Overexpression of Cyclin D1 Messenger RNA Predicts for Poor Prognosis in Estrogen Receptor-positive Breast Cancer[1]

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ABSTRACT

Cyclin D1 is a key cell cycle regulatory protein with demonstrated oncogenic activity in a variety of malignancies. Cyclin D1 mRNA and protein are overexpressed in approximately 50% of primary breast carcinomas; however, the pathophysiological consequences of increased expression remain unclear. To investigate the functional sequelae of cyclin D1 mRNA overexpression, we analyzed clinical outcome in relation to the cyclin D1 mRNA level in 253 primary breast cancer patients (median follow-up, 75 months) with particular reference to estrogen receptor (ER) status and endocrine response. Overall, with the exception of the relationship between cyclin D1 mRNA expression and the ER, cyclin D1 mRNA was not associated with other clinicopathological features such as age, menopausal status, axillary lymph node status, vascular invasion, tumor size, type, and grade. However, in patients with ER-positive tumors ($n = 182$), high levels of cyclin D1 mRNA were associated with increased risk of relapse ($P = 0.0016$), local recurrence ($P = 0.025$), metastasis ($P = 0.019$), and death ($P = 0.025$). In contrast, there were no clinical correlations with cyclin D1 expression in ER-negative disease ($n = 71$). In 33 patients who received endocrine therapy for their primary or recurrent breast cancers, there was an apparent association between a high cyclin D1 mRNA level and a shorter response duration within the ER-positive subgroup ($P = 0.04$). Our findings indicate that overexpression of cyclin D1 mRNA correlates with a worse prognosis within the ER-positive breast cancer phenotype and may be a contributing factor to the development of endocrine resistance in ER-positive disease.

INTRODUCTION

The cyclins are a family of key regulatory proteins that drive the ordered progression of mammalian cells through critical transition points in the cell division cycle. A number of classes of cyclins have been identified displaying sequential expression and activity throughout the cycle. The D-type (G_1) cyclins control the passage of cells through the G_1 phase, ultimately allowing entry into S phase (1). Cyclin D1 acts by forming complexes with Cdk4[4] and Cdk6, resulting in the phosphorylation of substrates including the retinoblastoma gene product, pRb. Unphosphorylated pRb restrains cell cycle progression by binding to the E2F family of transcription factors; phosphorylation releases this inhibition, inducing cells to enter S phase (2).

Cyclin D1 is essential for the formation of lobuloalveoli during normal mammary gland development (3, 4), whereas deregulated expression of cyclin D1 stimulates aberrant mammary epithelial cell proliferation and promotes tumorigenesis in transgenic mice (5). Overexpression of cyclin D1 renders the growth of normal cells less dependent on growth factors and accelerates passage through the G_1 phase of the cell cycle (6, 7), suggesting that increased expression may lead to the loss of normal regulatory constraints and confer a growth advantage. Cyclin D1 expression is elevated to similar levels in human mammary ductal carcinoma *in situ* and invasive breast cancer (8, 9) and is increased above normal levels in preneoplastic hyperproliferative lesions (9), implying that molecular alterations leading to cyclin D1 overexpression occur relatively early during breast carcinogenesis.

Human cyclin D1 is encoded by the *CCND1* gene, which is located at chromosome 11q13. This chromosomal locus, which encompasses several proto-oncogenes, is consistently amplified in a variety of epithelial cancers including breast, lung, esophageal, and bladder carcinomas and squamous cell carcinomas of the head and neck (10, 11). Whereas coamplification of adjacent oncogenes at this large locus may occur (12, 13), the fundamental role played by cyclin D1 in regulation of normal cell cycle control and the consistent amplification and overexpression of this gene in a range of malignancies imply that cyclin D1 is one of the dominant oncogenes in this region.

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[4] The abbreviations used are: Cdk, cyclin-dependent kinase; ER, estrogen receptor; DFI, disease-free interval; pRb, retinoblastoma protein.

Several publications have reported that 11q13 amplification in breast cancer is associated with poor prognosis (14–16). However, there is now a general consensus that whereas 11q13 is amplified in approximately 13% of breast cancers (10), the frequency of cyclin D1 mRNA and protein overexpression is at least 3-fold higher (45–50% ; Refs. 17–20), indicating that the latter may result from mechanisms other than gene amplification. Thus, assessment of 11q13 amplification is likely to identify a minority of cyclin D1-overexpressing tumors, whereas examining the relationship between cyclin D1 overexpression and prognosis is likely to more accurately reflect the pathophysiological consequences of aberrant cyclin D1 expression. In contrast to most gene amplification studies, two groups using immunohistochemical assessment of cyclin D1 expression reported an improved outcome with cyclin D1 overexpression in primary breast cancer (21, 22), whereas others failed to find an association (23–25). Thus, the true prognostic significance of cyclin D1 overexpression in human breast cancer has yet to be resolved.

The three largest published studies (15, 16, 26) examining 11q13 amplification found an association between cyclin D1 gene copy number and poor outcome, especially in the ER-positive phenotype. Moreover, a number of studies have reported a positive correlation between 11q13 amplification, cyclin D1 expression, and ER positivity in breast cancer (11, 16, 22, 24, 27), suggesting that the two genes may be functionally related. Estrogens induce cyclin D1 expression in human breast cancer cell lines (28–31), whereas antiestrogenic inhibition of proliferation is preceded by a reduction in cyclin D1 mRNA and protein synthesis in G_1 (32, 33), implicating cyclin D1 as a target for estrogen-stimulated mitogenesis in this disease. Furthermore, ectopic overexpression of cyclin D1 reverses the growth-inhibitory effect of antiestrogens in ER-positive breast cancer cells (34). These *in vitro* experiments demonstrate a close functional interplay between ER and cyclin D1 gene expression at the molecular level, but the potential clinical implications of such a relationship remain to be elucidated.

We have previously demonstrated a strong positive correlation between cyclin D1 mRNA and ER mRNA levels ($P = 0.0001$) in a large series of 364 primary breast cancers (27). To investigate issues of potential functionality between the two genes and their relationship to established prognostic features, we subsequently analyzed the relationship between cyclin D1 mRNA expression and various clinicopathological parameters in a subset of 253 patients. In particular, we assessed the clinical outcome of patients within the ER-positive and ER-negative subgroups and the degree of responsiveness to endocrine therapy according to their tumor cyclin D1 mRNA levels.

MATERIALS AND METHODS

Patient Characteristics. Tumor samples were obtained from surgical specimens of 364 breast cancer patients treated at the Nottingham Breast Unit over the period from February 1987 to December 1993. Clinical data were available from computerized databases on 253 of the patients with stage I-II disease ($n = 217$ for patients <70 years; $n = 36$ for elderly patients, *i.e.*, >70 years). Median age was 55 years (range, 24–93 years). The 217 primary cases <70 years old were treated with either simple/s.c. mastectomy or wide local excision followed by intact breast irradiation. All of these 217 patients underwent axillary lymph node sampling. Adjuvant systemic therapy protocols were introduced in 1989, based on the Nottingham Prognostic Index (35). Patients falling into the moderate and poor prognostic groups received adjuvant cyclophosphamide, methotrexate, and 5-fluorouracil chemotherapy ($n = 44$) or adjuvant tamoxifen ($n = 67$), depending on age and ER positivity.

Twenty-one of the 36 elderly stage I-II cases (>70 years old) underwent surgical treatment in the form of simple or wedge mastectomy with no axillary intervention unless palpable lymph nodes were present ($n = 8$). These patients did not receive routine adjuvant therapy. The remaining 15 elderly patients were treated with primary tamoxifen (a tumor specimen had been obtained via operative endocrine biopsy before the commencement of treatment). Four locally advanced cases treated with initial hormone therapy were included in the assessment of hormone response in relation to cyclin D1 level but were excluded from the overall survival analyses due to their inherently worse prognosis.

Measurement of Cyclin D1 and ER Expression. Total RNA was extracted from the primary breast cancer samples, Northern blotted, and analyzed as described previously (27). In brief, in the presence of 50% (v/v) deionized formamide, 2× saline-sodium phosphate-EDTA, 1% SDS, 0.5% Blotto (10% skim milk powder in 0.2% azide), 10% dextran sulfate, 0.04 mg/ml polyadenylic acid, and 0.5 mg/ml salmon sperm DNA, the filters were probed with full-length human cyclin D1 and ER cDNA (27) and also with cDNA for 36B4, a non-estrogen-regulated ribosomal protein that served as a control for RNA loading (36), at 50°C. After hybridization, the filters were washed at a highest stringency of 0.2× SSC and 1% SDS for 30 min at 65°C. Each filter contained four control cell lines for normalization between the 22 filters. The ratio of cyclin D1:36B4 mRNA signal intensity for each sample was normalized to that of MDA-MB-231 cells, which was defined arbitrarily as 1.0 to yield the relative expression of cyclin D1 mRNA. Breast tissue sections were analyzed for the presence of ER using the monoclonal ER-ICA kit (Abbott, North Chicago, IL) as discussed elsewhere (37).

Statistical Analysis of Clinical Data. Follow-up data were taken from the time of the last clinic appointment or the date of death (median follow-up, 75 months; range, 2–9 years). To date, 95 (38%) of the primary patients have relapsed, 64 (25%) have developed metastases, and 51 (20%) have died from breast cancer. Deaths from unrelated causes were censored for the purposes of survival analyses. All statistical calculations were performed using the SPSS Data Analysis Program (SPSS for Windows 6.1.3; SPPS United Kingdom Ltd.). The χ^2 test was used to assess differences in categorical variables using Fisher's exact (two-tailed) test where appropriate. Survival outcomes were assessed using univariate Cox regression analysis for comparison of continuous variables and life table analysis using the Wilcoxon Gehan statistic (38) for comparison of categorical variables. Multivariate analysis of survival was performed using the Cox proportional hazards model.

Table 1 Clinicopathological parameters according to cyclin D1 mRNA expression and ER status in stage I-II breast cancer

	Overall (n = 253)			ER-positive group (n = 182)			ER-negative group (n = 71)		
	Cyclin D1			Cyclin D1			Cyclin D1		
	Low	High		Low	High		Low	High	
Features	(n = 126)	(n = 127)	P	(n = 74)	(n = 108)	P	(n = 52)	(n = 19)	P
Age (yrs)									
<50	56	43		30	33		26	10	
50–70	55	64		35	58		20	6	
>70	15	20	0.241	9	17	0.427	6	3	0.820
Menopausal status									
Not known	3	2		2	2		1	0	
Premenopausal	49	43		27	34		22	9	
Postmenopausal	74	82	0.431	45	72	0.454	29	10	0.792
Tumor size (cm)									
Not known	6	1		3	1		3	0	
<1	8	2		5	1		3	1	
1–2	48	48		33	45		15	3	
>2	64	76	0.106	33	61	0.055	31	15	0.438
Tumor type									
Not known	8	4		3	3		5	1	
Ductal	79	73		49	61		30	12	
Lobular	0	4		0	4		0	0	
Others	39	46	0.095	22	40	0.127	17	6	1.000
Tumor grade									
Not known	7	3		3	3		4	0	
1	56	43		37	39		19	4	
2	52	66		29	54		23	12	
3	11	15	0.144	5	12	0.132	6	3	0.354
Vascular invasion									
Not known	20	32		11	27		9	5	
Negative	59	41		34	33		25	8	
Possible	13	15		9	15		4	0	
Positive	34	39	0.208	20	33	0.288	14	6	0.444
Lymph node status									
Not known	15	16		9	13		6	3	
Negative	19	21		16	20		3	1	
≤3 positive nodes	31	33		25	32		6	1	
>3 positive nodes	61	57	0.862	24	43	0.575	37	14	0.756
ER status									
Negative	52	19							
Positive	74	108	0.0001[a]						

[a] Statistically significant.

RESULTS

Measurement of ER and Cyclin D1. All samples expressed detectable cyclin D1 mRNA. In contrast, 28% of tumors had a complete absence of ER mRNA. Statistical analysis of the 253 patients indicated a significant positive correlation between relative expression of cyclin D1 mRNA and ER mRNA (P = 0.0001) or ER status (P = 0.0001) as reported previously (27). In addition, we found a strongly positive correlation between ER mRNA and immunocytochemical measurement of ER by ER-ICA H-score assessed independently on the same samples (P = 0.0001). A positive correlation was also found between cyclin D1 mRNA expression and ER H-score (P = 0.019). Having confirmed that conventional measurement of ER by ER-ICA was highly consistent with ER mRNA expression, we used the latter to define ER positivity for all subsequent analyses. In this population of 253 patients, 182 (72%) were ER positive and 71 (28%) were ER negative.

Clinical Outcome. For analysis of clinicopathological parameters in relation to cyclin D1, the 253 primary cases were divided into two equal groups of high and low cyclin D1 expression as defined by the median level of cyclin D1 mRNA. Overall, there was no association between the level of cyclin D1 and clinicopathological features, except ER, or survival outcome (Tables 1 and 2); however when the ER-positive and -negative subgroups were analyzed separately, there was a significant correlation between high cyclin D1 mRNA levels and worse outcome in patients with ER-positive tumors, *i.e.,* reduced DFI (P = 0.0016), reduced time to local recurrence (P = 0.025), reduced time to metastasis (P = 0.019), and reduced overall survival (P = 0.025; Fig. 1; Table 2). In addition, there was a near-significant trend for larger ER-positive tumors to have a high cyclin D1 level (P = 0.055; Table 1). The significant associations with reduced DFI (P = 0.016) and overall survival (P = 0.044) were upheld (with time to metastasis approaching significance, P = 0.058) on analysis of cyclin D1 level as a continuous variable within ER-positive disease. There were no corresponding associations with survival outcome when the level of ER expression was analyzed independently as a

Table 2 Relationship between cyclin D1 mRNA level and patient outcome

	Overall (n = 253)	ER-positive group (n = 182)	ER-negative group (n = 71)
Low cyclin D1	n = 126 (50%)	n = 74 (41%)	n = 52 (73%)
High cyclin D1	n = 127 (50%)	n = 108 (59%)	n = 19 (27%)
DFI	P = 0.122	P = 0.0016[a]	P = 0.969
Local recurrence	P = 0.549	P = 0.025[a]	P = 0.959
Regional recurrence	P = 0.913	P = 0.177	P = 0.645
Metastasis-free interval	P = 0.373	P = 0.019[a]	P = 0.883
Overall survival	P = 0.485	P = 0.025[a]	P = 0.509

[a] Statistically significant.

continuous variable within the ER-positive subgroup. In contrast to the result in ER-positive cases, we failed to establish an association between clinical outcome and cyclin D1 level in the 71 ER-negative cases, nor was there any relationship of cyclin D1 levels with prognostic groups as measured by the Nottingham Prognostic Index (35).

Axillary lymph node status is generally accepted as the single most important prognostic indicator in primary breast cancer. In this series of patients, Cox multivariate analysis of ER status, cyclin D1 mRNA level, tumor size, tumor grade, and lymph node status in relation to overall survival demonstrated that only lymph node status remained independently significant (P = 0.018 overall; P = 0.037 for the ER-positive subgroup). Because an association between cyclin D1 gene amplification and worse prognosis has been reported in lymph node-negative disease (15, 16, 26), we examined whether such an association was observed in tumors overexpressing cyclin D1 mRNA. Histological information was available on 222 of the 225 primary cases who underwent axillary lymph node dissection (n = 214 patients < 70 years; n = 8 patients > 70 years). Although no association between cyclin D1 mRNA expression and clinical outcome was apparent within the lymph node-positive or -negative groups as a whole, a significant association did exist between high cyclin D1 level and poorer survival in the lymph node-positive, ER-positive subgroup (P = 0.031; n = 124; Fig. 2; Table 3).

Endocrine Response. Given the tight positive correlation between cyclin D1 mRNA and ER mRNA and the *in vitro* evidence that cyclin D1 is an estrogen-regulated gene (28, 29, 31), we examined the hypothesis that cyclin D1 overexpression may exert an influence on response to antiestrogen therapy. Disease-free survival was compared in relation to cyclin D1 level in the 67 patients who received adjuvant tamoxifen therapy and within the ER-positive (n = 57) and ER-negative (n = 10) subgroups. Similar analyses were performed on the 44 patients (24 ER-positive patients and 20 ER-negative patients) who received adjuvant chemotherapy. There was no correlation between DFI and cyclin D1 level for any of the hormone or chemotherapy subgroups, although the number of cases in each group was relatively small.

Finally, clinical response (as assessed by UICC criteria) to primary tamoxifen treatment was compared in relation to the level of cyclin D1 in 33 patients (15 elderly patients, 4 patients with locally advanced breast cancer, and 14 patients with recurrent breast cancer). There was no correlation between cyclin D1 and 6-month clinical response, best response, or responsive/static *versus* progressive disease; however, the duration of the response to tamoxifen was significantly longer in ER-positive patients with low cyclin D1 mRNA levels (n = 14) than in those with high cyclin D1 mRNA levels (n = 9; P = 0.04; Fig. 3), implying that overexpression of cyclin D1 may confer a degree of resistance to antiestrogen therapy.

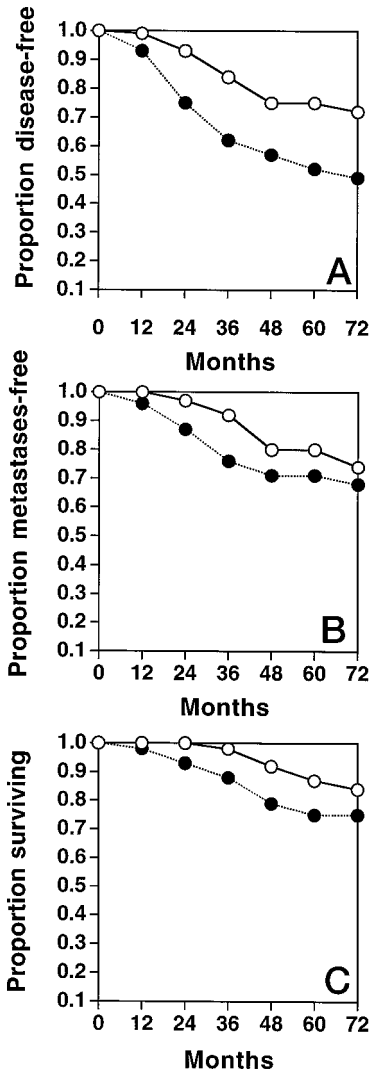

Fig. 1 Life table analysis showing the cumulative proportion of disease-free survival (*A*), metastasis-free survival (*B*), and overall survival (*C*) in 182 patients with ER-positive primary breast cancer in relation to low (○) or high (●) cyclin D1 mRNA levels.

DISCUSSION

Cyclin D1 is a well-characterized cell cycle regulatory protein with demonstrated oncogenic properties in the mammary gland; however, several clinical studies relating cyclin D1 gene expression, as measured by immunohistochemistry, to

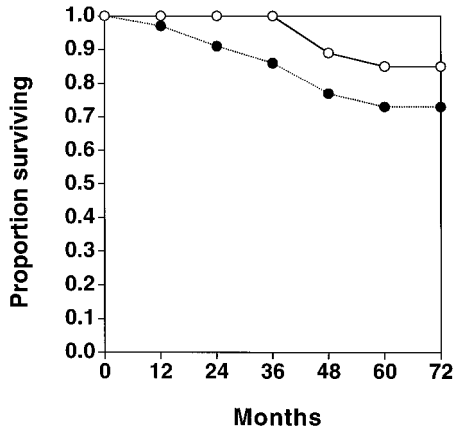

Fig. 2 Life table analysis showing the cumulative proportion of overall survival in patients with ER-positive, axillary lymph node-positive primary breast cancer in relation to low (○) or high (●) cyclin D1 mRNA levels.

disease outcome have failed to draw definitive conclusions as to its prognostic significance. The current study is the first to use mRNA levels as a measure of cyclin D1 expression and prognosis in a large population with a median follow-up of more than 6 years. Similarly, measurement of ER gene expression using mRNA as the parameter has not been commonly used in studies of disease outcome in breast cancer, but the demonstrated tight correlation between ER mRNA and the immunohistochemical ER-ICA H-score in our series of patients ($P = 0.0001$) validates the use of this parameter. In contrast, cyclin D1 gene expression is regulated by both transcriptional and posttranscriptional mechanisms (39) that may result in disparities between mRNA and protein levels (40). The present study demonstrated that cyclin D1 mRNA was a marker of poor prognosis with shorter relapse-free survival, time to local recurrence, time to distant metastasis, and overall survival in 253 patients with ER-positive primary breast cancers.

CCND1 encoding cyclin D1 is considered to be the predominant oncogene in the 11q13 amplicon. There have been several publications reporting a relationship between 11q13 amplification and poor prognosis in a number of different human malignancies including breast cancer (14–16). However, there was inconsistency among earlier smaller studies in defining the phenotype of breast cancer in which 11q13 amplification was associated with poor outcome (16). Nevertheless, the three largest studies demonstrated an earlier relapse in patients with 11q13-amplified, lymph node-negative, or ER-positive disease (15, 16, 26). Although 11q13 amplification can be used as a surrogate for cyclin D1 overexpression, we and others have shown that amplification greatly underestimates the frequency of overexpression. Both cyclin D1 mRNA and protein are overexpressed at a frequency two to three times greater than gene amplification (17–20), and this must mean that only about one in three overexpressing tumors can be accounted for by gene amplification as assessed by Southern blotting. One would therefore expect that mRNA data or immunohistochemical detection of cyclin D1 protein expression would be a more reliable estimate of overexpression. However, recent *in vitro* studies could not demonstrate a clear relationship between cyclin D1 expression and the activity of its major kinase partner Cdk4 in a series of breast cancer cell lines (41). Thus, possibilities exist that cyclin D1 may be involved in functions other than the control of proliferation through Cdk-activated mechanisms and may be exerting some of its oncogenic potential through Cdk4-independent pathways, *e.g.*, through binding to and activating the transcriptional activity of ER (42, 43). Nonetheless, the worse prognosis in patients with an ER-positive phenotype and high levels of cyclin D1 mRNA documented in the current series is consistent with the previously published data from an earlier independent population measuring *CCND1* amplification (16).

In contrast to the finding of earlier relapse in 11q13-amplified, axillary lymph node-negative disease (15, 16, 26), the present study failed to establish a relationship between high levels of cyclin D1 mRNA and prognosis in relation to nodal status alone. However, high levels of cyclin D1 mRNA predicted for an increased risk of death within the the ER-positive, lymph node-positive subgroup. Two previous studies employing approximately 1000 patients indicated that the two DNA markers *CCND1* and *EMS1* at 11q13 confer different phenotypes in ER-positive and ER-negative tumors (13, 16). *CCND1* amplification was associated with an increased risk of relapse in the ER-positive group, whereas amplification of *EMS1*, which is 800 kb telomeric to *CCND1*, was associated with an increased risk of relapse in patients with ER-negative disease. However, amplification of either *CCND1* or *EMS1* predicted for worse prognosis in the node-negative subgroup. The apparent inconsistency between these findings may well indicate that unlike the prognosis in patients with the ER-positive phenotype, in which there is a strong relationship with cyclin D1, the outcome within the node-negative subgroup may be governed by another as yet undefined gene located between *CCND1* and *EMS1* and coamplified with either *CCND1* or *EMS1*. Of the 222 patients with known lymph node status in this cohort, only 40 patients had node-negative disease; thus, the numbers are likely to be too small to detect any relationship with outcome within this group.

Several published studies using cyclin D1 immunohistochemistry have addressed the potential role of cyclin D1 as a prognostic indicator in breast cancer. Three studies failed to demonstrate a significant relationship between cyclin D1 protein overexpression and prognosis (23–25), whereas two other groups reported an improved survival in patients with cyclin D1 overexpression (21, 22). However, there is now convincing evidence to suggest that cyclin D1 expression is closely correlated with ER positivity (21, 22, 24, 25, 27). Unfortunately, the two former studies did not address the relationship between cyclin D1 expression and prognosis in patients with primary breast cancer within the ER subgroups (21, 22). The tumors with high expression of cyclin D1 are more likely to be ER positive and are consequently more likely to have a better clinical outcome than the ER-negative tumors that might well have low cyclin D1 expression. Moreover, these two studies noted a worse prognosis in the group with no cyclin D1 staining, a significant proportion of whom are likely to harbor mutations leading to dysfunctional pRb. Aberrations of other components of the cyclin D1 pathway such as loss of pRb or of the Cdk inhibitory proteins (44, 45) may exert a greater influence on

Table 3 Relationship between cyclin D1 mRNA expression and patient outcome according to pathological lymph node status

Survival by lymph node status	Overall (n = 222)			ER-positive group (n = 160)			ER-negative group (n = 62)		
	Cyclin D1			Cyclin D1			Cyclin D1		
	Low	High	P	Low	High	P	Low	High	P
Negative	19	21	0.811	16	20	0.420	3	1	0.564
Positive	92	90	0.557	49	75	0.031[a]	43	15	0.687

[a] Statistically significant.

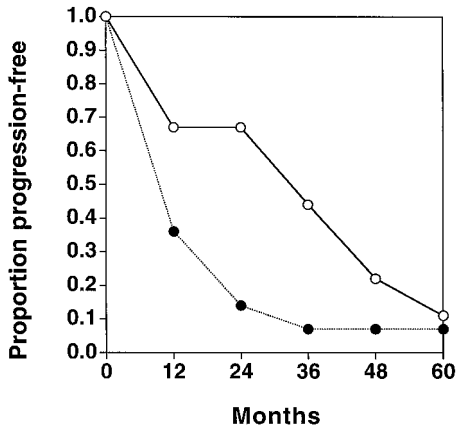

Fig. 3 Life table analysis showing the cumulative proportion of progression-free survival after tamoxifen treatment in patients with ER-positive breast cancer in relation to low (○) or high (●) cyclin D1 mRNA levels.

long-term outcome than cyclin D1 itself. Any comparison between the present cyclin D1 mRNA data and published data on cyclin D1 protein levels is potentially complicated by recently defined mechanisms of posttranscriptional control that likely result in disparities between these two parameters.

The strong positive correlation between high expression of cyclin D1 and ER positivity (22, 24, 27) suggests that these two genes are functionally related. There is accumulating evidence that this relationship is at least partly due to estrogen induction of cyclin D1 expression (28, 29, 31) and cyclin D1 stimulation of ER transcriptional activity (42, 43). Those tumors with higher cyclin D1 levels may be more likely to have functional ER and may therefore have a superior response to endocrine therapy. Conversely, there is also published evidence that inhibition of cyclin D1 gene expression with a concurrent decline in cyclin D1 mRNA and protein levels is an early and critical event in antiestrogen action (32, 33), suggesting that overexpression of cyclin D1 in ER-positive tumors may lead to insensitivity to antiestrogens. Subsequent results from *in vitro* studies have also been controversial. One study clearly demonstrated that ectopic induction of cyclin D1 expression in ER-positive breast cancer cell lines (T-47D and MCF-7) can overcome the inhibition of cell cycle progression induced by antiestrogen (34), whereas another study showed that tet-inducible cyclin D1 overexpression in MCF-7 breast cancer cells does not prevent the inhibition of cell growth by antiestrogens (46). Although the sample size in the subgroup treated with antiestrogen in this study was small and the analyses must therefore be interpreted with caution, the current finding of a shorter response duration in the ER-positive group of patients who had high tumor levels of cyclin D1 mRNA is compatible with the view that the resistance to hormonal therapy seen in some ER-positive breast cancers may be conferred by cyclin D1 overexpression, as hypothesized previously (34). However in this small series of women, apart from response duration, no significant correlation of cyclin D1 mRNA expression with other response parameters including 6-month response, best response, or responsive/static *versus* progressive disease was observed. Nonetheless, the current data encourage further investigation of the potential role of cyclin D1 as a predictive marker for endocrine responsiveness in larger studies.

In conclusion, this study has shown that high expression of cyclin D1 mRNA is an adverse prognostic marker specifically within ER-positive breast cancer. The tight relationship between cyclin D1 and ER expression precludes meaningful interpretation if ER subgroup analysis is not performed. Additional large studies examining the relationship between cyclin D1 protein expression and prognosis within the ER subgroups and comparing the mRNA and protein expression of cyclin D1 may well further clarify the apparent inconsistency between published studies. The significance of elevated cyclin D1 expression in relation to therapeutic responsiveness is currently being addressed by additional *in vitro* and clinical studies.

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