Correlation of E-cadherin expression with clinicopathological parameters in breast carcinoma

Umit Cobanoglu, *MD,* ***Safak Ersoz,*** *MD,* ***Havvanur Turgutalp,*** *MD,* ***Abdulkadir Reis,*** *MD,* ***Yavuz Ozoran,*** *MD.*

ABSTRACT

Objective: To investigate the correlation between the E-cadherin (E-CD) expression and clinicopathological parameters including tumor grade, patient age, tumor size, necrosis, peritumoral lymphovascular invasion and lymph node status in breast carcinomas.

Methods: The specimens were surgically obtained from 51 female patients with breast carcinoma between 1997 and 2001 in Karadeniz Technical University Medicine Faculty Farabi Hospital, Trabzon, Turkey. Histologic grading was according to the Bloom and Richardson methods. Tumors were classified as grade I (well differentiated), grade II (moderately differentiated) and grade III (poorly differentiated). Necrosis was graded as (-), (+), (++) and (+++).

Results: Grade 1 breast carcinomas (n=17) showed greater immunoreactivity than grade 2 (n= 22) and grade 3 (n=12) carcinomas. None of the infiltrating lobular carcinomas expressed E-CD. Statistically, significant difference has been noticed between E-CD expression and the histological grade. In contrast, no association were found between E-CD expression and metastatic potential, tumor size, tumor necrosis and patients' age.

Conclusion: Results in the present report suggest that E-CD expression in breast carcinoma is more related to histological type and differentiation grade than with metastatic potential, tumor size, tumor necrosis and patients' age.

Saudi Med J 2004; Vol. 25 (8): 1024-1027

The cadherin are a family of glycoproteins which are calcium dependent cell-cell adhesion proteins. The cadherin found in the epithelia [E-cadherin (E-CD)] has been demonstrated in a wide variety of tissues, including the breast. Due to their important role in cell-cell and cell-matrix adhesion, these molecules are important in tumor invasion and metastasis. Several studies have demonstrated an association between loss of expression and tumor metastasis. Other series have failed to show such relationships.[1-3]

Breast carcinoma is an important cause of morbidity and mortality among women. In this report, we describe the results of our studies concerning the E-CD expression in breast carcinoma in an attempt to correlate these data with the clinicopathological features.

Methods. The specimens were surgically obtained from 51 female patients with breast carcinoma between 1997 and 2001 in Karadeniz Technical University Medicine Faculty Farabi Hospital, Trabzon, Turkey. The pathology data included tumor size, histologic grade, necrosis, peritumoral lymphovascular invasion and number of lymph node metastases. Histologic grading was according to the Bloom and Richardson methods.

From the Department of Pathology, Faculty of Medicine, Karadeniz Technical University, Trabzon, *Turkey.*

Received 6th January 2004. Accepted for publication in final form 10th March 2004.

Address correspondence and reprint request to: Dr. Umit Cobanoglu, Karadeniz Teknik Universitesi, Tip Fakultesi, Patoloji Anabilim Dali, Trabzon, *Turkey.* Tel.+90 (462) 3775409. E-mail: ucoban@meds.ktu.edu.tr

Tumors were classified as grade I (well differentiated), grade II (moderately differentiated) and grade III (poorly differentiated). Necrosis was graded as (-), (+), (++) and (+++). Formalin fixed, paraffin embedded specimens were cut, dewaxed and stained with hematoxylin and eosin.

Monoclonal antibody against E-CD (4A2C7, Zymed, South San Francisco, CA) was used. The standard avidin biotin indirect immunoperoxidase method (Zymed) was used for immuno histochemistry. Briefly, 4 mm sections were cut from the paraffin embedded blocks. The glass slides were previously coated with poly L-lysine (Zymed). The sections were then incubated at 37^{o}C overnight. Thereafter, the sections were deparaffinized in xylene (30 minutes, twice) sequentially dehydrated by incubating in 100% alcohol, 90% alcohol, 70% alcohol, 50% alcohol, 30% alcohol and in phosphate buffered saline (10 minutes each). Antigen unmasking by pressure heating in 0.1 M citrate buffer (pH 6) for 5 minutes. After cooling and washing, the endogenous peroxidase activity was blocked by incubating in 3% H_20_2 for 30 minutes to prevent nonspecific binding of antibodies. Hence, the sections were incubated overnight with the primary antibody, washed, incubated with biotinylated secondary antibodies (30 minutes), washed and incubated with avidin peroxidase complex (30 minutes). The peroxidase reaction was visualized by incubating with 3-amino-9-ethylcarbazole for 10 minutes. Known positive cases (benign breast tissue) and negative controls (omission of the primary antibody) were included in each run and were shown to be positive and negative. Normal breast epithelial elements in the tumor sections were used as internal positive controls. All sections were counterstained using Mayer's hematoxylin.

At a high magnification (x 40), the extent and distribution of membrane staining of E-CD was recorded in each case using a semi quantitative scoring system. The approximate proportion of positive tumor cells was evaluated. Staining was classified as 4 if >80% of the tumor showed reactivity, 3 if 50-80% of the tumor showed staining, 2 if 20-50% of the tumor showed staining, one for patchy, focal reactivity and 0 if there was no evidence of staining. E-cadherin expression was compared with tumor type and grade, tumor necrosis, tumor size, lymph node status, patients' age and lymphovascular invasion. For statistical analysis, the analysis of variance (ANOVA) and Kuruskall Wallis Variant test were used for correlation with all parameters used. The staining score was expressed as mean value ± standard deviation (SD).

Results. The patients were aged 26-77 years (mean 46.5) and none of them had received irradiation and chemotherapy pre-operatively. Histologically, the 51 tumor were categorized as 45 (88%) infiltrating ductal carcinoma (IDC), 3 (6%) invasive lobular carcinoma (ILC), one (2%) atypic medullary carcinoma, 1(2%) medullary carcinoma and one (2%) tubular carcinoma.

Tumors ranged in size from 1-9 cm (mean 3.52, SD=1,72). Eighty percent (n=41) were node positive and 20% (n=10) were node negative. Metastatic nodes ranged from 1-27 in number. Seventeen of 41 (41%) node positive patients showed extracapsular invasion.

Tumors of grade I accounted for 35% (16/45), tumors of grade II accounted for 35% (16/45), tumors of grade III accounted for 30% (13/45). Infiltrating lobular carcinoma, atypic medullary carcinoma and medullary carcinoma were categorized as grade II and tubular carcinoma as grade I. Forty-two percent (19/45) demonstrated (+) necrosis, 13% (6/45) demonstrated (++) necrosis, 15% (7/45) demonstrated (+++) necrosis. No necrosis was observed in 18 cases . The number of infiltrating ductal carcinoma with a peritumoral intraductal component was 16 (16/45).

The mean value of E-CD expression in 51 cases was 44.9, SD=35.7. The mean value of E-CD expression in 45 infiltrating ductal carcinoma was 47.2, SD=34,6 (**Figure 1**). In contrast to the IDC, none of the 3 cases of ILC included in the study showed any evidence of reactivity for E-CD (**Figure 2**). In all evaluated ILCs, normal breast epithelium was present and showed preserved E-CD expression. Like ILCs, atypical medullary carcinoma and tubular carcinoma showed no E-CD reactivity. Medullary carcinoma showed 100% reactivity.

The immunoreactivity scores estimated in the breast carcinomas are shown in **Table 1**. There was a significant association between grade I - grade II breast carcinomas (p=0.013) and grade I - grade III breast carcinomas (p=0.005) when compared according to E-CD reactivity. The intensity of membranous E-CD expression was higher in grade I carcinomas than grade II and III carcinomas. In contrast, there was no statistically significant difference between grade II and grade III carcinomas (p=0.237) according to E-CD expression. Tumors associated with a peritumoral intraductal component were more often E-CD positive than others (53.0 ± 34.6; 38.7 ± 35.6). No correlation was observed when comparing E-CD expression with patient age, tumor size, necrosis, peritumoral lymphovascular invasion and lymph node status.

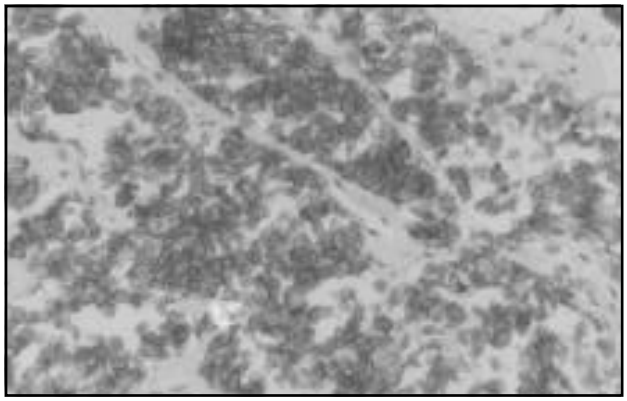

Figure 1 - Moderately differentiated infiltrating ductal carcinoma displaying membrane reactivity for E-cadherin on the majority of tumor cells (x 100).

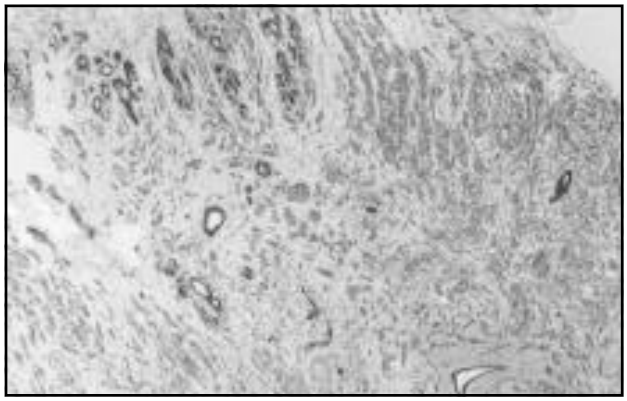

Figure 2 - No immunostaining with anti E-cadherin antibody in infiltrating lobular carcinoma. As internal control, normal tissue was immunostained (x 40).

Table 1 - E-cadherin (E-CD) expression compared with histological grade in infiltrating carcinoma of the breast (%).

Histological grade	**N = 51**	**E-CD expression**
I	17	64.2 ± 36.1
II	22	38.9 ± 34.9
III	12	30.2 ± 27.1

Analysis of variance test: p=0.013 between grade I–II breast carcinomas, p=0.005 between grade I–III breast carcinomas, p=0.237 between grade II-III carcinomas when compared according to E-CD reactivity.

Discussion. The cadherin are a family of molecules which have been named according to their distribution in various tissues as E for epithelial, P for placental, N for neural and R for retinal cadherin.[1] A survey of normal tissues has shown E-CD to be present on the cell surface in practically all epithelia where they are found scattered over the cell surface but are concentrated at zonula adherens intercellular junctions.[4,5] E-cadherin is a prominent factor in maintaining the epithelial architecture and consequently, loss of its normal function is thought to be a key element in cancer invasion. E-cadherin has been analyzed in a wide range of tumors to characterize clinical correlations potentially associated with a prognosis value. More specifically, in breast cancer, mutations may affect its expression, correlations have been reported with several morpho clinical parameters and the clinical course. The most marked correlation was observed with histological type. Association with other parameters remained uncertain.[6-9]

A number of studies have established an inverse correlation between E-CD expression and tumor differentiation.[10] In IDCs of the breast, the relationship of E-CD expression to clinicopathological parameters is inconsistent.[5] Oka et al[9] and Moll et al[10] found that reduced E-CD related to poor differentiation. The latter group also demonstrated a significant association between reduced E-CD staining and the presence of lymph node metastasis.[8-9] In contrast to this, Lipponen et al[11] examined 179 IDC and found no correlation with tumor grade or lymph node status. In our study, 45/45 (100%) of IDC showed some evidence of staining but while there was a trend towards grade I IDC restaining E-CD expression. Highly differentiated tumors generally maintained strong and homogenous E-CD expression, whereas in poorly differentiated carcinomas, E-CD positive and negative cells can be mixed. However, in contrast to the observation of Oka et al[9] and Jones et al,[6] no significant correlation was obtained between reduced membrane staining for E-CD and a positive lymph node status. Although, invasiveness and metastasis can be enhanced by the down regulation of E-CD expression, the alternation of other adhesion processes, such as cell substrate adhesion or other genetic events such as low 23 nm expression might be necessary for the invasiveness and metastasis development.[12-15]

In this study, while E-CD reactivity was maintained in 100% of IDC, none of the ILCs (n=3) showed evidence of membrane E-CD. These findings are in keeping with those of other workers and suggest distinct modes of invasion in these 2 cancer types. The malignant cells in lobular tumors of the breast typically have a pattern of infiltration as single cells. This dissociated appearance may

arise as a result of reduced intercellular adhesion mediated by E-cadherin.[1,6,9,11] Interestingly, a similar pattern of staining was seen in diffuse type gastric carcinomas, which are also characterized by lack of glandular differentiation and single, non-polarized cells infiltrating stroma.[16] Furthermore, mutations in the E-CD gene have been discovered in this form of gastric carcinoma.[7] In ILC of breast, loss of messenger ribonucleic acid (mRNA) for E-CD has been demonstrated.[17]

In conclusion, results in the present report suggest that E-CD expression in breast carcinoma is more related to histological type and differentiation grade than with metastatic potential, tumor size, tumor necrosis and patients' age. Thus, E-CD expression in human breast cancer appears to have minimal prognostic value, but may have a role as a phenotypic marker.

References

1. Rasbridge SA, Gillett SA, Sampson SA, Walsh FS, Millis RR. Epithelial (E-) and placental (P-) cadherin cell adhesion molecule expression in breast carcinoma. ***J Pathol*** 1993; 169: 245-250.
2. Soler AP, Knudsen KA, Salazar H, Han AC, Keshgegian AA. P-cadherin expression in breast carcinoma indicates poor survival. ***Cancer*** 1999; 86: 1263-1272.
3. Gonzales MA, Pinder SE, Wencyk PM, Bell JA, Elston CW, Nicholson RI, et al. An immunohistochemical examination of the expression of E-cadherin, and α− and β/γ− catenin, and α2- and β1 integrins in invasive breast cancer. ***J Pathol*** 1999; 187: 523-529.
4. Shimoyama Y, Hirohashi S, Hirano S, Noguchi M, Shimosato Y, Takeichi M, et al. Cadherin cell adhesion molecules in human epithelial tissues and carcinoma. ***Cancer Res*** 1989; 49: 2128-2133.
5. Gamallo C, Palacios J, Suarez A, Pizarro A, Navarro P, Quintanilla M, Cano A. Correlation of E-cadherin expression with differentiation grade and histological type in breast carcinoma. ***Am J Pathol*** 1993; 142: 987-993.
6. Jones JL, Royall JE, Walker RA. E-cadherin relates to EGFR expression and lymph node metastasis in primary breast carcinoma. ***Br J Cancer*** 1996; 74: 1237-1241.
7. Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, et al. E- Cadherin gene mutation s provide clues to diffuse type gastric carcinomas. ***Cancer Res*** 1994; 554: 3845-3852.
8. Oka H, Shiozaki H, Kobayashi K, Inove M, Tahara H, Kobayashi T, et al. Expression of E-cadherin cell adhesion molecules in human breast cancer tissues and its relationship to metastasis. ***Cancer Res*** 1993; 53: 1696-1701.
9. Moll R, Mitze M, Frixen UH, Birchmeier W. Differential loss of E-cadherin in infiltrating ductal and lobular carcinoma. ***Am J Pathol*** 1993; 143: 1731-1742.
10. Kinsella AR, Green B, Lepts GC, Hill CL, Bowie G, Taylor BA. The role of cell adhesion molecule E-cadherin in large bowel tumour cell invasion and metastasis. ***Br J Cancer*** 1993; 67: 904-909.
11. Lipponen P, Saarelainen E, Ji H, Aaltoma S, Syrjanen K. Expression of E- cadherin (E-CD) as related to other prognostic factors and survival in breast carcinoma. ***J Pathol*** 1994; 174: 101-109.
12. Shiozaki H, Tahara H, Oka H, Miyata M, Kobayashi K, Tamura S, et al. Expression of immunoreactive E-cadherin adhesion molecules in human cancers. ***Am J Pathol*** 1991; 139: 17-23.
13. Zutter MM, Mazoujian G, Santoro S. Decreased expression of integrin adhesive protein receptors in adenocarcinoma of the breast. ***Am J Pathol*** 1990; 137: 862-870.
14. Barnes R, Masood S, Barker E, Rosengad AM, Cognin DL, Crowell T, et al. Low 23 nm protein expression in infiltrating ductal breast carcinomas correlates with reduced patient survival. ***Am J Pathol*** 1991; 139: 245-250.
15. Hennessy C, Henry JA, May FEB, Westly BR, Angus B, Lennard TW. Expression of the anti metastatic gene 23 nm in human breast cancer: an association with good prognosis. ***J Natl Cancer Inst*** 1991; 83: 281-285.
16. Mayer B, Johnson JP, Leitl F, Jaunch KW, Heiss MM, Schildberg FW, et al. E-cadherin expression in primary and metastatic gastric cancer: down regulation correlates with cellular dedifferentiation and glandular disintegration. ***Cancer Res*** 1993; 53: 1690-1695.
17. Rebridge SA, Poulsom R, Millis RR. In-situ hybridization for E-cadherin in breast carcinoma. ***J Pathol*** 1995; 175: 142A.