RESEARCH COMMUNICATION

E-Cadherin Expression Correlates With Histologic Type But Not Tumour Grade in Invasive Breast Cancer

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Abstract

The traditional classification of infiltrating breast carcinomas into ductal and lobular can be diagnostically challenging in a small proportion of cases with equivocal histological features and in *in-situ* lesions with overlapping features. Distinguishing between the infiltrating ductal (IDC) and lobular (ILC) carcinomas is clinically important because of the different pattern of systemic metastases and prognostic evaluation. E-cadherin is a potentially useful immunohistochemical marker which may serve to differentiate between the two tumour types. We therefore studied E-cadherin expression in 32 cases of breast carcinomas comprising 16 IDCs and 16 ILCs. The correlation between E-cadherin expression and the histological grade of IDCs was also analysed. Our results showed complete loss of E-cadherin expression in all ILCs, while the IDCs consistently showed variable E-cadherin positivity. No significant correlation was found between E-cadherin expression and the histological grade of IDCs. We conclude from this study that E-cadherin is a useful marker to differentiate between IDC and ILC of the breast. A larger study of IDCs is now needed to further evaluate the correlation between E-cadherin and tumour grade to estimate its prognostic potential.

Key Words: E-cadherin - infiltrating ductal carcinoma - infiltrating lobular carcinoma

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Introduction

Traditionally, breast cancer is classified as infiltrating ductal (IDC) and lobular carcinoma (ILC) based on the recognition of characteristic histopathological features. In the majority of breast cancer cases, distinguishing between the two can be achieved easily if the classical histological features of both types are readily observed (Tavassoli and Devilee, 2003). However, in poorly differentiated carcinomas with equivocal histological features and the pleomorphic variant of lobular carcinoma, the diagnosis can be challenging (Acs et al., 2001; Qureshi et al., 2006). Similar diagnostic problems may be encountered in in-situ carcinomas of the breast (Acs et al., 2001; Maluf et al., 2001; Wahed et al., 2002). Low grade ductal carcinoma in-situ with a solid growth pattern involving the terminal ducts and lobules may resemble lobular carcinoma in-situ. Large areas of comedo-type necrosis can be seen in lesions with typical lobular cytology, hence are difficult to distinguish between ductal carcinoma-in-situ (Acs et al., 2001).

Clinically, the division between ductal and lobular carcinomas has its practical implications (Lehr et al., 2000). Infiltrating lobular carcinomas often do not form definite masses that can be diagnosed by palpation or mammography making early diagnosis difficult. The occurrence of tumour at resection margins, bilaterality,

and the rate of recurrence are higher in lobular carcinomas compared to ductal carcinomas due to its distinctive growth pattern (Lehr et al., 2000; Yoder et al., 2007). In addition, lobular carcinomas have a predilection to metastasise to leptomeninges and peritoneal surface, a feature rarely seen in ductal carcinomas (Lehr et al., 2000; Goldstein et al., 2002; Yoder et al., 2007). Overall, ILC is associated with better prognosis compared with IDC (Yoder et al., 2007). Hence, there is a need for a biomarker which can reliably differentiate the two tumour types.

E-cadherin is one of the biomarkers which have potential to serve as an adjunct to aid pathologists in these problems. E-cadherin is a transmembranous glycoprotein that participates in calcium-dependent intercellular adhesion (Takeichi, 1991; Maluf et al., 2001; Kowalski et al., 2003; Yoder et al., 2007). It has an important function in embryonic development and it is postulated that abnormal E-cadherin function or expression in carcinomas facilitates the detachment process that mediate metastasis (Takeichi, 1991; Acs et al., 2001; Maluf et al., 2001). Normal breast ductal epithelial cells strongly express E-cadherin protein in the cytoplasmic membrane. Many studies have shown reduced expression of E-cadherin in about 50% of breast carcinomas while other studies have shown complete loss of E-cadherin expression in infiltrating lobular carcinomas (Gamallo et al., 1993; Moll et al., 1993; De Lew et al., 1997; Qureshi

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et al., 2006).

The objective of this study was to analyse correlations of E-cadherin expression with histological type and grade in infiltrating ductal carcinomas, and to further evaluate E-cadherin as a potential differentiating immuno-histochemical marker for IDC and ILC.

Materials and Methods

Materials

We performed a retrospective study to determine the expression of E-cadherin in newly diagnosed infiltrating ductal carcinomas (IDC) and infiltrating lobular carcinomas (ILC) in Hospital Kuala Lumpur (HKL) from the year 2002 until 2006. Thirty two breast cancer cases comprising 16 IDC and 16 ILC were retrieved from the files of the Department of Pathology, HKL. All histopathological slides of the cases were reviewed by two independent pathologists and graded histologically according to the modified Bloom and Richardson Grading System. Discrepancies in histological grading were resolved by consensus with simultaneous viewing. The most representative slides and their corresponding paraffin blocks were selected for immunohistochemical staining.

Immunohistochemical Staining

Immunohistochemical staining was performed on formalin-fixed paraffin-embedded tissue (PET) sections. Three-four micrometer thick sections were cut and deparaffinised in xylene and rehydrated in alcohols in decreasing concentrations. The sections were placed in target retrieval solution at 100˚C for 20 minutes and then left to cool at room temperature for 20 minutes. Endogenous peroxidise activity was blocked by 3% hydrogen peroxide for 5-10 minutes and washed with water. They were then placed in Tris-Buffer Solution (TBS) for 3 changes at 3 minutes each and the excess TBS was dabbed off. A drop of primary monoclonal mouse anti-human E-cadherin antibody, clone NCH-38 in optimised dilution (1:50) was added and incubated at room temperature for 30 minutes. The slides were subsequently washed with 3 changes of TBS at 3 minutes each. A drop of di-amino-benzidine (DAB) was then spread over the sections for 7 minutes, after which they were rinsed in water. The sections were counterstained with Haematoxylin for 20-30 minutes, dehydrated in increasing concentrations of alcohol, and mounted.

Histological Grading of the Infiltrating Ductal Carcinomas

Histological grading was performed on formalin-fixed PET sections of infiltrating ductal carcinoma cases according to the Modified Bloom and Richardson's Grading System. The combined histological grade was obtained using the scale 1-3 assigned to three features; percentage of tubular formation, degree of nuclear pleomorphism and number of mitotic figures.

Evaluation of Immunohistochemical Staining

A semi-quantitative estimation was made on the staining intensity and the relative abundance of E-cadherin immunoreactive cells. E-cadherin expression was positive when cells demonstrated linear membranous staining. The staining intensity was graded from 0 - +3 (0 for background staining of acellular stroma, +1 for focal sparse intercellular staining, +2 for markedly reduced and heterogenous staining with predominantly finely-dotted intercellular staining pattern or continuous linear staining if present, was restricted to less than 50% and +3 for strong, continuous and intense staining equivalent to the normal breast epithelium). Normal breast epithelial cells were used as internal positive controls. The abundance of E-cadherin positive cells was graded from 0-4 by counting at least 100 tumour cells in areas of heterogenous E-cadherin expression (0=less than 5% positive cells; 1=5-25%; 2=26-50%; 3=51-75% and 4=76-100%). To assess the preservation of E-cadherin expression, a composite score was obtained by adding the values of the immunoreactions intensity and relative abundance. The E-cadherin expression was preserved when the composite score was 6-7 and scores 0-5 indicated reduced E-cadherin expression. Immunostains were evaluated independently by two pathologists and any differences in interpretation were resolved by simultaneous viewing.

Data were analysed using SPSS program version 12.0.1. Chi-square test was used for statistical analysis. The statistical significance was set at $p<0.05$.

Results

Our results showed all 16 IDCs (100%) were immunopositive for E-cadherin but with variable degrees of expression; 9 (56.3%) showed preserved E-cadherin expression (Figures 1 and 2) while in the remaining 7 (43.8%) E-cadherin expression was reduced. All 16 (100%) ILCs showed complete loss of E-cadherin expression (Figure 3). There was a statistically significant correlation between E-cadherin expression and histological type (Fischer's exact test; p value = 0.01) as shown in Table 1.

Of the 9 IDCs with preserved E-cadherin expression, 4 (44.4%) were grade III and 5 (55.6%) were grades I and II collectively. Table 2 depicts the results of E-cadherin expression with histological grades of IDCs. There was no significant link.

Discussion

The classification of breast carcinomas is currently

Table 1. Cases of IDCs and ILCs with Preserved or Reduced E-cadherin Expression

Expression	IDCs	ILCs	P value
Preserved	9 (56%)	0 (0.0%)	0.01
Reduced	7 (44%)	16 (100%)	

Table 2. The Relationship between E-cadherin Expression and Histological Grade of IDCs

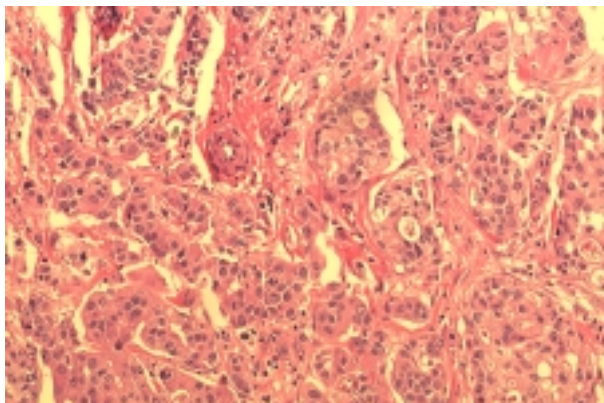| Expression	Grades I and II	Grade III	P value
Preserved	5 (71.4%)	4 (44.4%)	0.358
Reduced	2 (28.6%)	5 (71.4%)	

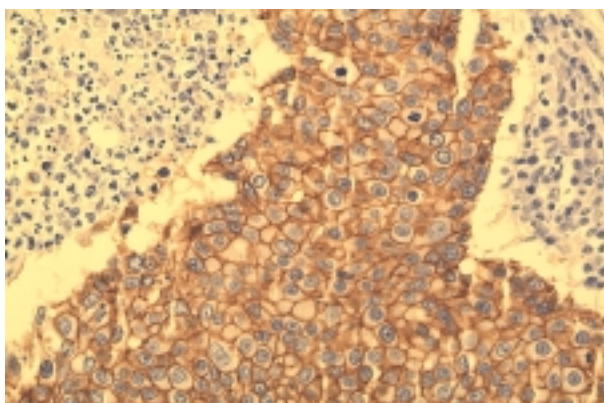**Figure 1. Infiltrating Ductal Carcinoma of the Breast, Grade 3: H&E x 20**

Figure 2. Preserved E-cadherin Expression on Infiltrating Ductal Carcinoma Cells as Strong Diffuse Membranous Staining: X 20
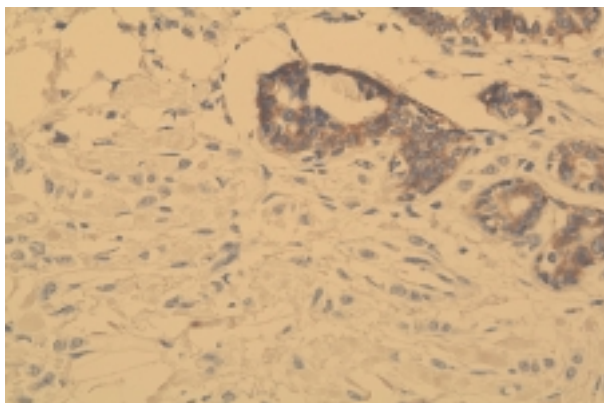

Figure 3. Complete Loss of E-cadherin Expression in an Infiltrating Lobular Carcinoma. Adjacent Normal Breast Epithelial Cells Serve as an Internal Control Positive for E-cadherin: X 20

based purely on the recognition of specific histological features on microscopic examination. Infiltrating ductal carcinomas which comprised the majority (75-80%) of breast carcinomas are histologically characterised by the formation of tubules, or ducts that infiltrate throughout the breast parenchyma. Approximately 20% of IDC can be further subcategorised based on unique histopathological features into medullary, tubular, papillary, mucinous, micropapillary and metaplastic. The majority (80%) of IDC are not subclassifiable and are designated as ductal carcinomas of 'No Special Type' (NST) or 'Not Otherwise Specified'(NOS). Classic ILC

comprises 10-15% of all breast carcinomas. Histologically, it is composed of small, uniform cells invading the breast parenchyma in a linear, single file pattern or concentrically around benign ducts in a target-like arrangement. It has ill-defined margins and does not form microcalcifications. Although well-defined histological features have served to classify the majority of tumours, a definite histological classification into ductal or lobular types proved to be difficult in some cases due to overlapping features, ductal and lobular growth patterns within the same tumour or the presence of equivocal histological features. In this study, we have analysed the expression of E-cadherin in IDC and ILC to study its utility as a biomarker to help distinguish between the two tumour types. Our results showed complete loss of E-cadherin immunoreactivity in all 16 (100%) ILCs while all IDCs retained at least some expression of E-cadherin. Similar results were previously reported by other authors(Acs et al., 2001; Qureshi et al., 2006; Lehr et al., 2000; Moll et al., 1993; Gamallo et al., 1993).

Some studies have attributed the loss of E-cadherin expression in lobular carcinomas to mutation of the E-cadherin gene (Jacobs et al., 2001). The gene for E-cadherin, CDH1, maps to chromosome 16q22. Loss of cellular adhesion is a critical step in tumour progression and metastasis (Yoder et al., 2007; Bashyam, 2002; Chamber et al., 2002). Most IDC tumours maintain some degree of cell adhesion as evidenced in histological differentiation (Yoder et al., 2007). In contrast, ILC tumours are very dishesive in their histological appearance and have single cell morphology. Therefore, the loss of E-cadherin expression reflects the histological appearance of ILC. Loss of adhesion also probably explains the tendency of ILC to metastasise to more remote locations such as the leptomeninges and peritoneum (Yoder et al., 2007). As demonstrated in previous series and our study, it seems reasonable to utilise E-cadherin as an adjunct diagnostic tool to classify breast carcinomas in difficult cases (Acs et al., 2001; Qureshi et al., 2006; Lehr et al., 2000; Moll e al., 1993; Gamallo et al., 1993).

The different immunostaining patterns observed in IDC and ILC cases in our study also suggest that ductal and lobular carcinomas develop through different genetic pathways. Molecular studies on E-cadherin gene will be helpful to further understand the tumourigenesis of these tumours and design genetic-based treatment in future.We observed variable quantitative E-cadherin expression in all the IDCs in this study.

Interestingly, we did not find significant correlation between E-cadherin expression and histologic grade for the IDCs in this study which is similar to that of Acs et al (2001) and Lipponen et al (1994). However, this contrasted with the findings of other authors who reported reduced and heterogenous E-cadherin immunostaining in poorly-differentiated IDCs while preservation of E-cadherin expression in well and moderately differentiated carcinomas (Moll et al., 1993; Gamallo et al., 1993; Oka et al., 1993). This was probably due to the small number of cases included in this study. Further studies on E-cadherin expression of IDCs comprising more cases representing each tumour grade would be beneficial to

evaluate the role of E-cadherin in predicting tumour aggressiveness. Future studies on E-cadherin can be done to explore the possibility of extending its utility on differentiating in-situ lesions of ductal or lobular phenotype as the clinical management of these two lesions are different.

Our results confirmed the strong correlation between histologic type of breast carcinoma with E-cadherin expression by the tumour cells. In tumours that show equivocal histological features, immunohistochemical detection of E-cadherin expression can be used as a diagnostic tool to differentiate between ductal and lobular carcinomas. Tumours that show complete absence of E-cadherin membrane staining most likely represent lobular carcinomas while most ductal carcinomas will demonstrate diffuse membrane expression of E-cadherin.

Acknowledgements

References

Acs G, Lawton TJ, Rebbeck TR, Livolsi VA, Zhang PJ (2001). Differential expression of E-cadherin in lobular and ductal neoplasm of the breast and its biologic and diagnostic implication. *Am J Clin Pathol*, **115**, 85-98.

Bashyam MD (2002). Understanding cancer metastases. *Cancer*, **94**, 1821-9.

Chamber AF,Groom AC, MacDonald IC (2002). Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer*, **2**, 563-72.

De Leeuw WJF, Berx G, Vos CBJ, et al (1997). Simultaneous loss of E-cadherin and Catenins in invasive lobular breast cancer and lobular carcinoma in situ. *J Pathol*, **183**, 404-11.

Gamallo C, Palacios J, Suarez A, et al (1993). Correlation of E-cadherin expression with differentiation grade and histologic type in breast carcinoma. *Am J Pathol*, **142**, 987-93.

Goldstein NS (2002). Does the level of E-cadherin expression correlate with the primary breast carcinoma infiltration pattern and type of systemic metastases? *Am J Clin Pathol*, **118**, 425-34.

Jacobs TW, Pliss N, Kouria G, Schnitt SJ (2001). Carcinoma in situ of the breast with indeterminate features. Role of E-cadherin staining in categorisation. *Am J Surg Pathol*, **25**, 229-36.

Kowalski PJ, Rubin MA, Kleer CG (2003). E-cadherin expression in primary carcinomas of the breast and its distant metastases. *Breast Cancer Res*, **5**, R217-R222.

Lehr HA, Folpe A, Yaziji H, Kommoss F, Gown AM (2000). Cytokeratin 8 immunostaining pattern and E-cadherin expression distinguish lobular from ductal breast carcinoma. *Am J Clin Pathol*, **114**, 190-6.

Lipponen P, Saarelainen E, Ji H, et al (1994). Expression of E-cadherin(E-CD) as related to other prognostic factors and survival in breast cancer. *J Pathol*, **174**, 101-9.

Maluf HM, Sanson PE, Koerner FC (2001). Solid low-grade in situ carcinoma of the breast. Role of associated lesions and E-cadherin in differential diagnosis. *Am J Surg Pathol*, **25**, 237-44.

Moll R, Mitze M, Frixen UH, Birchmeier W (1993). Differential loss of E-cadherin expression in infiltrating ductal carcinoma and lobular breast carcinomas. *Am J Pathol*, **143**, 1731-41.

Oka H, Shiozaki H, Kobayashi K, et al (1993). Expression of E-cadherin cell adhesion molecules in human breast cancer tissue and its relationship to metastasis. *Cancer Res*, **53**, 1696-701.

Qureshi HS, Linden MD, Divine G, Raju UB (2006). E-cadherin status in breast cancer correlates with histologic type but does not correlate with established prognostic parameters. *Am J Clin Pathol*, **125**, 377-85.

Takeichi M (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science*, **251**, 1451-5.

Tavassoli FA and Devilee P (Eds) (2003). World Health Organisation classification of tumours. Pathology and genetics tumours of the breast and female genital organs. IARC Press: Lyon, France.

Wahed A, Conelly J, Reese T (2002). E-cadherin expression in pleomorphic lobular carcinoma: an aid to differentiation from ductal carcinoma. *Ann Diagn Pathol*, **6**, 349-51.

Yoder BJ, Wilkinson EJ, Masol NA (2007). Molecular and morphologic distinctions between infiltrating ductal and lobular carcinoma of the breast. *Breast J*, **13**, 172-9.