Placental Cadherin and the Basal Epithelial Phenotype of *BRCA1*-Related Breast Cancer

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Abstract

Purpose: *BRCA1*-related breast cancer frequently has a basal epithelial phenotype, and P-cadherin is a basal marker. We undertook a detailed evaluation of the relationship among P-cadherin, prognostic markers in breast cancer, and outcome.

Experimental Design: This study was restricted to 292 cases of first primary invasive breast cancer diagnosed in Ashkenazi Jewish women between 1980 and 1995. All available blocks were stained for P-cadherin, and 261 were included in the final statistical analyses, including 27 germ line *BRCA1* mutation carriers and 8 *BRCA2* mutation carriers. Descriptive analyses were done followed by survival analyses and a Poisson regression analysis.

Results: P-cadherin was present in 80 of the 261 breast cancers (31%) and was more frequently present in tumors that have a basal epithelial phenotype [i.e., high-grade, estrogen receptor – and KIP1 ($p27^{Kip1}$) – negative tumors, with expression of cytokeratin 5/6, cyclin E, TP53, and presence of *BRCA1* mutations and vascular nests (all $P < 0.001$)]. In a univariate survival model, expression of P-cadherin was associated with a relative risk (RR) of death from breast cancer at a 10-year follow-up of 2.9 (95% confidence interval, 1.8-4.7; $P < 0.0001$) and was a predictor of poor univariate survival in both lymph node – negative and – positive breast cancers. In a multivariate analysis, the effect of P-cadherin levels was not independent of other basal-related markers. Multivariable interaction modeling showed that P-cadherin positivity was highly predictive of a poor prognosis in small, node-negative breast cancers (RR, 7.1; $P = 0.006$).

Conclusions: P-cadherin is a marker for basal-like breast cancers and is strongly associated with the presence of a *BRCA1* mutation. It is an adverse prognostic factor, particularly in small, node-negative breast cancers.

Recent microarray studies have shown that invasive ductal breast cancer is divisible into many subgroups (1–5), not all of which are apparent on conventional histopathologic examination. One of the most interesting phenotypes that has reemerged in the past few years is the basal epithelial phenotype, characterized by positive staining for antibodies raised against cytokeratins such as cytokeratin 5/6 and cytokeratin 14, which are known restricted to basal cells within the breast (6, 7). Expression of these keratins has been associated with a poor outcome (8, 9). We (10) and others (4) recently showed that *BRCA1*-related breast cancers are much more likely to express these basal keratins than are nonhereditary breast cancers. We subsequently showed that the "core" basal phenotype [estrogen receptor (ER) – negative, HER2-negative, cytokeratin 5/6 – positive breast cancer] is associated with elevated levels of cyclin E and TP53 and low levels of KIP1 (previously known as $p27^{Kip1}$; ref. 11). Whether the core basal phenotype reflects the cell of origin of *BRCA1*-related breast cancer (12), or is simply a marker of a differentiation pathway that is selected from a common precursor cell (13) is presently unknown. Notably, a recent model suggests that undifferentiated cytokeratin 5/6 – positive cells could represent adult breast stem cells (14). In this model, the cytokeratin 5/6 – positive undifferentiated phenotype is retained in a subset of breast cancers. Consequently, *BRCA1* may have some role in determining the normal progression to a luminal phenotype of mature breast cells (12).

Placental cadherin (P-cadherin) is a member of the cadherin gene family. Other members include epithelial cadherin (E-cadherin) and neural cadherin (N-cadherin). A great deal is known about N- and E-cadherin (15). From the cancer standpoint, loss of E-cadherin in lobular breast cancer and in

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diffuse gastric cancer is a well-established tumor-initiating or promoting event (16). N-cadherin expression in mammary cell lines can stabilize fibroblast growth factor, resulting in an invasive phenotype (17). Until recently, P-cadherin was less studied with respect to cancer. It was originally identified as having an important role in implantation and embryogenesis in mice (18). In a development setting, Daniel et al. showed that P-cadherin is localized to the cap cells in the terminal end buds of the developing murine mammary gland. These cap cells are relatively undifferentiated mammary cells and could represent mammary stem cells (19). Whether or not cap cells are true stem cells, it is interesting that they are ER negative (20). In normal epidermis, P-cadherin expression is limited to the basal layer. Interestingly, P-cadherin expression is preserved in basal cell carcinomas (21), whereas its expression is usually not maintained in most epithelial cancers, including breast cancer (22, 23).

P-cadherin is a transmembrane glycoprotein and is implicated in cell-cell adhesion (24). The intracellular domains physically associate with both the actin cytoskeleton and with the catenin family of cytoplasmic proteins (25), which themselves influence transcription following their relocalization to the nucleus (26). At the level of transcription, there seems a functional convergence of wingless (Wnt), β-catenin, and cadherin pathways (27), and all three proteins are implicated in regulation of morphogenesis and tissue polarity.

P-cadherin is located on chromosome 16q. Physical loss of this chromosome is much more common in low-grade, nonbasal cancers than in high-grade cancers (including basal subtypes; refs. 28–30). If chromosome 16q alterations are found in these latter cancers, then the mechanism observed is mitotic recombination (29). This would not affect the dosage of P-cadherin; thus, high-grade breast cancers are likely to have two copies of chromosome 16q, even if loss of heterozygosity is observed.

In normal human breast tissue, P-cadherin is a highly sensitive marker for myoepithelial cells and radial scars (22, 31). In breast cancers, P-cadherin expression is most common in high-grade, ER-negative tumors (23, 32, 33) and has been shown an independent marker of poor prognosis in some (34) but not in all studies (35). Notably, medullary breast cancers commonly express P-cadherin (36). Tumors that overexpresss the ubiquitin ligase Skp2 also tend to overexpress P-cadherin and other markers associated with the basal phenotype and have a poor prognosis (37). Recent data have shown that P-cadherin expression is significantly more frequent in *BRCA1*-related breast cancers (15 of 19) than in either *BRCA2* (1 of 15) or non-*BRCA1/2* breast cancer (0 of 29; ref. 38). Given the recent work defining the basal epithelial phenotype of *BRCA1*-related breast cancer (and data presented above, showing that P-cadherin is expressed in the basal layer of the normal breast), we set out to establish in detail the relationships between P-cadherin and traditional prognostic indicators, such as tumor size, histologic grade, and nodal status, as well as between P-cadherin and the basal epithelial phenotype in general, and germ line *BRCA1* mutations in particular. The effect of P-cadherin expression on a 10-year breast cancer survival in both univariate and multivariate models were studied. In addition, a Poisson regression model was fitted to study how P-cadherin status influenced the increase in the number of positive lymph nodes with increasing size of the primary tumor.

Materials and Methods

Clinicopathologic review and immunohistochemistry. The study design is an ethnically restricted single hospital-based retrospective cohort study, as described in previous publications (11, 39). The study was approved by the hospital's Institutional Review Board. Of 309 consecutive cases of Ashkenazi Jewish women ages ≤65 years diagnosed with a first primary, nonmetastatic, invasive breast cancer between January 1, 1980 and November 1, 1995 at the Sir Mortimer B. Davis-Jewish General Hospital, Montreal, Quebec, 17 (5.5%) were excluded because of (a) inability to locate pathology blocks, (b) only carcinoma *in situ* was present on the available pathology blocks, or (c) DNA could not be adequately amplified after repeated attempts, thus leaving 292 cases. Breast cancer blocks were identified from each of these women and clinicopathologic and follow-up information were obtained by chart review. Specimens were reviewed for histologic type, nuclear grade (data not presented here), and lymph node status and were stained for ER and TP53 using immunohistochemistry, as previously described (40, 41). Histologic grade was determined using the Nottingham criteria (42). The cyclin E, KIP1, HER2, cytokeratin 5/6, and factor VIII immunohistochemistry assays were done and scored as described in previous publications (10, 39, 43, 44).

For P-cadherin, immunohistochemistry was done on available 5-μm-thick slides from formalin-fixed, paraffin-embedded tissue blocks. Antigen retrieval was done by boiling for 10 minutes at 750 W and 15 minutes at 350 W in TRIS EDTA buffer (pH 9) in a microwave oven. The slides were incubated at room temperature for 60 minutes using a monoclonal antibody against P-cadherin (clone 56) from BD Transduction Laboratories (Lexington, KY), 250 μg/mL, diluted 1:400. The staining procedures were done using the DAKO Techmate 500 autostainer and the DAKO Envision staining system detection kit (K5007) and counterstaining with Harris hematoxylin (Dako S2020).

Staining was recorded using a semiquantitative and subjective grading system, considering the intensity of staining and the proportion of tumor cells showing a positive reaction. Intensity was recorded from 0 (no staining) to 3 (strong staining; scores 0-3). Weak and moderate staining intensities showed membranous positivity only, but in addition, some cytoplasmatic positivity could be detected with strong overall staining. The proportion of positive cells was recorded as 1 (<10%), 2 (10-50%), and 3 (>50%; scores 1-3). A staining index (SI) was obtained by multiplying the scores of staining intensity (scores 0-3) with the scores of staining area (scores 1-3). Cases were then categorized as negative when they were completely negative or with only minimal staining (SI, 0-1) or positive (SI, 2-9); the cutoff value was based on the distribution plot of the present sample set. Immunohistochemistry scoring was done blinded for patient characteristics and outcome.

BRCA1 and BRCA2 mutation status. Specimens were coded and DNA was extracted from the paraffin wax–embedded blocks using standard techniques. Mutation analysis was carried out as previously described (39), looking specifically for the recurrent mutations in the Ashkenazi Jewish population (*BRCA1*: 185delAG, 5382insC; *BRCA2*: 6174delT).

Statistical analysis. Clinical, pathologic, and molecular data were collected in a mutually blinded fashion. Patient characteristics were compared using nonparametric Wilcoxon's test and Fisher's exact test. Test for trends were assessed using Cochran-Armitage. Statistical significance was assessed at the two-sided 5% level, whereas borderline statistical significance was defined as *P*s between 5% and 10%. Survival rates were calculated from the date of primary surgery until death from breast cancer (breast cancer–specific survival). The mean follow-up of those who did not die of breast cancer was 9.8 years (n = 189); the mean overall follow-up was 8.4 years (n = 261). Ten-year survival curves were estimated using the Kaplan-Meier method and significance was assessed with the log-rank test.

To estimate the relative risk (RR) of death from breast cancer, a Cox proportional hazards model was used where all the measured prognostic factors were examined in a full multivariate model: tumor

size, axillary lymph node status, histologic grade, age at diagnosis, ER, P-cadherin, TP53, HER2, cyclin E, KIP1, cytokeratin 5/6, vascular nests (also called glomeruloid microvascular proliferation, GMP) and, mutation status (noncarrier, *BRCA1* and *BRCA2*). All data was censored at 10 years and significance was assessed at the 5% level using two-sided tests. The final most parsimonious model was built using the log-likelihood ratio test, employing a backward and forward approach in which variables with the highest contribution to the likelihood function were kept in the model. This final model contained only six variables: tumor size, histologic grade, nodal status, HER2, cyclin E, and vascular nests. Where possible, the multivariate models were adjusted for missing values. To do this, we included a dichotomized variable to identify whether or not the variable of interest was missing. This allowed us to include 259 of the 261 subjects in the final model. To investigate possible interactions among tumor size, lymph node status, and P-cadherin, the parsimonious model was refitted to also include terms for all of these as well as second- and third-degree interaction terms among those three factors.

Finally, a Poisson regression model was built to examine the effect of P-cadherin on the relationship between the number of positive lymph nodes and tumor size: $\ln(\mu) = \ln(N_{exam}) + \alpha + \alpha_{PCad+} + (\beta \times T_{size}) + (\beta_{PCad+} \times T_{size})$, where μ = average number of positive nodes, α = overall intercept, α_{PCad+} = extra intercept for P-cadherin + patients, β = overall slope, β_{PCad+} = extra slope for P-cadherin + patients, and the natural logarithm of the number of nodes examined was used as an offset. To take into account overdispersion, the dispersion variable was estimated as the deviance divided by the number of degree of freedoms. Patients with tumor size of >10 cm ($n = 2$) were excluded from this analysis.

Results

Individuals with no information on recurrences or outcome ($n = 4$) and patients for whom sufficient tissue was not available, or where the P-cadherin staining was not of sufficient quality to be interpretable were excluded ($n = 27$), leaving 261 patients in this historical cohort. There were 27 *BRCA1*-positive cases and 8 *BRCA2*-positive cases in the series.

Expression of P-cadherin was recorded as positive in 80 cases (31%) and negative in 181 (69%) of cases (Table 1). There was no difference in the age distribution between P-cadherin–positive and P-cadherin–negative cases ($P = 0.89$). Tumors that expressed P-cadherin, were more likely to be of higher histologic grade (grade 3 versus grade 1, $P < 0.0001$) than were nonexpressing cancers. Despite the clear association between P-cadherin staining and increasing grade, there was no significant relationship with lymph node status [odds ratio (OR), 0.76; $P = 0.40$]. The P-cadherin–positive tumors were more often ER negative than were P-cadherin–negative tumors (74% versus 19%, $P < 0.001$). They were also more likely to be HER2/erbB-2 positive than the P-cadherin–negative tumors [OR, 2.2; 95% confidence interval (95% CI), 1.1-4.7; $P = 0.04$]. P-cadherin–positive tumors were highly significantly more likely to be TP53 positive ($P < 0.0001$), KIP1 negative ($P = 0.0009$), cytokeratin 5/6 positive, cyclin E positive, and glomeruloid microvascular proliferation positive (all $P < 0.001$). In particular, we found that the combination of low KIP1 staining and high cyclin E levels were highly associated with P-cadherin positivity (OR, 24.5; 95% CI, 9.1-66.0; $P < 0.0001$). A strongly positive association with *BRCA1* mutation carrier status (OR, 6.7; 95% CI, 2.8-16.2; $P < 0.0001$) was also observed. No such association was seen for *BRCA2* mutation carrier status (OR, 0.9; 95%CI, 0.2-4.8; $P = 0.99$). Of note, the "core" basal phenotype (ER negative, HER2 negative, cytokeratin 5/6 positive) was seen in 39 of 250 cancers with data for all three markers and the OR for P-cadherin positivity in association with this core basal phenotype was 19.7 (95% CI, 7.8-50; $P = 0.0001$), which is substantially greater than the single associations observed with ER negativity (OR, 12.5), cytokeratin 5/6 positivity (OR, 6.1), or HER2 negativity (OR, 0.45). In the latter case, it is interesting that overall, P-cadherin is positively associated with HER2 status (OR, 2.2) but is negatively associated with HER2 status when HER2 is included in the core basal phenotype. No difference was seen in the association between P-cadherin and HER2 when the data were dichotomized by cytokeratin 5/6 status alone (data not shown).

Univariate survival analysis by Kaplan-Meier, dichotomized by P-cadherin status, showed a significantly worse 10-year breast cancer–specific survival in the positive group (P-cadherin negative, 79%; P-cadherin positive, 50%, $P < 0.0001$). This relationship was present in both lymph node–negative patients (P-cadherin negative, 89%; P-cadherin positive, 65%; $P = 0.0007$; Fig. 1B) and node-positive patients (P-cadherin negative, 64%; P-cadherin positive, 37%; $P = 0.009$; Fig. 1C).

In a univariate proportional hazards model, P-cadherin positivity was associated with a poor prognosis (RR, 2.91; 95% CI, 1.8-4.7; $P < 0.0001$). To evaluate if P-cadherin positivity had independent prognostic value, a multivariate model was constructed (Table 2). P-cadherin, TP53, KIP1, cytokeratin 5/6, and *BRCA1* were not included in the final, most parsimonious, model. From this, we can conclude that P-cadherin is closely associated with the presence of several conventional adverse prognostic factors and with markers related to the basaloid phenotype described previously (Table 1) but does not in itself have a multivariate prognostic effect. Nevertheless, when P-cadherin was added to the basic Nottingham Prognostic Index model (tumor size, lymph node status, and histologic grade), P-cadherin was remained an independent marker of a poor breast cancer–specific survival (RR, 2.2; 95% CI, 1.3-3.8; $P = 0.004$). Of other variables included in the parsimonious multivariate model, HER2 (RR, 1.8; $P = 0.06$), cyclin E (RR, 2.0; $P = 0.03$) and GMP (RR, 1.9; $P = 0.02$) showed stronger correlation with breast cancer–related deaths than did P-cadherin (Table 2).

The data were also evaluated in models divided by the lymph node status (Table 2; Fig. 1B and C). In the node-negative patients, the parsimonious model contained the following variables: age of diagnosis (RR, 0.4; $P = 0.08$), histologic grade (grade 2: RR, 8.2; $P = 0.05$; grade 3: RR, 3.9; $P = 0.23$), KIP1 (RR, 0.2; $P = 0.02$), and cyclin E (RR, 4.0; $P = 0.01$; Table 2). In the node-positive patients, the parsimonious model contained the variables tumor size (RR, 3.2; $P = 0.006$), TP53 (RR, 2.2; $P = 0.02$), and GMP (RR, 3.0; $P = 0.003$; Table 2). Notably, no variables were common to the best-fitting models for node-negative and node-positive breast cancer.

The observation that the prognostic factor of interest, P-cadherin, was strongly associated with high histologic grade ($P < 0.0001$) but was less strongly associated with tumor size ($P = 0.05$) and not associated with nodal status ($P = 0.40$) led us to wonder whether there might be an interaction among P-cadherin status, tumor size, and nodal status in predicting outcome. We refitted the univariate and parsimonious multivariate proportional hazards model (Table 2) to include terms

Table 1. Individual prognostic factors

	P-cadherin negative (n = 181)	P-cadherin positive (n = 80)	OR (95% CI)	P	10-year % survival	No. events	P
Age at diagnosis (y)							
<50	72	31			64.5	32	0.06
≥50	109	49	1.04 (0.61-1.79)	0.89	73.9	34	
Tumor size (cm)							
<2	99	33			83.2	18	<0.0001
≥2	73	43	1.77 (1.02-3.05)	0.05	57.9	43	
Histologic grade*							
1	76	10			92.2	5	
2	78	26	2.53 (1.14-5.61)	0.03	65.2	32	<0.0001
3	25	44	13.38 (5.88-30.43)	<0.0001	50.9	28	<0.0001
Lymph node status							
Negative	86	43			81.5	20	<0.0001
Positive	79	30	0.76 (0.44-1.33)	0.40	56.8	41	
ER							
Negative	34	59			51.1	39	<0.0001
Positive	147	21	0.08 (0.04-0.15)	<0.0001	80.4	27	
p53							
Negative	153	47			76.2	39	<0.0001
Positive	27	33	3.98 (2.17-7.28)	<0.0001	50.7	27	
HER2							
Negative	164	65			73.6	51	0.001
Positive	17	15	2.23 (1.05-4.72)	0.04	45.0	15	
KIP1							
Negative	93	57			64.1	46	0.002
Positive	75	16	0.35 (0.18-0.66)	0.0009	80.6	14	
Cyclin E							
Negative	148	35			76.0	37	<0.0001
Positive	22	44	8.46 (4.50-15.89)		49.8	28	
Cytokeratin 5/6							
Negative	128	26		<0.0001	75.8	32	0.004
Positive	43	53	6.07 (3.39-10.87)		59.0	32	
GMP							
Negative	149	50		<0.0001	73.6	44	0.0002
Positive	15	27	5.36 (2.64-10.89)		48.3	20	
BRCA1/2							
Negative	167	59		<0.0001	72.4	53	
BRCA1 mutation	8	19	6.72 (2.79-16.17)	<0.0001	57.5	10	0.08
BRCA2 mutation	6	2	0.94 (0.19-4.80)	0.99	53.6	3	0.29
KIP1 and Cyclin E †							
Cyclin E negative, KIP1 negative	63	9			84.5	9	
Cyclin E negative, KIP1 positive	77	21	1.91 (0.82-4.47)	0.16	71.6	24	0.03
Cyclin E positive, KIP1 negative	10	7	4.90 (1.49-16.14)	0.01	61.8	5	0.10
Cyclin E positive, KIP1 positive	10	35	24.50 (9.10-65.99)	0	43.2	21	<0.0001

*P < 0.0001, increasing trend in OR.
†P < 0.0001, increasing trend in OR of KIP1 by Cyclin E.

for P-cadherin status as well as all second- and third-degree interaction terms among those three factors. In the univariate analysis, P-cadherin was an adverse prognostic factor in small, node-negative breast cancers (RR, 11.6; P = 0.0004) and in large, node-positive cancers (RR, 2.2; P = 0.03; Table 3). The significant effect of P-cadherin in prognosis in small, node-negative breast cancers persisted in the multivariable model (RR, 7.1; P = 0.006). By contrast, in all other combinations of tumor size and nodal status, P-cadherin status had no significant independent effect on outcome (P > 0.21). These findings indicate that there is a significant interaction among tumor size, nodal status, and P-cadherin immunohistochemical staining in our data set and help to explain why, overall, P-cadherin is not an independent prognostic factor.

To explore this result further, we constructed a Poisson regression model to test if the association between the number

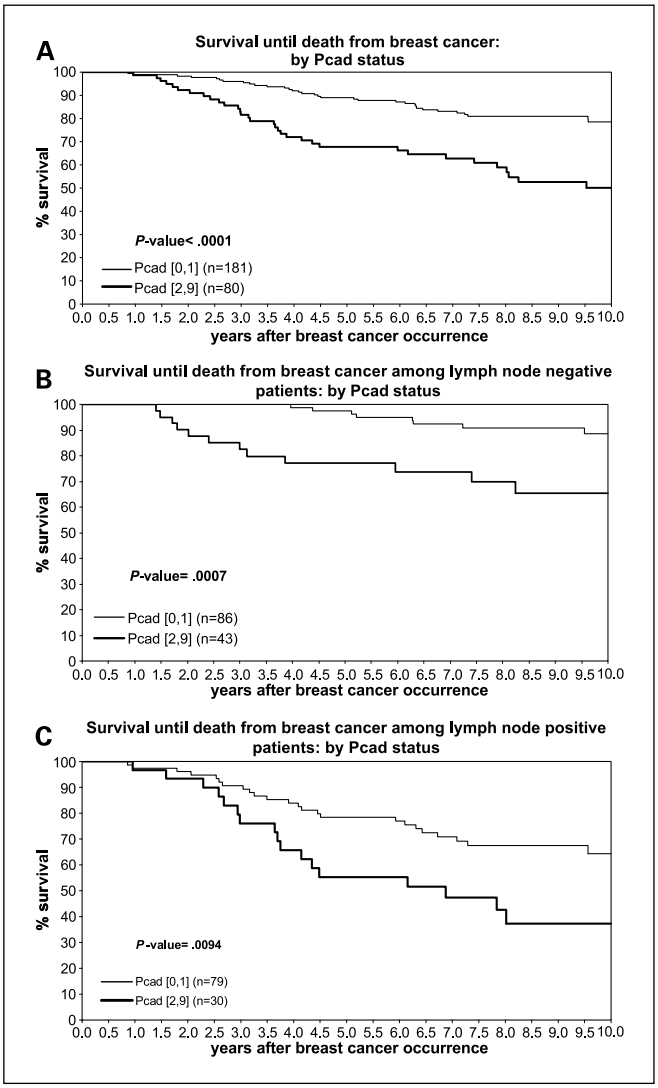

Fig. 1. Survival curves (Kaplan Meier) showing cumulative survival differences between patients whose tumors were (*A*) P-cadherin negative (10-year survival 78.6%) or positive (10-year survival, 50.1%; $P < 0.0001$, log-rank test); (*B*) lymph node negative and P-cadherin negative (10-year survival, 88.7%), or lymph node negative and P-cadherin positive (10-year survival, 65.5%; $P = 0.0007$, log-rank test); (*C*) lymph node positive and P-cadherin negative (10-year survival, 64.3%), or lymph node positive and P-cadherin positive (10-year survival, 37.3%; $P = 0.009$, log-rank test).

of positive axillary lymph nodes at diagnosis and tumor size is altered by the P-cadherin status (see Materials and Methods). In Fig. 2, the upward slope of the P-cadherin–negative curve (dashed) is significantly different ($\beta_{\text{interaction slope}} = -0.31$, $P = 0.0008$) from the nearly horizontal P-cadherin–positive curve (solid). The findings are similar to our previous analysis of *BRCA1* and cytokeratin 5/6 but are more pronounced. It seems plausible that this disassociation between tumor size and nodal status is a distinguishing nonmorphologic trait that connects the basaloid phenotype with *BRCA1*-positive breast carcinomas.

Discussion

The aim of this study was to evaluate the expression of the cell adhesion molecule P-cadherin in a series of breast carcinomas, using immunohistochemistry, and correlate this with other, previously gathered data in respect to prognosis and connection with a basaloid phenotype of breast carcinoma. Here we have shown that the basal-like phenotype of *BRCA1*-related breast cancer can be extended to include P-cadherin. This basal marker is associated with almost all of the clinicopathologic features that we (11) and others (1, 4, 30, 37) have previously identified as being characteristic of the basal phenotype. The presence of P-cadherin has prognostic value, although this is only seen in univariate analysis (Fig. 1), as other markers, such as cyclin E and vascular nests are more powerful predictors of outcome and are independent of traditional indicators such as tumor size, histologic grade, and lymph node status (Table 2).

Notably, there is no association between P-cadherin staining and the presence of a *BRCA2* mutation, although our sample size is very small. Nevertheless, this observation fits with several previous studies that have indicated that *BRCA2*-related breast cancers are not obviously distinguishable from nonhereditary breast cancers. To emphasize this point, we repeated all the analyses presented in this paper after excluding *BRCA2*, and the results were largely unchanged. The only notable difference is that in the most parsimonious final model (Table 2), inclusion of the eight *BRCA2*-related breast cancers resulted in the retention of HER2 as a variable and the exclusion of KIP1.

The Wnt, β-catenin, and cadherin pathways seem functionally interrelated (27), and there has been a resurgence in interest in the role of Wnt signaling in breast cancer (45). The results presented here support recent data suggesting that Wnt-related pathways may be particularly implicated in the basal subtype of breast cancer (5). Mouse models of breast cancer also suggest that activation of the Wnt pathway causes mammary cancer by deregulating mammary stem cells (46), providing an intriguing link to the concept that BRCA1 may play a similar role in breast stem cell regulation (12). Recent analysis of histologic differences between different transgenic mammary tumors has provided indirect support for a role of the Wnt pathway in determining a *BRCA1*-related phenotype of breast cancer. A comparison of the histopathologic features of erbB-2/neu– or ras-induced murine mammary tumors was made with those produced by Wnt- and Wnt-related transgenes (47). It was found that most Wnt-induced mammary tumors had inflammatory infiltrates and pushing margins and at least 15% had a myoepithelial component. These are all features of *BRCA1*-related breast cancer (4, 10, 48). Notably, these tumor characteristics were rarely, if ever, seen in erbB-2/neu/ras–induced cancers, which were free of inflammatory infiltrates and contained morphologic structures not seen in Wnt-induced cancers.

As a member of the cadherin family, P-cadherin has an important role in maintaining the structural integrity of the epithelium (49), and it is notable that in the epidermis, only the cells in the basal layer proliferate. P-cadherin expression is limited to the basal layer of the normal breast (31); therefore, its expression in *BRCA1*-related breast cancer suggests that loss of the normal controls that regulate basal cell proliferation may be a key feature of such cancers.

The available evidence suggests that loss of *BRCA1* results in a defined pathway to breast cancer. This signature is conserved from initiation to progression. It is not known to what extent

Table 2. Parsimonious Cox models for survival until death from breast cancer

		Univariate		Multivariate	
Variable	Definition	RR (95% CI)	*P*	RR (95% CI)	*P*
Tumor size (cm)	<2	1	0.0001	1	
	≥2	3.02 (1.74-5.24)		1.78 (0.99-3.22)	0.06
Histologic grade	1	1		1	
	2	6.12 (2.38-15.70)	0.0002	3.76 (1.36-10.45)	0.01
	3	9.06 (3.50-23.48)	0.0001	4.14 (1.44-11.91)	0.008
Lymph nodes	Negative	1	0.0001	1	
	Positive	2.84 (1.66-4.86)		2.38 (1.34-4.21)	0.003
HER2	Negative	1	0.002	1	
	Positive	2.52 (1.42-4.49)		1.80 (0.97-3.35)	0.06
Cyclin E	Negative	1	0.0001	1	
	Positive	2.68 (1.64-4.38)		2.00 (1.16-3.43)	0.01
GMP	None	1	0.0004	1	
	0	2.60 (1.53-4.41)		1.94 (1.12-3.37)	0.02

NOTE: Multivariate model: 65 events in 259 subjects.

this represents a signature derived from the cell of origin of these cancers (12), or more is the result of differentiation from a precursor that is common to all breast cancers and therefore does not reflect histogenesis (13). For example, it is possible that the particular cytokeratin signature seen in *BRCA1*-related breast cancer reflects a change in gene expression that occurs in both normal (50) and transformed epithelial cells (51) and does not imply that there is a particular cell of origin for these cancers. However, if this change in differentiation was to regularly occur at a relatively late stage in the evolution of the cancer, then prognostic determinants based on the genes expressed in primary tumors would not be reliable, and additionally, the molecular profile of such cancers would change during the process of metastasis. In fact, the molecular signature of primary breast cancers seems stable (52) and prognostically important (1, 2). These observations support the view that the phenotype of *BRCA1*-related breast cancer is determined early on in the life of the cancer, whether or not the cell of origin of these cancers is actually basal, luminal, both or either. This view is supported by work on different mechanisms of chromosome 16q loss of heterozygosity, referred to above, where 16q loss of heterozygosity resulting from physical loss of the chromosome was much less likely to occur in high-grade cancers than in low-grade cancers (28–30). These findings suggest that these two groups of cancers arose from different types of cells, rather than one becoming the other by acquiring further genetic alterations. In our data set, grade 3 cancers were almost 15 times more likely to be P-cadherin positive than were grade 1 cancers (Table 1), and from numerous previous publications, the basal phenotype is nearly always more commonly seen in high-grade cancers than in low-grade cancers.

If we assume that the "basal phenotype" is determined early and is selected for during clonal development, then it is important to establish the biological significance of each feature of the basal phenotype. Here, we have focused on P-cadherin and how its presence may directly, or indirectly, influence prognosis. We have recently shown that several

Table 3. Risk of death from breast cancer due to P-cadherin positivity stratified by lymph node status and tumor size

			Univariate		Multivariate	
Lymph nodes	Tumor size (cm)	P-cadherin	RR (95% CI)	*P*	RR (95% CI)	*P*
Negative	<2	–	1		1	
		+	11.55 (2.98-44.73)	0.0004	7.08 (1.78-28.23)	0.006
	≥2	–	1		1	
		+	1.30 (0.38-4.48)	0.68	0.65 (0.16-2.70)	0.55
Positive	<2	–	1		1	
		+	1.30 (0.25-6.68)	0.76	0.32 (0.05-1.90)	0.21
	≥2	–	1		1	
		+	2.21 (1.09-4.50)	0.03	1.10 (0.48-2.49)	0.82

NOTE: The multivariable model also included terms for histologic grade, KIP1, Cyclin E, and GMP. Subjects with missing tumor size or lymph node status are excluded.

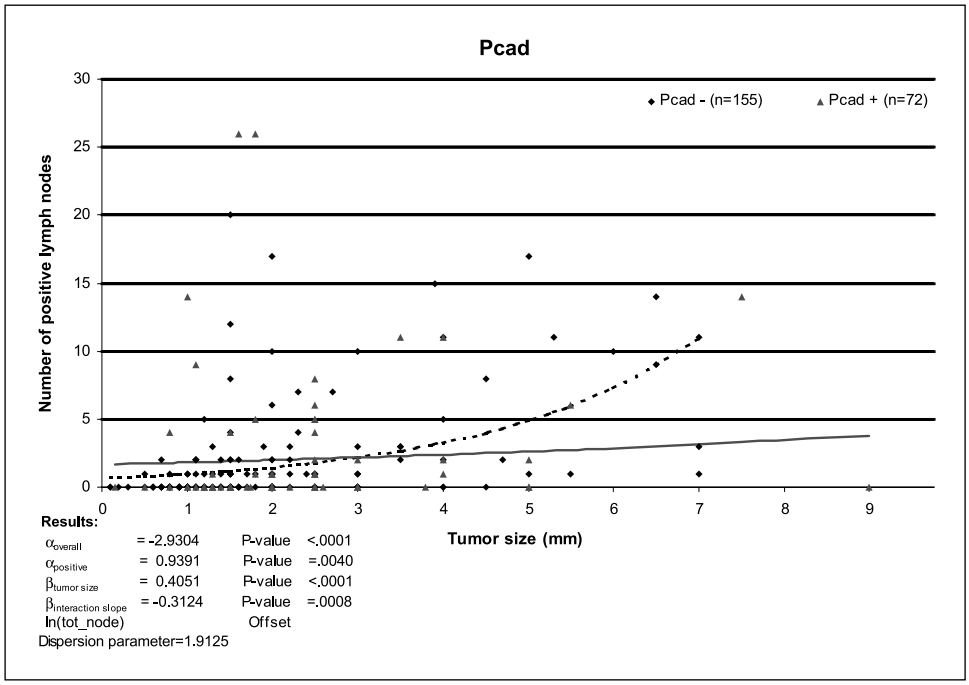

Fig. 2. Poisson regression curves to predict the number of positive lymph nodes for patients with P-cadherin – negative and P-cadherin – positive status, respectively (see Materials and methods for the model used). P-cadherin – negative patient (*dark diamond*) or P-cadherin – positive patient (*pale triangle*). Estimated curves for the P-cadherin negative cases (*dashed line*) and for the P-cadherin positive cases (*solid line*). The difference between the two log-transformed slopes is significant. α (overall) = −2.9304, $P < 0.0001$; α (P-cadherin positive) = 0.9391, $P = 0.004$; β (tumor size) = 0.4051, $P < 0.0001$; β (interaction slope) = −0.3124, $P = 0.0008$.

features of the extended basal phenotype (i.e., cyclin E, KIP1, TP53, and GMP) all can independently influence prognosis following breast cancer (11). We show here that when several basal-associated markers are included in the model, P-cadherin levels do not maintain their prognostic significance. Previous studies of P-cadherin (34, 35) did not include other basal-related markers in the multivariable model, and this could explain the finding in one study (34) that P-cadherin had an independent prognostic value. As shown above, when we created a multivariable model, including tumor size, nodal status, histologic grade, and P-cadherin status, P-cadherin was an independent prognostic marker in our data set as well. Therefore, we conclude that P-cadherin is a reliable marker of the basal epithelial phenotype and is strongly associated with the presence of germ line *BRCA1* mutations but does not by itself add prognostic value beyond basal markers previously investigated. From this and other analyses of our data (11, 39), protein levels of cyclin E when combined with KIP1 may prove to be a useful indicator of prognosis, particularly in node-negative breast cancer.

We previously showed that the expected relationship between increasing tumor size and increasing number of axillary nodes involved by metastatic tumor does not hold equally for *BRCA1*-related and other types of breast cancer (53). We later showed that this might be a general property of basal breast cancers (11), and this conjecture is supported by the data shown here. In fact, the slope for P-cadherin positive tumors is almost horizontal and is clearly statistically significantly different from the P-cadherin – negative slope (Fig. 2). This result confirms and extends our original observations discussed above (53, 54), and given that basal breast cancers are associated with a worse prognosis than nonbasal breast cancers (8, 9, 55), suggests that the mechanism of metastatic spread may be different for this subtype of breast cancer. This view is supported by the multivariable proportional hazards model, including terms for interactions among P-cadherin, tumor size, and lymph node status (Table 3). The results of this model showed that among lymph node – negative breast cancers with small tumors, P-cadherin positivity was an independent adverse prognostic factor (RR, 7.1). The model we developed included terms for histologic grade, HER2, cyclin E, and GMP status. The observation that P-cadherin is an independent prognostic factor in small, node-negative cancers but not in larger node-negative, or in any node-positive, cancers suggests that the expression of P-cadherin on the surface of these small tumors has particular significance beyond its association with these established risk factors [grade (56), cyclin E (43), KIP1 (43, 57), and GMP (44)]. Perhaps, the presence of P-cadherin increases "through-put" in the cadherin-β-catenin-Wnt pathway and drives downstream targets that result in increased microvascular invasion and this elevates the probability of distant metastases. Another possibility is that P-cadherin increases the "stickiness" of the rare, systemically metastatic cells that are released into the circulation by these small, node-negative cancers. It is conceivable that in larger tumors, or those that are node-positive at diagnosis, there is a greater degree of tumor heterogeneity and many other pathways are deregulated, so that the specific effect of P-cadherin is obscured or lost altogether.

Several different sources of data all point towards a specific pathway for *BRCA1*-related breast cancer, which likely involves expression of basal epithelial markers such as cytokeratin 5/6(10), epidermal growth factor receptor (58), and P-cadherin (38), in association with perturbations of the cadherin-β-catenin-Wnt signaling pathway (5, 46). The downstream consequences of these alterations include elevated cyclin E (59) and lowered KIP1 protein levels (39) and amplification of c-MYC (60). Both elevated cyclin E protein levels and amplification of c-MYC are by themselves sufficient to cause aneuploidy (61, 62). In our data set, the association

between the combination of KIP1 and cyclin E and P-cadherin was found to be particularly strong. That is, tumors that were KIP1 negative and cyclin E positive were more than 25 times more likely to be P-cadherin positive than were tumors with the opposite phenotype (Table 1). Taken as a whole, these data suggest that abnormalities in P-cadherin levels, particularly when combined with expression of basal-associated markers, such as cyclin E, are key determinants of the clinicopathologic features and natural history of *BRCA1*-related breast cancer. Understanding the earliest events that define this pathway will be an important goal.

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