumours | Among estrogen receptor negative tumours | <i>P</i> -value |
|----------------|--|--|-----------------|
| | Grade 3 tumours (%) | Grade 3 tumours (%) | |
| High cyclin D1 | 115 (67%) | 39 (21%) | <0.00005* |
| Low cyclin D1 | 57 (33%) | 148 (79%) | |
| | Tumours with moderate or strong Ki67 (%) | Tumours with moderate or strong Ki67 (%) | |
| High cyclin D1 | 147 (66%) | 34 (23%) | <0.00005* |
| Low cyclin D1 | 77 (34%) | 124 (84%) | |

* Chi-square

**Fig. 1** A scattergram showing the correlation of cyclin D1 and E expression among estrogen receptor positive tumours (a) and among estrogen receptor negative tumours (b)

The crucial role of cyclin D1 in driving proliferation among ER positive tumours may explain the role of cyclin D1 in tamoxifen resistance. In ER positive tumours with high cyclin D1 expression the activation of ER by cyclin D1 may increase tamoxifen's agonistic effect on ER. Cyclin D1 showed a strong correlation with positive ER and PR status, which is in accordance with earlier data, and confirms the important role of cyclin D1 in ER positive breast cancer [3–6, 8, 11]. The low expression of cyclin D1 among medullary cancers is also in line with previous findings, and indicates the idea that cyclin D1 does not have a significant role among basal subtype, or BRCA1 cancers [8].

In our previous report (Aaltonen et al., unpublished data), cyclin D1 expression differed in sporadic and familial breast cancers. To test whether heredity might have affected our results the analyses were carried out among familial non-BRCA1/2, sporadic, BRCA1 and BRCA2 positive patients separately and the results were similar in all patient groups. Thus familial background does not seem to explain the different cyclin D1 association among ER positive and negative tumours.

The findings of this study clearly strengthen the role of cyclin E in aggressively behaving breast cancer. Most studies evaluating the association of cyclin E with histopathological markers have found correlation with aggressive features such as high grade and negative hormone receptor status [17, 19, 27], and some studies furthermore a correlation with high Ki67 expression, younger age at diagnosis and large tumour size [22, 23, 43]. The associations in our study were similar; high cyclin E correlated with high grade, high Ki67 expression and cyclin A expression, ER and PR negativity, large tumour size and younger age at disease onset. In this study high cyclin E also correlated with negative p53 immunohistochemistry. This confirms an earlier finding of Lindahl et al. [23], showing that tumours with high cyclin E had p53 insertions or deletions, and undetectable p53 expression. One potential explanation may be that chromosomal

Table 4 Correlation of cyclin E with other tumour characteristics (Spearman's Rho)

| Variable | Correlation coefficient | 95%CI | P-value |
|--|-------------------------|------------------|----------|
| <i>A. Among all tumours</i> | | | |
| T | 0.077 | 0.019 to 0.134 | 0.009 |
| N | -0.004 | -0.062 to 0.054 | 0.88* |
| M | -0.011 | -0.069 to 0.047 | 0.71* |
| Grade | 0.391 | 0.341 to 0.439 | <0.0005 |
| Estrogen receptor | -0.348 | -0.399 to -0.295 | <0.0005* |
| Progesterone receptor | -0.288 | -0.341 to -0.233 | <0.0005* |
| HER2 | 0.176 | 0.119 to 0.232 | <0.0005* |
| Ki67 | 0.389 | 0.339 to 0.437 | <0.0005 |
| Cyclin A | 0.402 | 0.352 to 0.449 | <0.0005 |
| p53 | 0.242 | 0.186 to 0.296 | <0.0005 |
| Age at onset | -0.146 | -0.201 to -0.090 | <0.0005 |
| Cyclin D1** | 0.000 | -0.057 to 0.057 | 0.99 |
| <i>B. Among estrogen receptor positive tumours</i> | | | |
| T | 0.031 | -0.037 to 0.098 | 0.37 |
| N | 0.026 | -0.042 to 0.094 | 0.45* |
| M | -0.025 | -0.093 to 0.044 | 0.48* |
| Grade | 0.247 | 0.183 to 0.309 | <0.0005 |
| Progesterone receptor | -0.059 | -0.126 to 0.008 | 0.09 |
| HER2 | 0.141 | 0.074 to 0.207 | <0.0005* |
| Ki67 | 0.259 | 0.195 to 0.321 | <0.0005 |
| Cyclin A | 0.261 | 0.197 to 0.323 | <0.0005 |
| p53 | 0.137 | 0.069 to 0.203 | <0.0005 |
| Age at onset | -0.114 | -0.180 to -0.047 | 0.001 |
| Cyclin D1** | 0.231 | 0.166 to 0.294 | <0.0005 |
| <i>C. Among estrogen receptor negative tumours</i> | | | |
| T | 0.053 | -0.070 to 0.175 | 0.40 |
| N | -0.055 | -0.176 to 0.068 | 0.38* |
| M | 0.003 | -0.120 to 0.126 | 0.96* |
| Grade | 0.372 | 0.261 to 0.473 | <0.0005 |
| Progesterone receptor | -0.191 | -0.305 to -0.071 | 0.002 |
| HER2 | 0.082 | -0.044 to 0.206 | 0.200 |
| Ki67 | 0.396 | 0.287 to 0.495 | <0.0005 |
| Cyclin A | 0.423 | 0.317 to 0.519 | <0.0005 |
| p53 | 0.116 | -0.007 to 0.236 | 0.06 |
| Age at onset | -0.088 | -0.207 to 0.034 | 0.156 |
| Cyclin D1** | -0.178 | -0.294 to -0.056 | 0.004 |

* P-value from Mann–Whitney test

** Correlation is different among all tumours, or estrogen receptor positive and negative tumours analyzed separately

instability caused by cyclin E leads to inactivation of the tumour suppressor p53. Medullary and ductal histology were more common among cyclin E positive than negative

tumours. The association with medullary histology has been described earlier, and may be due to cyclin E's ability to modulate the infiltrative behavior of the tumour [44]. Tumours with high cyclin E were significantly more often HER2 positive than tumours with low cyclin E. To our knowledge, only one smaller study has evaluated the correlation of cyclin E and HER2 expression, and found a positive correlation [45]. This association supports the role of high cyclin E in aggressive breast cancer phenotype. Importantly, cyclin E was also found as an independent marker of poor metastasis free survival.

In conclusion, this study confirms that cyclin D1 correlates with high proliferation among ER positive but with low proliferation among ER negative breast cancer, suggesting that proliferation in ER positive and negative breast cancers is driven by different mechanisms, and supporting the important role of cyclin D1 in tumourigenesis of estrogen receptor positive breast cancer. Furthermore, cyclin E associated with aggressive breast cancer phenotype and was found as an independent marker of poor metastasis free survival. These findings reveal new biological data on the mechanisms of proliferation and cell cycle control as well as pathogenesis of breast cancer, with also clinical implications for prognostic evaluation and possibly even for developing new targeted therapies for breast cancer in the future.

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