Reduction of E-cadherin expression is associated with non-lobular breast carcinomas of basal-like and triple negative phenotype

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ABSTRACT

Aim: E-cadherin inactivation in breast cancer has been shown to be strongly associated with lobular breast cancer. However, little is known about the levels of E-cadherin expression according to the breast cancer "molecular" subtypes. The aim of this study was to address the distribution of E-cadherin expression according to the different molecular subtypes of breast cancer.

Methods: E-cadherin expression was immunohistochemically analysed in a tissue microarray containing duplicate cores of 245 invasive breast carcinomas, of which 182 cases were of non-lobular histology, using a semi-quantitative scoring system based on the percentage of cells showing membrane immunopositivity.

Results: In non-lobular breast carcinomas, reduced and/or negative E-cadherin expression was significantly associated with lack of oestrogen receptor expression, low levels of *CCND1* expression, positivity for cytokeratins 5/6 and 17, epidermal growth factor receptor and caveolins 1 and 2, p53 expression, high MIB-1 proliferation indices, basal-like phenotype and triple negative phenotype.

Conclusion: This study demonstrates that in the group of non-lobular breast cancers, reduction/lack of E-cadherin expression is preferentially found in basal-like breast carcinomas.

E-cadherin is a transmembrane glycoprotein coded by the *CDH1* gene, which maps to chromosome 16q22.1 It is localised on the surface of epithelial cells and mediates adhesion through Ca^{2+}-dependent homotypic binding. Based on its biological functions, E-cadherin is regarded as an invasion and metastasis suppressor. Loss of E-cadherin expression or function correlates with increased invasiveness and metastasis in carcinomas of several anatomical sites.[1 2]

In breast carcinomas, there are several lines of evidence to suggest that *CDH1* gene inactivation leading to E-cadherin inactivation is strongly associated with lobular breast cancer, both in situ and invasive. It has also been demonstrated that loss of E-cadherin expression leads to the so characteristic discohesiveness and pattern of invasion and metastatic spread of this breast cancer subtype.[1]

The biological significance of lack of E-cadherin expression in non-lobular breast cancers remains unclear. Although lack of E-cadherin expression in non-lobular breast cancers has been reported to correlate with biomarkers of aggressiveness, including larger tumour size, higher tumour grade, higher prevalence of recurrence and metastasis, inflammatory breast cancer, a remarkably aggressive type of breast cancer is reported to express high levels of E-cadherin.[2–4]

Molecular profiling is reshaping breast cancer taxonomy. The seminal microarray-based class discovery studies performed by the Stamford group have demonstrated that breast cancers can be systematically classified into luminal, basal-like, normal-like and ERBB2 subgroups.[5 6] Interestingly, it has been demonstrated at the protein and mRNA levels that the basal-like subtype consistently expresses P-cadherin.[7] However, little is known about the levels of E-cadherin expression according to breast cancer molecular subtypes.

The aims of this study were to address the distribution of E-cadherin expression according to the different molecular subtypes of breast cancer and its correlation with the expression of key biomarkers and amplification of key oncogenes in breast cancer samples.

METHODS

A tissue microarray (TMA) was constructed with primary breast cancer samples with replicate 0.6 mm cores of 245 invasive breast carcinomas. These samples were obtained from consecutive patients who were diagnosed and treated at the Royal Marsden Hospital, London, UK, with therapeutic surgery followed by anthracycline-based adjuvant chemotherapy. All patients with oestrogen receptor (ER)-positive tumours also received adjuvant endocrine therapy. Follow-up data were available for 244 patients, ranging from 0.5 to 125 months (median, 67 months; mean, 67 months). Patient information, pathological characteristics of the tumours, detailed TMA preparation and the expression of a number of biomarkers have been reported previously[8] This study was approved by The Royal Marsden Hospital Research Ethics Committee.

Expression of E-cadherin was analysed using the mouse monoclonal antibody clone HECD-1 (1:200) (Invitrogen/Zymed, Carlsbad, California, USA) by immunohistochemistry (IHC) as previously described.[3] E-cadherin expression was semi-quantitatively analysed by two of the authors (BM-A and JSR-F) according to the percentage of cells showing membrane positivity: 0, 0–10%; 1, 10 to <25%; 2, 25 to 50%; 3, 50 to 75%; and 4, >75%. Expression of E-cadherin was considered normal when scores were $\geqslant 3$, reduced when equal to 2, and negative when scores were $\leqslant 2$.

E-cadherin expression was correlated with the expression of ER, progesterone receptor (PgR), MIB-1, human epidermal growth factor receptor-2 (HER2), epidermal growth factor receptor (EGFR), cytokeratin (Ck)14, Ck 5/6, Ck 17, cyclin

Table 1 Correlations between E-cadherin expression and clinicopathological parameters and immunohistochemical markers in 182 non-lobular invasive breast carcinomas

Parameter		No.	E-cad normal (%)	E-cad reduced (%)	E-cad negative (%)	p Value
Size: TNM		179				0.1477
	T1		72 (74.2)	11 (11.3)	14 (14.4)	
	T2		57 (79.2)	1 (1.4)	14 (19.4)	
	T3		8 (80)	1 (10)	1 (10)	
Grade		178				0.1624
	1		12 (63.2)	3 (15.8)	4 (21.1)	
	2		39 (88.6)	1 (2.3)	4 (9.1)	
	3		86 (74.8)	9 (7.8)	20 (17.4)	
Type		180				0.0204
	IDC		125 (80.1)	11 (7.1)	20 (12.8)	
	Mixed		10 (62.5)	1 (6.3)	5 (31.3)	
	Other		3 (37.5)	1 (12.5)	4 (50)	
LVI		179				0.5898
	−		46 (76.7)	3 (5)	11 (18.3)	
	+		92 (77.3)	10 (8.4)	17 (14.3)	
LN metastasis		175				0.2014
	−		49 (72.1)	4 (5.9)	15 (22.1)	
	+		85 (79.4)	9 (8.4)	13 (12.1)	
ER		180				0.0029
	−		23 (60.5)	2 (5.3)	13 (34.2)	
	+		115 (81)	11 (7.7)	16 (11.3)	
PgR		180				0.2854
	−		33 (70.2)	3 (6.4)	11 (23.4)	
	+		105 (78.9)	10 (7.5)	18 (13.5)	
HER2		180				0.3267
	−		113 (74.8)	11 (7.3)	27 (17.9)	
	+		25 (86.2)	2 (6.9)	2 (6.9)	
HER2 CISH		175				0.6916
	Not amp		111 (76)	11 (7.5)	24 (16.4)	
	Amp		24 (82.8)	2 (6.9)	3 (10.3)	
EGFR		180				0.0010
	−		128 (80)	12 (7.5)	20 (12.5)	
	+		10 (50)	1 (5)	9 (45)	
Ck 14		179				0.0931
	−		127 (78.9)	12 (7.5)	22 (13.7)	
	+		11 (61.1)	1 (5.6)	6 (33.3)	
Ck5/6		172				0.0449
	−		121 (80.1)	11 (7.3)	19 (12.6)	
	+		13 (61.9)	1 (4.8)	7 (33.3)	
Ck17		178				0.0009
	−		122 (79.7)	13 (8.5)	18 (11.8)	
	+		15 (60)	0 (0)	10 (40)	
Basal Ck		179				0.0492
	−		118 (79.2)	12 (8.1)	19 (12.8)	
	+		20 (66.7)	1 (3.3)	9 (30)	
Basal Ck or EGFR		179				0.0002
	−		118 (81.4)	12 (8.3)	15 (10.3)	
	+		20 (58.8)	1 (2.9)	13 (38.2)	
Nielsen groups		175				0.0043
	Basal		15 (55.6)	1 (3.7)	11 (40.7)	
	Luminal		95 (79.8)	9 (7.6)	15 (12.6)	
	HER2		25 (86.2)	2 (6.9)	2 (6.9)	
Triple negative		180				0.0193
	No		123 (79.9)	11 (7.1)	20 (13)	
	Yes		15 (57.7)	2 (7.7)	9 (34.6)	
P53		178				0.0499
	−		96 (80)	10 (8.3)	14 (11.7)	
	+		40 (69)	3 (5.2)	15 (25.9)	
MIB-1		176				0.0429
	<10%		59 (85.5)	4 (5.8)	6 (8.7)	
	10–30%		53 (67.1)	9 (11.4)	17 (21.5)	
	>30%		22 (78.6)	0 (0)	6 (21.4)	

Continued

Table 1 Continued

Parameter		No.	E-cad normal (%)	E-cad reduced (%)	E-cad negative (%)	p Value
TOP2A		175				0.5025
	Low		55 (72.4)	7 (9.2)	14 (18.4)	
	High		79 (79.8)	6 (6.1)	14 (14.1)	
TOP2A CISH		177				0.7871
	Not amp		123 (77.8)	10 (6.3)	25 (15.8)	
	Amp		14 (73.7)	2 (10.5)	3 (15.8)	
Cyclin D1		177				0.0204
	Low		13 (61.9)	0 (0)	8 (38.1)	
	Intermediate		26 (70.3)	3 (8.1)	8 (21.6)	
	High		96 (80.7)	10 (8.4)	13 (10.9)	
CCND1 CISH		162				0.2749
	Not amp		106 (75.2)	9 (6.4)	26 (18.4)	
	Amp		18 (85.7)	2 (9.5)	1 (4.8)	
MYC CISH		162				0.9404
	Not amp		13 (76.5)	1 (5.9)	3 (17.6)	
	Amp		109 (75.2)	12 (8.3)	24 (16.6)	
CAV1		180				0.0010
	−		128 (80)	12 (7.5)	20 (12.5)	
	+		10 (50)	1 (5)	9 (45)	
CAV2		175				0.0008
	−		128 (79)	12 (7.4)	22 (13.6)	
	+		5 (38.5)	7 (53.8)	1 (7.7)	

E-cad, E-cadherin; IDC, invasive ductal carcinoma; Ck, cytokeratin; ER, oestrogen receptor; PgR, progesterone receptor; EGFR, epidermal growth factor receptor; TOP2A, topoisomerase IIα; CISH, chromogenic in situ hybridisation; LN, lymph node; LVI, lympho-vascular invasion; HER2, human epidermal growth factor receptor-2; CAV, caveolin; Nielsen groups,[6] immunophenotypic groups defined based upon the expression of ER, HER2, Ck 5/6 and EGFR.

D1 expression, caveolin 1 and 2 (CAV1 and CAV2) expression, topoisomerase IIα (TOP2A) expression, and with amplification of *HER2*, *TOP2A*, *CCND1* and *MYC* genes. Details on the expression of the above proteins and the prevalence of *HER2*, *TOP2A*, *CCND1* and *MYC* gene amplification, as defined by chromogenic in situ hybridisation, are described elsewhere.[8] All cases were classified into luminal, HER2, basal-like and undetermined groups according to the IHC panel described Nielsen *et al*.[6]

The StatView 5.0 software package (SAS Institute Inc., Cary, North Carolina, USA) was used for all calculations. Correlations between categorical variables were performed using the χ^2 test. Metastasis-free and breast cancer specific survival was expressed as the number of months from diagnosis to the occurrence of an event (distant metastasis or disease-related death, respectively). Cumulative survival probabilities were calculated using the Kaplan–Meier method. Differences between survival rates were tested with the log-rank test. All tests were two-tailed, with a confidence interval of 95%.

RESULTS AND DISCUSSION

Briefly, 28 cores were either lost or did not have invasive tumour. Out of the 217 remaining cases, E-cadherin expression in neoplastic cells was negative in 61 cases, reduced in 14 cases, and normal in 142 cases. As expected, when lobular and non-lobular breast cancers were analysed, a strong correlation between lack of E-cadherin expression and lobular histotype ($p<0.0001$) was found. No other significant correlations between E-cadherin expression and other clinicopathological features were observed (data not shown).

After excluding all lobular carcinomas from the analysis, a significant correlation between normal E-cadherin expression and ductal histological type ($p = 0.0204$) was observed (table 1). E-cadherin expression showed no correlation with PR, HER2 overexpression and *HER2* amplification, CK14, TOP2A expression and amplification, *CCND1* gene amplification and *MYC* amplification. In this subset of cancers, reduced and/or negative E-cadherin expression was significantly associated with lack of ER expression, low levels of CCND1 expression, positivity for Ck5/6, Ck17, EGFR, basal-like phenotype and triple negative phenotype (table 1, fig 1). Furthermore, E-cadherin reduced and/or negative tumours more frequently showed p53 positivity, high proliferation indices as defined by MIB-1 expression, and expression of CAV1 and 2 (table 1). Taken together, the above features are characteristic of basal-like breast cancers.[6] We have also performed a meta-analysis of publicly available microarray mRNA expression array data that revealed a statistically significant correlation between reduction of E-cadherin gene (*CDH1*) expression levels and basal-like phenotype ($p<0.0001$, t test), further corroborating our results.[9] Furthermore, basal-like breast cancer cell lines show significantly lower *CDH1* mRNA levels than luminal breast cancer cell lines ($p<0.05$, t test).[10]

As the vast majority of basal-like and triple negative phenotype cancers are of histological grade III, one could argue that the above associations would be a mere reflection of the associations between high histological grade and reduction of E-cadherin expression. However, when only grade III non-lobular breast carcinomas were analysed, we observed statistically significant correlations between reduction or lack of E-cadherin and ER negativity and expression of EGFR, CK17 and CAV1 and 2. In addition, in this subgroup, reduction or lack of E-cadherin expression was significantly associated with basal-like carcinomas as defined by Nielsen *et al*.[6] and triple negative phenotype (table 2).

There is evidence to suggest that retained E-cadherin expression is associated with better survival in women diagnosed with invasive ductal carcinoma[2] and non-lobular cancer.[3] In contrast with that, we did not observe a significant correlation between E-cadherin expression and disease-free interval and overall survival. These discrepant results may be due to distinct scoring systems for E-cadherin expression and the composition of our cohort, whose selection criteria were patients subjected to therapeutic surgery followed by adjuvant

Table 2 Correlations between E-cadherin expression and clinicopathological parameters and immunohistochemical markers in 130 grade III non-lobular invasive breast carcinomas

Parameter		No.	E-cad normal (%)	E-cad reduced (%)	E-cad negative (%)	p Value
Size: TNM		115				0.1313
	T1		44 (72.1)	8 (13.1)	9 (14.8)	
	T2		37 (78.7)	0 (0)	10 (21.3)	
	T3		5 (71.4)	1 (14.3)	1 (14.3)	
Type		115				0.0067
	IDC		84 (78.5)	8 (7.5)	15 (14)	
	Mixed		1 (33.3)	0 (0)	2 (66.7)	
	Other		1 (20)	1 (20)	3 (60)	
LVI		115				0.7425
	−		30 (76.9)	2 (5.1)	7 (17.9)	
	+		56 (73.7)	7 (9.2)	13 (17.1)	
LN metastasis		112				0.5605
	−		33 (70.2)	4 (8.5)	10 (21.3)	
	+		51 (78.5)	5 (7.7)	9 (13.8)	
ER		115				0.0408
	−		23 (63.9)	2 (5.6)	11 (30.6)	
	+		63 (79.7)	7 (8.9)	9 (11.4)	
PgR		115				0.4377
	−		30 (69.8)	3 (7)	10 (23.3)	
	+		56 (77.8)	6 (8.3)	10 (13.9)	
HER2		115				0.0759
	−		63 (70)	8 (8.9)	19 (21.1)	
	+		23 (92)	1 (4)	1 (4)	
HER2 CISH		112				0.1662
	Not amp		63 (71.6)	7 (8)	18 (20.5)	
	Amp		21 (87.5)	2 (8.3)	1 (4.2)	
EGFR		115				0.0016
	−		76 (80)	8 (8.4)	11 (11.6)	
	+		10 (50)	1 (5)	9 (45)	
Ck14		115				0.1501
	−		75 (77.3)	8 (8.2)	14 (14.4)	
	+		11 (61.1)	1 (5.6)	6 (33.3)	
Ck5/6		110				0.1859
	−		71 (78.9)	7 (7.8)	12 (13.3)	
	+		13 (65)	1 (5)	6 (30)	
Ck17		114				0.0071
	−		70 (77.8)	9 (10)	11 (12.2)	
	+		15 (62.5)	0 (0)	9 (37.5)	
Basal Ck		115				0.1788
	−		66 (76.7)	8 (9.3)	12 (14)	
	+		20 (69)	1 (3.4)	8 (27.6)	
Basal Ck or EGFR		115				0.0023
	−		66 (80.5)	8 (9.8)	8 (9.8)	
	+		20 (60.6)	1 (3)	12 (36.4)	
Nielsen groups		115				<0.0001
	Basal		15 (57.7)	1 (3.8)	10 (38.5)	
	Luminal		45 (75)	6 (10)	9 (15)	
	HER2		23 (92)	1 (4)	1 (4)	
Triple negative		115				0.0295
	No		71 (79.8)	7 (7.9)	11 (12.4)	
	Yes		15 (57.7)	2 (7.7)	9 (34.6)	
P53		114				0.0581
	−		54 (80.6)	6 (9)	7 (10.4)	
	+		31 (66)	3 (6.4)	13 (27.7)	
MIB-1		112				0.0724
	<10%		15 (93.8)	0 (0)	1 (6.3)	
	10–30%		46 (67.6)	9 (13.2)	13 (19.1)	
	>30%		22 (78.6)	0 (0)	6 (21.4)	
TOP2A		112				0.9051
	Low		31 (72.1)	4 (9.3)	8 (18.6)	
	High		52 (75.4)	5 (7.2)	12 (17.4)	
TOP2A CISH		112				0.3528

Continued

Table 2 Continued

Parameter		No.	E-cad normal (%)	E-cad reduced (%)	E-cad negative (%)	p Value
	Not amp		72 (75)	6 (6.3)	18 (18.8)	
	Amp		13 (81.3)	2 (12.5)	1 (6.3)	
Cyclin D1		113				0.0850
	Low		11 (61.1)	0 (0)	7 (38.9)	
	Intermediate		15 (71.4)	2 (9.5)	4 (19)	
	High		58 (78.4)	7 (9.5)	9 (12.2)	
CCND1 CISH		104				0.0916
	Not amp		65 (72.2)	5 (5.6)	20 (22.2)	
	Amp		12 (85.7)	2 (14.3)	0 (0)	
MYC CISH		104				0.9290
	Not amp		66 (72.5)	8 (8.8)	17 (18.7)	
	Amp		9 (69.2)	1 (7.7)	3 (23.1)	
CAV1		115				0.0079
	−		76 (79.2)	8 (8.3)	12 (12.5)	
	+		10 (52.6)	1 (5.3)	8 (42.1)	
CAV2		112				0.0079
	−		78 (78)	8 (8)	14 (14)	
	+		5 (41.7)	1 (8.3)	6 (50)	

E-cad, E-cadherin; IDC, invasive ductal carcinoma; Ck, cytokeratin; ER, oestrogen receptor; PgR, progesterone receptor; EGFR, epidermal growth factor receptor; TOP2A, topoisomerase IIα; CAV, aveolin; CISH, chromogenic in situ hybridisation; LN, lymph node; LVI, lympho-vascular invasion; HER2, human epidermal growth factor receptor-2; Nielsen groups,[6] immunophenotypic groups defined based upon the expression of ER, HER2, Ck 5/6 and EGFR.

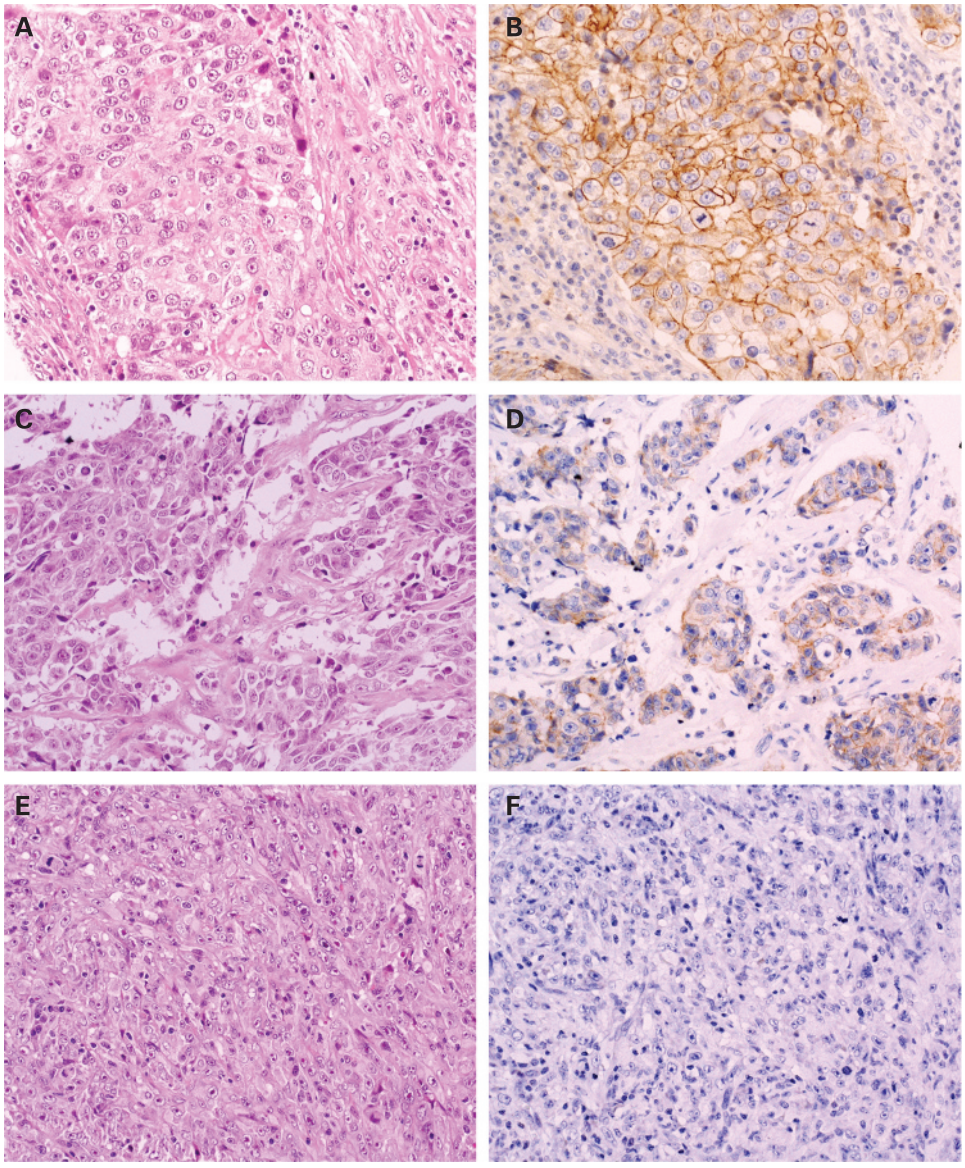

Figure 1 E-cadherin expression in basal-like breast carcinomas as defined by Nielsen *et al* criteria.[6] Grade III invasive ductal carcinoma of no special type (A), and with basal-like phenotype with strong membranous E-cadherin expression (B). Grade III invasive ductal carcinoma of no special type (C), and with basal like phenotype with reduced E-cadherin expression (D). Invasive metaplastic breast cancer (E), with basal-like phenotype lacking E-cadherin expression (F).

anthracycline chemotherapy (these are less often small, of grade I, without lymph node metastasis and vascular invasion than cases from a population-based study). On the other hand, given the size of our cohort, we cannot rule out type II or β errors.

The significance of E-cadherin deficiency as a predictive factor in metastatic spread is not clear. We did not find a statistically significant correlation between E-cadherin expression patterns and metastasis free and breast cancer specific survival. Our results corroborate those reported by Rakha *et al* 2005,[3] who found no association between E-cadherin expression and vascular invasion and lymph node status. Interestingly, there are several lines of evidence to suggest that basal-like breast cancers less often show lymph node metastasis and have a peculiar proclivity to disseminate to brain when compared with non-basal-like grade III tumours.[11] Given the experimental evidence linking E-cadherin with patterns of invasion and metastasis, our findings suggest a possible role of E-cadherin in the metastastic pattern of basal-like cancers.

In conclusion, E-cadherin expression is either reduced or lost in >40% of basal-like breast carcinomas. Further studies to investigate the mechanism of E-cadherin downregulation in basal-like cancers and its impact on invasion and metastasis patterns of these tumours are warranted.

Take-home messages

- E-cadherin downregulation is associated with lobular histo-type.
- In non-lobular breast cancers, E-cadherin expression is significantly more often reduced in basal-like and triple negative breast cancers.
- E-cadherin reduced/negative non-lobular breast cancers have higher proliferation rates and more often display p53 nuclear expression.

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