JKAU: Med. Sci., **Vol. 18** No. 4, pp: 17-28 (2011 A.D. / 1432 A.H.)
DOI: 10.4197/Med. 18-4.2

Differential Expression of E-Cadherin and High Molecular Weight Cytokeratin in Invasive Lobular and Ductal Breast Carcinoma

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Abstract. This study included 62 breast biopsy cases; 39 invasive breast carcinoma, 11 non-invasive carcinoma cases and 12 benign breast lesions. Immunohistochemical cases revealed; ductal and mucinous carcinoma, epithelial component in metaplastic carcinoma, and benign breast lesions were positive to E-cadherin. All lobular carcinomas, sarcomatoid component of metaplastic carcinoma and neuroendocrine carcinoma were totally negative to E-cadherin. However, 45.5% of invasive ductal carcinoma, all lobular carcinomas, plus all benign breast lesions showed positive reaction to high molecular weight cytokeratin. Cases of ductal carcinoma *in situ* were all negative to high molecular weight cytokeratin. Previous results concluded that immunohistochemical study of E-cadherin expression was recommended as a tool to differentiate between challenging cases of lobular and ductal carcinoma lesions as it has no role in differentiating ductal hyperplastic cases from ductal carcinoma *in situ*. In contrast, high molecular weight cytokeratin has no role in discriminating lobular from ductal carcinoma, thus it can differentiate between ductal hyperplasia and ductal carcinoma *in situ*.

Keywords: E-cadherin, High molecular weight cytokeratin, ductal carcinoma, lobular carcinoma, immunohistochemistry.

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Accepted for publication: 18 June 2011. Received: 05 October 2010.

Introduction

Invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) are the most common malignancies of the breast, accountable for 80% and 15% of all invasive breast tumors, respectively[1,2]. Both tumor subtypes are distinguished on the basis of their histology, with ductal tumors tending to form glandular structures, whereas lobular tumors are less cohesive and tend to invade in a single file.

The distinction of ductal and lobular types of invasive mammary carcinoma is clinically important; lobular tumors often grow slowly than ductal tumors, and are more often estrogen and progesterone receptor positive. Plus, they have a lower vascular endothelial growth factor expression, different metastatic patterns and are less response to neoadjuvant therapy[3-9].

The differentiation between these two tumor entities may present a challenge in breast core needle biopsies, and poorly differentiated carcinomas showing equivocal histological features with a diffuse infiltrating pattern as well as in the pleomorphic variant of invasive lobular carcinoma. A similar diagnostic challenge also occur *in situ* carcinomas of the breast. Although, some *in situ* carcinomas composed of distinct lobular and ductal components have been categorized as truly mixed lesions, other tumors cannot be classified owing to the presence of equivocal histological features. Lobular carcinoma *in situ* (LCIS) may be mimicked by low-grade ductal carcinoma *in situ* (DCIS) with a solid growth pattern involving terminal ducts and lobules[1,2,10].

E-cadherin is a member of a family of transmembrane glycoproteins responsible for the Ca2+-dependent cell-cell adhesion mechanism, and has been demonstrated to be involved in organogenesis and morphogenesis[11-13]. In epithelial cells, E-cadherin is considered one of the key molecules for the formation of the intercellular junctional complex and for the establishment of cell polarization[13]. Expression of reduced numbers of functionally active E-cadherin molecules has been observed in aggressive tumors of the esophagus, ovary, and stomach[14-16]. Moreover, a decreased expression of E-cadherin is thought to be associated with invasiveness of the tumor cells. Previous investigators studied its value in discriminating subtypes of mammary carcinoma[17]. Additionally, many studies suggest the usefulness of cytokeratin in

differentiating histological subtypes of breast carcinomas, whether invasive or non-invasive[18].

The aim of the present study is to examine the pattern of E-cadherin and high molecular weight cytokeratin expression in a series of ductal and lobular carcinomas of the breast in lumpectomy, and mastectomy specimens as to evaluate their role in the classification of carcinomas with equivocal features.

Materials and Methods

Fifty retrospective breast biopsy specimens containing mammary carcinoma, and 12 non-malignant biopsies from the surgical pathology files of the Department of Pathology, King Abdulaziz University Hospital, Jeddah were selected.

Hematoxylin & Eosin (H&E) stained slides were reviewed to establish a diagnosis with histological type and tumor grade based on an established criteria[1-3]. All invasive carcinomas were graded using the modified combined histological grading system as described by Bloom and Richardson[19]. After the initial histological examination, cases were classified into invasive ductal carcinoma (IDC; n = 22), ductal carcinoma *in situ* (DCIS; n = 7), invasive lobular carcinoma (ILC; n = 10), lobular carcinoma *in situ* (LCIS; n = 4), mucoid carcinomas (n = 3), metaplastic carcinoma with sarcomatoid features (n = 3), and small cell neuroendocrine carcinoma (n = 1). Additional 6 cases with fibrocystic changes and 6 fibroadenoma were also evaluated.

Immunohistochemical assay was performed on formalin-fixed paraffin-embedded sections and an automated immunostainer (Ventana Medical Systems, Inc., Tucson, AZ USA) as described in manufacturer manual. Five-micrometer thick sections were cut and deparaffinized in xylene and rehydrated in graded alcohols. Slides were steamed in a 0.01-mol/L concentration of sodium citrate buffer (pH 6.0) for 20 min. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 20 min. After blocking with 1.5% normal horse serum in automation, buffer slides were incubated with the monoclonal antibodies against E-cadherin (1:200 dilution), and a high molecular weight cytokeratin (HMWCK) (1:100 dilution) for 1 hr. Slides then were incubated for 30 min at 37°C with secondary antibody followed by Detection System, and counterstained with hematoxylin. Negative

control sections were made by exclusion of the primary antibody. Positive control sections were obtained from sections of normal breast tissue for E-cadherin and skin for HMWCK. Positive histological reaction for E-cadherin antibodies used was visualized as cell membrane brown linear staining; whereas, for high molecular weight cytokeratin positivity appeared as cytoplasmic and cell membrane brown stain. A semi quantitative scoring system was used to score immunohistochemical positivity. The intensity of immunohistochemical staining was graded on a scale of 0 to 3 (0 = no staining, 1 = equivocal, weak intensity, 2 = unequivocal, moderate intensity, and 3 = unequivocal, high intensity). Positive immunohistochemical staining was defined as unequivocal staining of at least 50% of the neoplastic cells.

Results

Invasive ductal carcinomas were histologically graded as GI; 3 cases, GII; 16 cases, and GIII; 3 cases. All grade I and II cases showed strong positive immunostaining (score+3) to the E-cadherin visualized in the cell membrane of tumor cells, whereas staining intensity and score was relatively reduced in high grade tumors (score+1-2) (Fig. 1a). All cases of DCIS showed a strong E-cadherin staining (Fig. 2a).

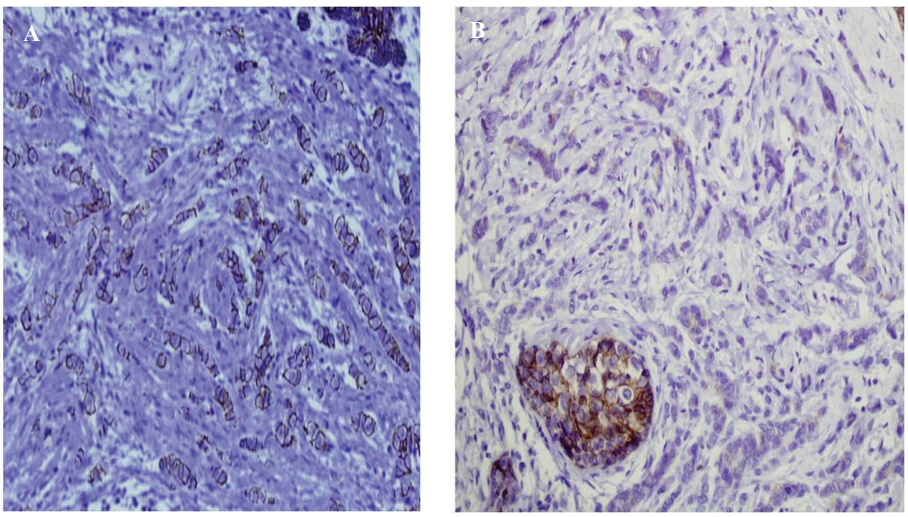

Fig. 1. Immunohistochemical staining for E–cadherin: A) Poor differentiated ductal carcinoma showing cord-like arrangement of malignant cells. Note, a moderate intensity staining of E-cadherin (ABC, X 400). B) Invasive lobular carcinoma totally negative to E-cadherin. Note, a positive staining ductal structures (ABC, X 400).

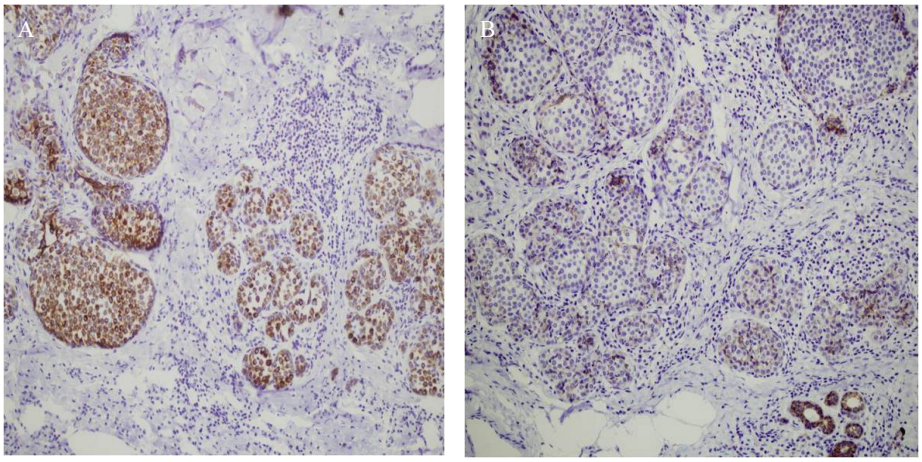

Fig. 2. Immunohistochemical staining for E–cadherin: A) DCIS showing solid pattern of non-invasive component strongly positive to E-cadherin (ABC, X 200). B) LCIS totally negative to E-cadherin. Note, positive staining ductal structures (ABC, X 200).

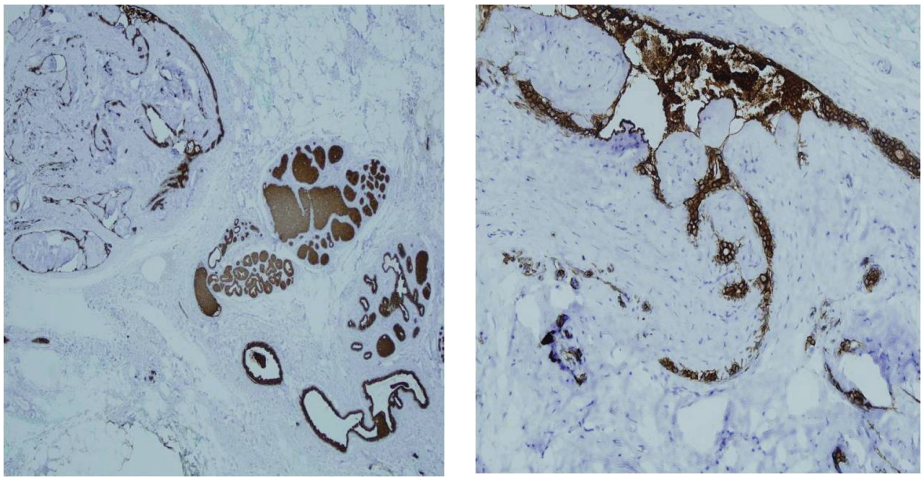

Fig. 3. Positive Immunohistochemical staining for E–cadherin in benign breast lesions (ABC, X 100).

Similarly, cases of mucinous, the epithelial component in metaplastic carcinoma as well as fibroadenoma cases and fibrocystic diseases, showed a strong positive cell membrane staining to E-cadherin (Fig. 3).

On the other hand, invasive lobular carcinomas [GII; 9 cases and GIII 1 case] and lobular carcinoma in situ were all negative to E-

cadherin. (Fig. 1b, and 2b, respectively). Sarcomatoid areas in metaplastic carcinomas and neuroendocrine carcinoma were also negative to E-cadherin.

High molecular weight cytokeratin showed positive cytoplasmic staining in 10 cases (45.5%) of IDC, while none of DCIS, mucinous carcinoma, neuroendocrine carcinoma or epithelial component in metaplastic carcinoma showed immunopositivity. Thus, all ILC, LCIS, fibroadenoma and hyperplastic ductal lesions (100%) showed strong positive HMWCK (Fig. 4).

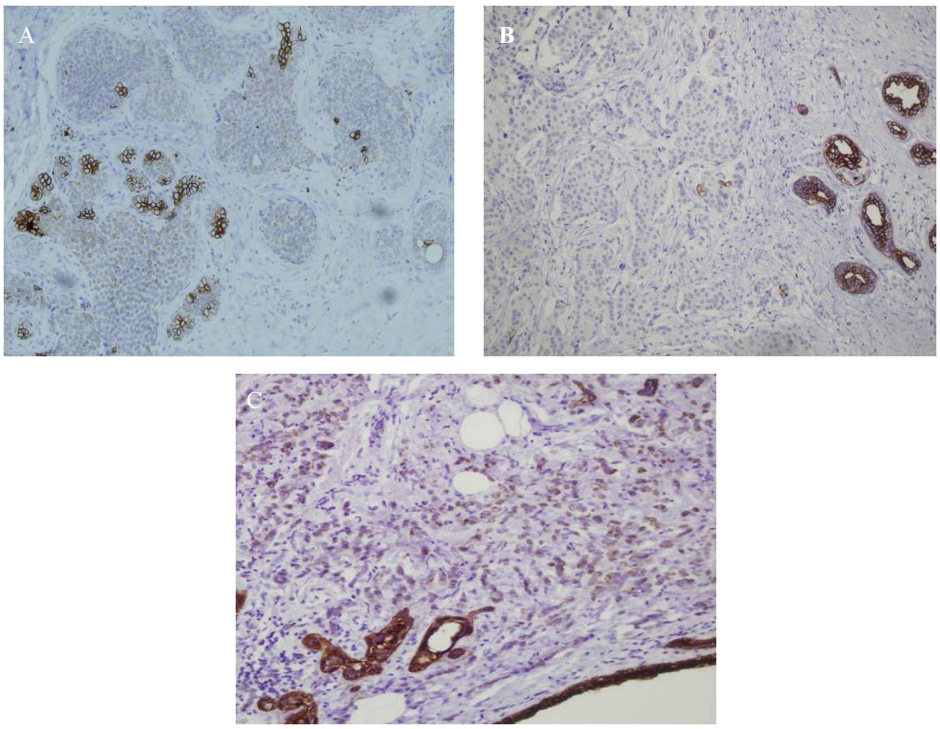

Fig. 4. Immunohistochemical staining of HMWCK*:* A) Negative staining in DCIS (ABC, X 100), B) Negative staining in IDC (ABC, X 100), and C) Positive staining in ILC (ABC, X 100).

Discussion

In the present series, the expression of E-cadherin in 62 breast lesions, including benign, *in situ* and invasive mammary carcinomas were analyzed. It was found that E-cadherin positivity was prominent

feature of ductal carcinomas (both invasive and non-invasive). However, it was negative in the lobular carcinomas.

All cases of DCIS and IDC cases showed moderate to strong membrane expression of E-cadherin, as seen in the non-neoplastic mammary epithelium including fibrocystic changes, and fibroadenoma. In contrast, membrane expression of E-cadherin was not identified in ILCs or LCIS. Previous studies have shown a reduced expression of E-cadherin in approximately 50% of mammary carcinomas in association with high histological grade, nodal metastases, and the loss of estrogen and progesterone receptors[20-26]. However, not all studies confirmed these findings. Geza *et al.*[27] reported that 2% of 42 ILCs showed a moderate reactivity to E-cadherin and 6% of cases of LCIS showed weak and focal reactivity. Hence, the only E-cadherin–positive ILC case was diagnosed histologically as pleomorphic lobular carcinoma and was associated with an intermediate-grade solid DCIS component. He suggested that because of the well-known difficulty of differentiating the pleomorphic variant of lobular carcinoma from ductal carcinoma with a dispersed infiltrating pattern, this tumor likely represents an example of the latter.

In concordance of our results, other studies observed that invasive lobular carcinoma (ILC) and LCIS, showed complete loss of expression of E-cadherin[20,21,28]. These studies showed that E-cadherin gene mutations and loss of the wild-type allele by loss of heterozygosity is the predominant mechanism. Thus, E-cadherin protein expression frequently is lost in the lobular carcinoma, indicating that E-cadherin acts as a classic tumor suppressor gene[29-31]. E-cadherin germline mutations in gastric and lobular breast carcinoma were reported recently, suggesting the importance of E-cadherin mutations in tumorigenesis. Hence, there is emerging evidence that E-cadherin is associated specifically with the lobular phenotype of breast carcinoma Furthermore, E-cadherin inactivation might have a crucial role in the dispersed and discohesive growth pattern in LCIS and ILC[28,29]. However, the practicality of using E-cadherin expression to differentiate between ductal and lobular carcinomas in a large series, including cases with equivocal features, has not been evaluated.

Some studies in breast cancer have demonstrated that aberrant E-cadherin expression is associated with high-grade, estrogen receptor

(ER)-negative, and metastatic breast carcinomas. However, other studies have failed to confirm any correlation between E-cadherin membrane expression and tumor size, grade, tubule formation, nuclear pleomorphism, mitotic activity, estrogen and progesterone receptor status, and CerbB-2 over expression in IDCs[27,32]. Contradictory finding among different research works denotes that no consistent correlation of E-cadherin staining emerged with any of clinicopathological features of ductal carcinomas.

As regards to HMWCK, no specific differential value between ductal and lobular carcinoma has been obtained in our series. In contradictory to previous reports, which concluded that HMWCK was mainly positive in lobular carcinoma while it was mostly negative in ductal carcinoma[18,33]. The main diagnostic value of HMWCK was between breast masses showing hyperplastic changes and ductal carcinoma in situ, and between DCIS and invasive ductal carcinoma[34,35].

Conclusion

From the present results, we conclude that immunohistochemical study of E-cadherin expression is recommended as a tool to differentiate between challenging cases of lobular and ductal carcinoma lesions, both non-invasive and invasive. Additionally, it has no role in differentiating ductal hyperplastic cases from ductal carcinoma in situ. In contrast, high molecular weight cytokeratin that has no role in discriminating lobular from ductal carcinoma, while it can differentiate between ductal hyperplasia and ductal carcinoma in situ.

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دراسة الظهور المتباين ل أ–كادهرين و سيتوكيراتين ذو الوزن الجزيئي العالي في حالات السرطان القنوى الجائر و حالات السرطان الفصيصى بالثدى

علي صادق صوان

قسم علم الأمراض، كلية الطب،

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جدة – المملكة العربية السعودية

المستخلص. اشتملت هذه الدراسة الاستعادية الأرشيفية على ٦٢ حالة من سرطانات الثدي، ٣٩ منها سرطانات من نوع السرطان الجائر، ١١ حالة من نوع السرطان غير الجائر، ١٢حالة من أورام الثدي الحميدة. حالات سرطان الثدي القنوي (من النوع الجائر وغير الجائر) وكذلك حالات السرطان المخاطيني، وكذلك الجزء الظهاري من الأورام المتغيرة الجبلة، والأورام الحميدة بالثدي كانت كلها موجبة الصبغة مع (إ– كادهرين)، أما حالات سرطان الثدي الفصيصي (الجائر وغير الجائر) والجزء نظيرالساركوما من الورم متغير الجبلة، كانت كلها سالبة الصبغة مع (إ– كادهرين). ومن ناحية أخرى كانت ٤٥,٥٪ من حالات السرطان القنوي، وكل حالات السرطان الفصيصي، وكذلك حالات الأورام الحميدة كانت موجبة الصبغة من (سيتوكيراتين عالي الوزن الجزيئي) ولكن حالات السرطان القنوي غير الجائر كانت كلها سلبية الصبغة مع (سيتوكيراتين عالي الوزن الجزيئي). من النتائج السابقة وجدنا أن تقنية الصبغة الكيميائية المناعية باستخدام (إ– كادهرين) من الممكن استخدامها للتمييز بين

حالات السرطان القنوي والسرطان الفصيصي الجائر وغير الجائر بالثدي، ولكنها غير مفيدة للتفريق بين حالات السرطان القنوي غير الجائر وحالة فرط النسيج القنوي. وعلى العكس من ذلك كان استخدام (سيتوكيراتين عالى الوزن الجزيئي) لم يكن ذو فائدة في التمييز بين السرطان القنوي والسرطان الفصيصي، ولكن ذو فائدة في التفريق بين حالات السرطان القنوي غير الجائر وحالات فرط النسيج القنوي.