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Relationship of P-cadherin Expression to Basal Phenotype of Breast Carcinoma

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Source of support: a grant from the Medical University in Lodz, Poland (No. 502-11-592), and grants from the Polish National Committee of Scientific Research (KBN, Warsaw, Poland; No. 2 P05E 099 28 and No. 2 P05A 015 29).

Background. P-cadherin (P-CD) is a molecule expressed mainly by basal cells involved in cell adhesion. We evaluated expression of P-CD in operable breast carcinomas and its relationship with immunohistochemical markers of the basal-like phenotype and with clinical outcome.

Material and Methods. Expression of P-CD was analyzed by immunohistochemistry in 194 tissue specimens of invasive operable ductal breast cancer.

Results. 112 cases (57.7%) were identified as being P-CD-positive. P-CD-positive tumors usually lacked steroid receptors (p=0.042), expressed basal type cytokeratins (p=0.001), and were positive for cyclin E (p=0.039). In a univariate analysis of cancer-specific survival with a median follow-up period of 58 months, P-CD expression was not associated with prognosis (5-year survival rate for positive vs. negative patients 67.0 vs. 77.0%, log rank p=0.121).

Conclusion. P-CD may be regarded as an additional immunohistochemical marker of basal-like breast carcinomas. However, P-CD expression is not an adverse prognostic factor.

Introduction

Cadherins are a family of Ca^{+2}-dependent glycoproteins involved in cell adhesion (24). Classic cadherins are divided into four subclasses: epithelial cadherin (E-cadherin), neural cadherin (N-cadherin), placental cadherin (P-cadherin, P-CD), and liver cell adhesion molecule (L-CAM), however, others have also been described [23]. Cadherins are differentially expressed in various cells and organs. During organogenesis, through mediation of contacts between cells, these molecules participate in tissue stratification. It has been suggested, that altered expression of cadherins and other adhesive molecules may play a role in carcinogenesis, tumor invasion and the development of metastases [2].

Many reports have been published regarding the role of E-cadherin in molecular oncology [6]. P-CD has been less extensively studied with respect to cancer [2]. The expression of P-CD in nonmalignant human epithelial tissues is confined only to the basal layers of stratified epithelia [21]. In normal human breast tissue P-CD is present in myoepithelial cells [10]. However, in cells of invasive breast carcinomas, P-CD is detected in 35-50% of cases [5, 9, 17]. Its aberrant expression is more often seen in estrogen receptor-negative (ER), high-grade cancers [9, 15, 16]. P-CD-positive tumors usually express cyclin E [1].

On the basis of cDNA microrray data, some breast cancer subtypes have been defined at the molecular level [18]. Tumors negative for ER form three groups: a basal-like subtype, HER2-positive subtype, and a normal breast-like subtype. With the use of immunohistochemistry technique identification of the basal-like phenotype may be possible [3, 14]. The essential part of the basal-like phenotype is the expression of cytokeratin 5/6 (CK5/6) or cytokeratin 17 (CK17). Tumors expressing these keratins are associated with poor prognosis and are usually negative for ER and positive for cyclin E [8, 19, 25]. Some authors have sug-

gested, that P-CD is a marker of basal epithelial phenotype, especially in BRCA1-related breast cancer [16].

Contrasting opinions exist about the prognostic role of P-CD in breast cancer. The majority of studies have shown that its expression was related to poor prognosis while some were less conclusive [1, 5, 17].

The aim of our study was to evaluate expression of P-CD in operable breast carcinomas and its relationship with immunohistochemical markers of the basal-like phenotype and with clinical outcome.

Material and Methods

Specimens of primary tumors were consecutively obtained from 194 women with operable invasive ductal carcinomas not otherwise specified at a time of routine surgery at the Oncology Department of Copernicus Memorial Hospital in Lodz, Poland, between 1997 and 2001. In all cases, surgical procedure was a radical mastectomy with axillary lymph node dissection. Serial sections of the tumor were obtained from archived paraffin embedded tissue blocks. The primary pathologic diagnosis was confirmed in haematoxylin and eosin staining. Subsequent slides were stained for P-CD cytokeratin 5/6 (CK5/6) and 17 (CK17), cyclin E, ER, progesterone receptor (PgR), HER2 and Ki-67. All operative and pathology reports were reviewed to confirm disease stage. Follow-up period was defined as a time from surgery to the last observation for censored cases or death for complete observations.

Immunohistochemistry and scoring

Paraffin embedded sections were routinely processed. Slides for immunostaining for ER, PgR, Ki-67, and CK17 (ER, PgR, Ki-67 from Dako, CK17 from Novocastra) were pretreated with citrate buffer in a microwave oven. HER2 expression was examined with the commercially available Herceptest kit from Dako. Antibodies for CK5/6 (Dako), cyclin E and P-CD (both from Novocastra) were applied following autoclaving with high pH buffer. Antibodies dilutions were as follows: ER – 1:35, PgR – 1:75, Ki-67 – 1:25, CK5/6 – 1:100, CK17 – 1:40, P-CD – 1:200, cyclin E – 1:40. All following procedures were done according to standard protocols with EnVision kit (Dako).

For P-CD a semiquantitative scoring system was used, taking into account both the intensity of staining and the proportion of tumor cells showing membranous positive reaction [1]. The scores of staining intensity were recorded from 0 (no staining) to 3 (strong staining). The scores of staining area were recorded as 1 (<10%), 2 (10–50%) or 3 (>50%). A staining index (SI) was obtained by multiplying the score of staining intensity by the score of staining area. Negative cases had SI=0-1, positive ones had SI=2-9 (Fig. 1). For CK5/6 and CK17 membranous staining results were classified as follows: negative - no staining seen in invasive tumor cells, positive - weak or strong staining seen in invasive cancer cells. ER and PgR nuclear staining scoring was done using the method described by McCarty et al. [13]. Tumors were considered positive for steroid receptors if Histo-score for ER or PgR was above 100. HER2 staining was scored according to Herceptest kit manufacturer's instructions and score 3+ denoted HER2 positive tumors. Ki-67 and cyclin E labeling indices were defined as the percentage of tumor cells displaying nuclear immunoreactivity and were calculated by counting nuclear stained tumor cells in 1000 tumor cells. For cyclin E, samples were classified as negative (<2%) or positive (≥2%).

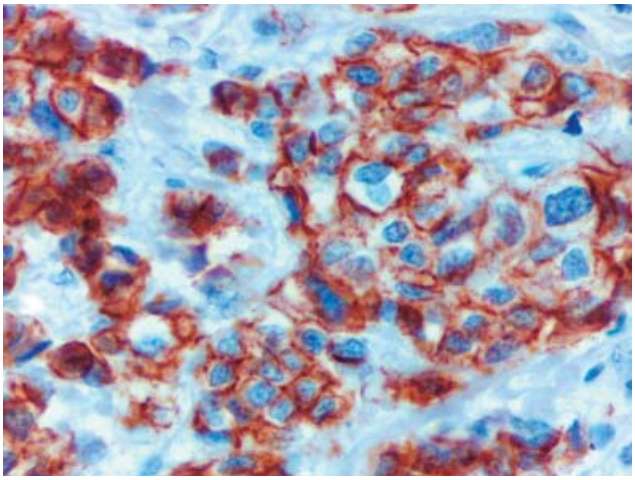

Fig. 1. Positive immunostaining for P-CD. Invasive breast carcinoma. Magnification 400 x.

Statistical analysis

Pearson's χ^2 test was used to test for contingency between dichotomized values of P-CD expression (negative and positive) and values of other histopathological findings. The Mann-Whitney U test was employed to evaluate significance of differences in age and Ki-67 expression between negative and positive patients. Cancer-specific survival was estimated according to Kaplan-Meier method from the date of primary surgery to the date of death or the last follow-up. Data for patients who died from other causes than breast cancer were censored at the time of death. Differences in survival distributions were evaluated by a log-rank test. The prognostic significance of P-CD in the subgroups was assessed with the use of Cox proportional hazard regression model. All results were considered statistically significant when two-sided p was less than 0.05. The analyses were performed using the StatsDirect software (StatsDirect Ltd, UK).

Results

Patient characteristics

The median follow-up period for 143 censored patients was 62 months (range 9-78). For the whole group it was 58 months (range 1-78). During follow-up six deaths due to other causes than breast cancer were observed.

P-cadherin expression

112 cases (57.7%) were identified as being P-CD-positive, whereas 82 (42.3%) were found negative (Table 1). P-CD-positive tumors were usually negative for steroid receptors, expressed basal type keratins, and were positive for cyclin E (Table 1). There was also a statistically insignificant but quite obvious tendency towards a higher proportion of node-positive cases in the P-CD-positive group.

TABLE 1

Associations between clinical and histopathological features and expression of P-cadherin (P-CD)

Feature	P-CD-negative (N=82)	P-CD-positive (N=112)	p value
Age, years (mean ± SD)	57.3 ± 12.6	57.0 ± 12.0	0.979
Tumor size			
T1	26	38	0.745
T2-4	56	74	
Nodal status			
Negative	47	50	0.081
Positive	35	62	
Grading			
G1-2	50	60	0.304
G3	32	52	
ER			
Negative	43	67	0.305
Positive	39	45	
PgR			
Negative	34	67	0.012
Positive	48	45	
Steroid receptors			
Negative	29	56	0.042
Positive	53	56	
CK5/6			
Negative	65	62	<0.001
Positive	17	50	
CK17			
Negative	67	79	0.075
Positive	15	33	
CK5/6 or 17			
Negative	63	60	0.001
Positive	19	52	
HER2			
Negative	70	89	0.386
Positive	12	23	
Cyclin E			
Negative	38	39	0.039
Positive	37	71	
Unknown	7	2	
Ki-67 expression, % (mean ± SD)	7.6 ± 7.9	10.0 ± 11.0	0.100

Numbers in the second and third columns denote numbers of patients, except from age and Ki-67 expression. Ki-67 expression was assessed in 81 P-CD-negative and in 111 P-CD positive tumors

TABLE 2
Individual prognostic factors and prognostic relevance of P-CD expression (positive vs. negative) in the subgroups

Factor and subgroups	No of patients	5-year % survival rate (95%CI)	p value	Hazard ratio (95%CI)	p value
Age					
<50 year	62	72.7 (59.3-82.4)	0.801	1.3 (0.5-3.6)	0.595
≥50 year	132	70.8 (61.7-78.2)		1.7 (0.8-3.5)	0.138
Tumor size					
T1	64	77.3 (63.9-86.2)	0.124	2.5 (0.7-9.1)	0.166
T2-4	130	68.6 (59.4-76.1)		1.4 (0.7-2.7)	0.312
Nodal status					
Negative	97	82.1 (72.3-88.7)	<0.001	1.7 (0.6-4.6)	0.328
Positive	97	60.1 (48.9-70.0)		1.3 (0.6-2.6)	0.508
Grading					
G1-2	110	73.9 (63.9-81.5)	0.408	1.5 (0.7-3.4)	0.286
G3	84	68.3 (56.7-77.3)		1.5 (0.6-3.7)	0.341
ER					
Negative	110	60.5 (50.3-69.2)	<0.001	1.1 (0.6-2.0)	0.848
Positive	84	86.5 (75.9-92.6)		3.9 (0.8-18.2)	0.088
PgR					
Negative	101	61.3 (50.7-70.3)	0.001	1.4 (0.7-2.8)	0.387
Positive	93	82.9 (72.6-89.6)		1.2 (0.4-3.3)	0.785
Steroid receptor	85	59.3 (47.8-69.1)	0.001	1.4 (0.7-3.0)	0.386
Negative	109	81.4 (71.9-87.9)		1.3 (0.5-3.2)	0.616
P-CD					
Negative	82	77.0 (65.4-85.2)	0.121	Not applicable	
Positive	112	67.0 (56.9-75.2)			
CK5/6					
Negative	127	76.1 (67.1-82.9)	0.095	1.8 (0.9-3.9)	0.124
Positive	67	62.4 (49.0-73.3)		0.9 (0.3-2.2)	0.767
CK17					
Negative	146	76.0 (67.8-82.3)	0.075	1.6 (0.8-3.2)	0.197
Positive	48	58.3 (42.0-71.5)		0.8 (0.3-1.7)	0.505
CK5/6 or 17					
Negative	123	78.3 (69.5-84.8)	0.025	2.1 (0.9-4.7)	0.077
Positive	71	59.7 (46.6-70.6)		0.8 (0.3-1.7)	0.505
HER2					
Negative	159	75.2 (67.3-81.5)	0.004	1.7 (0.9-3.4)	0.130
Positive	35	54.4 (35.9-69.6)		1.0 (0.4-3.0)	0.987
Cyclin E					
Negative	77	84.6 (73.9-91.2)	<0.001	1.2 (0.4-3.9)	0.767
Positive	108	60.7 (50.3-69.6)		1.4 (0.7-2.9)	0.306
Unknown	9				

Cancer-specific survival

For all cases, P-CD expression was not associated with prognosis (Table 2). P-CD-positive patients had slightly worse outcome when compared with the negative ones but this difference was not significant (5-year survival rate 67.0 vs. 77.0%, log rank p=0.121). In a univariate proportional hazards model, P-CD positivity was not associated with survival in any subgroup of patients (Table 2).

Discussion

With the advent of the cDNA microarray technology a new classification of breast malignant tumors has become possible. These molecular subtypes are strongly associated with patient survival. The shortest survival has been observed in the basal-like and HER2 subtypes [22, 26]. Basal-like tumors show high expression of genes for keratin 5 and 17, P-CD, and proliferation-related genes [22]. Thus, some efforts have been made to reproduce this molecular classification with use of immunohistochemistry. Till now, the basal-like subtype assessed by immunostaining has been defined as being ER- and HER2-negative, and positive for CK5/6. Some authors suggest also positivity for CK17, epidermal growth factor receptor (EGFR), and CD117 (c-kit receptor) [14, 25]. However, little is known about the relevance of the positive immunostaining for P-CD in the categorization of tumors into the basal-like subtype. Some studies, however, indicate such relationship [1, 12].

In our series of tumors, positive staining for P-CD was found in 58% of cases, the proportion which is a little higher than reported by others [1, 5, 9, 17]. Arnes et al. with the use of the same scoring system have found only 31% P-CD-positive cases [1]. Such disagreements between studies could be possibly explained by the subjectivity of the method and differences between scoring systems used. The results of scoring systems in immunostaining are not entirely reproducible, even with respect to routine assays [20]. Moreover, there are also differences in the prevalence of various cancer subtypes between human races [3].

We confirmed highly significant association between expression of P-CD and CK5/6 which had been observed earlier by others [1, 12]. The relationship of P-CD with CK17 was insignificant. Indeed, the positive staining for CK5/6 is considered to be critical for the basal-like phenotype determined by immunohistochemistry [14]. Tumors positive for P-CD were usually negative for steroid receptors and positive for cyclin E. The relationship between P-CD and cyclin E has been also recently reported by Arnes et al. [1]. The high level of cyclin E promotes uncontrolled cell divisions. Cell cycle regulation by cyclin E has been shown to be altered in breast cancer. High level of cyclin E protein has been demonstrated in association with higher disease stage, higher tumor grade, steroid receptors negativity and, finally, poor clinical outcome [7]. Such an aggressive behaviour of the tumor is also seen in basal-type cancers. This observation raises the question if cyclin E overexpression may be attributable to the basal-type subgroup of breast cancer, especially on the basis of data derived from immunostaining [4]. Interestingly enough, we did not find any significant association between P-CD and ER. It may be explained, at least partially, by the overlapping of the tumor subtypes separated immunohistochemically. Some tumors express molecular markers specific for different subgroups at the same time, i.e. ER and CK5/6 or HER2 and CK5/6. We have reported this observation already [11].

In a univariate survival analysis, the P-CD-positive patients had similar prognosis when compared with P-CD-negative group. This applied to all cases and to all subgroups. This observation remains in contrast to some reports [1, 5]. Again, a relatively higher proportion of P-CD-positive tumors in our series and the observation of the overlapping between subgroups may together contribute to the possible explanation. On the other hand, a tendency towards a higher proportion of node-positive tumors in P-CD positive group may also have a slight negative effect on survival. Beside axillary nodes involvement, ER expression was the strongest single prognostic factor. Thus, lack of association between ER and P-CD observed by us, may explain no relation between P-CD expression and cancer-specific survival. This is supported by the observation that in ER-positive tumors P-CD positivity almost reached statistical significance as a negative prognostic factor. Moreover, even if P-CD showed a prognostic value in a univariate analysis in the studies cited it did not retain significance as an independent prognostic factor in a multivariate analysis [1, 5, 17].

Summarizing, we report highly significant association between expression of P-CD and basal-type keratin CK5/6 and, to somehow less degree, cyclin E. P-CD may be regarded as an additional immunohistochemical marker of basal-like breast carcinomas. We did not confirm prognostic value of this marker. However, the absence of a standard staining scoring system for P-CD and the overlapping between subgroups defined on the basis of immunohistochemical data can possibly explain lack of relation to clinical outcome in a univariate survival analysis.

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