E-cadherin expression in estrogen receptor-positive and negative breast carcinomas of postmenopausal women

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Summary

Background: Preservation of E-cadherin expression is usually related to non-invasive and well differentiated breast carcinomas. *Purpose:* The aim of this study was to evaluate E-cadherin immunohistochemical expression in estrogen receptor (ER) positive and negative infiltrating ductal breast carcinomas. *Methods:* Twenty-three postmenopausal patients with Stage II, operable, infiltrating ductal breast carcinomas were divided into groups A (ER+; n = 13) and B (ER-; n = 10). E-cadherin immunohistochemical expression was assessed semiquantitatively according to membrane staining intensity and classified as negative (< 10% of cells with stained membranes), positive + (10-50% of cells stained) or positive ++ (> 50% of cells stained). Fisher's exact test was used to compare the distribution of staining intensity in the two groups ($p < 0.05$). *Results:* In group A (ER+), E-cadherin staining was positive in all cases: + (n = 3; 23%) and ++ (n = 10; 77%) compared to three cases (30%) in group B (ER-), + (n = 2; 20%) and ++ (n = 1; 10%). This difference was statistically significant ($p < 0.0005$). *Conclusions:* The present results indicate that E-cadherin expression loss is significantly associated with ER-negative tumors and therefore with a more aggressive phenotype of invasive ductal breast carcinoma.

Key words: Breast; Cancer; E-cadherin; Estrogen receptor; Cell adhesion molecules.

Introduction

The mammary alveoli and ducts that are formed cyclically during pregnancy and lactation consist of bilayered epithelial structures surrounding a central lumen [1]. Luminal cells adhere to each other via E-cadherin, which is also necessary for cell survival, whereas the myoepithelial cells surrounding the luminal layer adhere to each other via P-cadherin [1, 2]. E-cadherin (EC) is a calcium-regulated transmembrane glycoprotein that functions as an epithelium-specific, cell-cell adhesion molecule [3-7]. The physiology of the reproductive tissues, including the breast, is dependent on the preservation of appropriate cell-cell contact, cell adhesion molecules (CAMs) being important for regulating tissue architecture and maintaining tissue integrity [7].

The loss or down-regulation of E-cadherin has been associated with breast cancer progression, and although its practical application as a diagnostic and prognostic marker in breast cancer remains controversial [8], some studies have shown that a reduction in E-cadherin expression constitutes an adverse prognostic marker in breast cancer [8-10]. E-cadherin expression is irreversibly lost in more than 85% of invasive lobular carcinomas (ILC). The loss of EC occurs at onset of the disease, i.e., at the preinvasive stage of lobular carcinoma in situ (LCIS) [1, 8]. However, EC expression in invasive ductal carcinoma, unlike invasive lobular carcinoma, is highly variable [11]. In general, preservation of E-cadherin expression is related to non-invasive and well-differentiated breast carcinomas [12].

Estrogens receptors are expressed in around 60-65% of breast cancer cases, and in these cases, a relatively better prognosis can be expected compared with tumors that do not express them [13, 14]. Likewise, tissue response to estrogen may be implicated in EC regulation, which, via the estrogen receptor (ER), indirectly represses the Snail transcription factor that down-regulates EC [13]. Thus, in ER-negative breast tumors, the Snail transcription factor would predominate and there would be a corresponding decrease in EC. Nevertheless, despite current controversies, there are few studies comparing E-cadherin expression in ER-positive and ER-negative breast tumors, leading us to design the present study.

Materials and Methods

Patients

Twenty-three patients with operable Stage II infiltrating ductal breast carcinoma, receiving medical care at the Mastology Division, Department of Gynecology, *Getúlio Vargas* Hospital, Federal University of Piauí were included in the present study. The study was approved by the Internal Review Board of the Federal University of Piauí and all the patients signed informed consent forms prior to initiation of the study. All the patients had been menopausal for at least one year and had no history of any previous treatment for breast cancer. Tumor samples were obtained by incisional biopsy at the time of definitive surgery for the purpose of evaluating ER status and to perform immunohistochemistry for E-cadherin. Tumors in which the semiquantitative evaluation of estrogen receptors following immunohistochemical staining was classified as high (≥ 10% immunoreactive cells) were considered positive [14].

Study Design

This was an analytical, cross-sectional study in which patients were divided into two groups: A (ER+; n = 13) and B (ER-; n = 10). All the patients had Her-2 negative tumors. The groups were considered homogenous with respect to age, tumor size, stage, histological grade and axillary status (Table 1).

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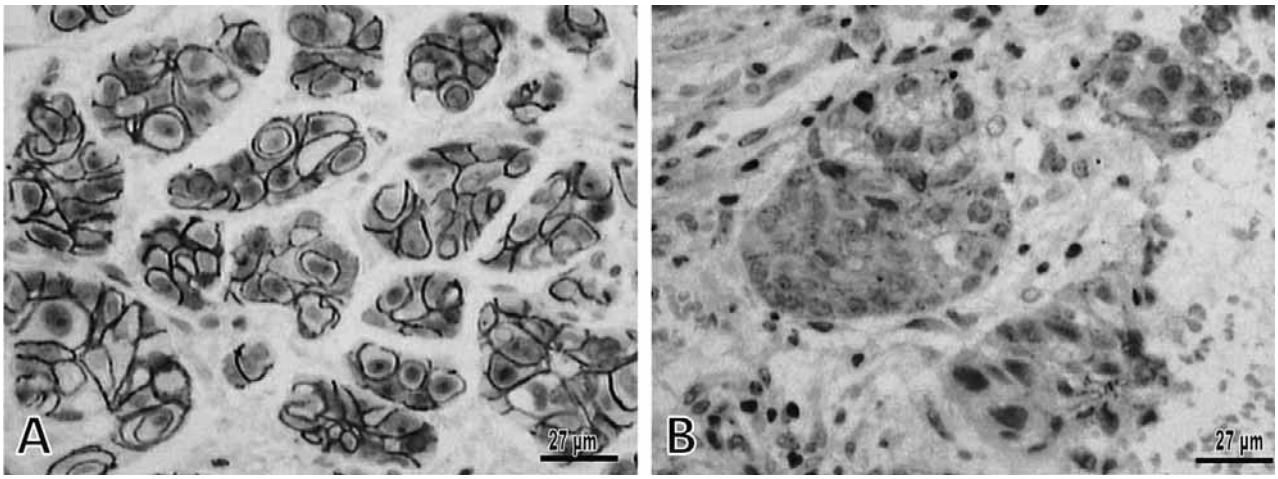

Figure 1. — Photomicrographs of histological sections of invasive ductal breast cancer: (A) In patient # 8, ER-positive, note the high concentration of cell membranes strongly stained brown by the anti-E-cadherin antibody, and (B) in patient # 6, ER-negative, note the sparse cells with plasma membranes weakly stained by the anti-E-cadherin antibody (original magnification 200 x).

Table 1. — *Patient characteristics in the ER-positive and ER-negative groups*.

	Group A (ER +)	Group B (ER-)	*p* value
n	13	10	
Age (years)			0.0581
Mean	63.2	58.8	
S.D	6.3	12.1	
Tumor Size (cm)			1.0000
Mean	3.7	3.3	
S.D	0.9	0.6	
Staging (%)			0.3788
IIa	53.8	70.0	
IIb	46.2	30.0	
Histological grade (%)			0.4674
G1	46.1	30.0	
G2	46.1	40.0	
G3	7.8	30.0	
Axillary status (%)			1.0000
N0	46.1	60.0	
N1	53.9	40.0	

Table 2. — *Immunohistochemical staining for E-cadherin in estrogen receptor-positive and negative invasive ductal breast carcinomas*.

Tumor	n	Staining intensity Negative	+	++
ER+	13	0 (0%)	3 (23%)	10 (77%)
ER-	10	7 (70%)	2 (20 %)	1 (10%)

Association between loss of E-cadherin expression and ER-negative breast carcinomas was statistically significant ($p < 0.0002$).

Immunohistochemistry for E-cadherin

All samples were fixed in 10% neutral buffered formalin for 24 hours and then embedded in paraffin. Sections measuring 5 µm were deparaffinized and antigenic recovery was performed using 0.21% citric acid (pH 6) in a pressure cooker for 8 min after pressure was initiated. Next, the slides were incubated with anti-E-cadherin (mouse) antibody, Clone 4A2C7 (Cat. No. 18-0223-Zymed, South San Francisco, CA)* at a dilution of 1:1200 and incubated overnight at 4-8°C. The slides were then washed with PBS containing Tween, excess PBS was aspirated and the secondary reagent (anti-mouse BA 200 – Vector, Burlingame, CA) was instilled, incubation following for 60 min at room temperature. After this, the slides were washed again with PBS-Tween, the excess PBS was aspirated, and the ABC Elite system (PK 6100 – Vector, Burlingame, CA) was instilled. Slides were then incubated for 45 min at room temperature, after which DAB (diaminobenzidine tetra-hydrochloride – REF.: D-5637, Sigma, St. Louis, MO) was instilled. Finally, the slides were washed in abundant distilled water, counterstained with hematoxylin, dehydrated in an absolute ethyl alcohol-xylol series and finally mounted in Permount resin. The cells expressing E-cadherin were identified by dark-brownish staining of the cytoplasmic membrane.

Quantification

E-cadherin expression was evaluated under light microscopy by two observers who were blinded with respect to group identification. These observers semiquantitatively counted the cells in which the membrane was positively stained (400 x magnification) using a system consisting of a light microscope (Nikon Eclipse E-400, optical microscope, Tokyo, Japan) connected to a videocamera (Samsung Digital Camera SCC-131, Seoul, Korea) with capture and transmission to a computer equipped with the Imagelab® software program (Softium Informatica LTDA, São Paulo, Brazil). Only tumor cells with obvious immunohistochemical labeling of the cytoplasmic membrane were considered positive. Immunopositivity was calculated as grade + if 10-50% of cells were positive or grade ++ if more than 50% of cells were positive. Tumors were graded as negative when less than 10% of the cells were stained [15].

Statistical analysis

The Student's t-test was used to test the homogeneity of the two groups with respect to age of the patients and tumor volume. Fisher's exact test was used to evaluate stage, lymph node status and histological grade between the two groups and to calculate the proportion of E-cadherin-positive cells in the estrogen receptor-positive and negative breast carcinomas [16]. Statistical significance was established at $p < 0.05$.

Results

Light microscopy detected a higher concentration of cells in which the membrane was strongly stained by the anti-E-cadherin antibody in the estrogen receptor-positive breast carcinomas compared to the estrogen receptor-negative tumors (Figure 1). Cells with E-cadherin-stained membranes were found in all the patients in group A (ER+), three (23%) being classified as grade + and ten (77%) as grade ++. In comparison, in group B (ER-), seven (70%) were found to be negative for E-cadherin, while only three (30%) were positive, two (20%) of which were classified as grade + and one (10%) as grade ++ (Table 2). This association between a reduction in E-cadherin expression and ER-negative tumors was statistically significant ($p < 0.0005$).

Discussion

Cell adhesion is a significant factor in containing cancer; hence, loss of intercellular adhesion may be associated with unfavorable prognoses [7]. The cadherins comprise a rapidly expanding superfamily of cell adhesion molecules that includes E-, N- and P-cadherin, known as type I cadherins because they were the first to be discovered. They all promote calcium-dependent cell-cell adhesion via homophilic intercellular interactions [5, 7]. The presence of cadherins in the reproductive tract, particularly in the breast, suggests an effect of estrogens in these tissues via cadherin expression [17]. Furthermore, some *in vivo* and *in vitro* studies have suggested a correlation between ER-negative status and the loss of E-cadherin [18, 19].

In the present study, there was a significant reduction in EC expression in the ER-negative, invasive ductal breast carcinomas compared to the ER-positive tumors. Unlike invasive lobular breast cancers in which EC expression is irreversibly lost in the majority of cases, in ductal carcinomas EC expression is highly variable and its relationship with respect to prognosis, histological grade and hormone receptor status is controversial [1, 8].

Some studies have reported preserved EC expression in almost all invasive ductal carcinomas but have found reduced expression to be associated principally with poor differentiation and high tumor grade [8-10, 19, 20]. Other studies have reported a correlation between reduced EC expression, lymph node status and ER status [18, 21]. On the other hand, other studies have failed to find any correlation between EC expression and tumor size, grade, mitotic activity, HER-2 overexpression or ER status [22, 23].

The patients who participated in the present study were homogenous with respect to age, tumor size, histological grade, stage and axillary status. This may have been due to the selection criteria adopted, since only post-menopausal patients with operable, stage II tumors over 3 cm in size were admitted to the study. Irrespective of these morphological prognostic factors, the loss of EC expression was significantly correlated with estrogen receptor-negative status. The association between ER-negative tumors and poorer prognosis may involve, in addition to the loss of EC expression, other molecular markers related to the angiogenesis, proliferation and apoptosis of tumor cells [24-29].

The connection between estrogens and cadherins has long been postulated from *in vivo* studies [7]. Factors that regulate EC expression, particularly the zinc-finger transcription factor Snail, an E-cadherin inhibitor, play an important role in the relationship between EC and prognosis, and may be regulated by steroid hormones [1, 7]. Moreover, several studies have shown that the E-cadherins expressed by the reproductive tissues are responsive to hormonal stimulus by which they control morphological changes in these tissues. The ER indirectly stimulates estrogen-dependent expression of metastatic tumor antigen 3 (MTA3), which in turn transcriptionally represses the cadherin transcription factor Snail [7, 30]. Therefore, it is proposed that estrogen maintains epithelial architecture by constraining Snail repression of E-cadherin [30], suggesting a mechanistic link between ER-negative status, tumor invasion and poor prognosis [1, 7, 30].

Some authors have demonstrated that in non-lobular breast carcinomas, reduced and/or negative EC expression was significantly associated with lack of ER expression and preferentially found in basal-like carcinomas [31, 32]. Therefore, ER-negative status is related to a loss of EC, which was confirmed by the findings of the present study, and this loss of EC may provide an explanation for the unfavorable prognosis of ER-negative breast cancers.

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