Expression of E-cadherin in primary endometrial carcinomas: clinicopathological and immunohistochemical analysis of 30 cases

M. Varras[1], E. Skafida[2], T. Vasilakaki[2], A. Anastasiadis[1], C. Akrivis[3], N. Vrachnis[4], G. Nikolopoulos[5]

[1]Third Department of Obstetrics and Gynecology, "Elena Venizelou" General Maternity State Hospital, Athens
[2]Department of Pathology, "Tzaneio" General State Hospital, Piraeus
[3]Department of Obstetrics and Gynecology, "G. Chatzikosta" General State Hospital, Ioannina
[4]Second Department of Obstetrics and Gynecology, University of Athens, Athens; [5]Hellenic Centre for Diseases Control and Prevention (Greece)

Summary

Introduction: Decreased expression of E-cadherin has been associated with poorly differentiated endometrial carcinomas and poorer outcomes. *Aim:* The purpose of this study was to examine the distribution of E-cadherin immunohistochemical expression in specimens from primary endometrial carcinomas and its relation to classical clinicopathological prognostic factors. *Materials and Methods:* Surgically-resected tissues of 30 patients with primary endometrial carcinomas were studied. Histological type and grade, depth of myometrial invasion, lymph-vascular space invasion, fallopian tube or ovarian invasion, and the presence of tumoral necrosis were evaluated. Immunohistochemical examination was performed on deparaffinized four-μm-thick sections. *Results:* The mean age of patients was 65 years (± 11.41). The 63.54% of carcinomas were moderately/poorly differentiated. No statistical correlation was found between the score or intensity of E-cadherin immunohistochemical staining (strong or moderate positive expression) and the clinicopathological factors tested. *Conclusions:* The association of E-cadherin immunoreactivity with the standard clinicopathological factors seemed to be contradictory. The classical clinicopathological factors remain the most important prognostic parameters.

Key words: E-cadherin; Carcinoma; Endometrial; Endometrioid; Immunohistochemistry; Pathology.

Introduction

Cell-to-cell adhesion is mediated by cell surface glycoproteins known as cadherins, through a Ca^{2+}-dependent mechanism [1-3]. Cadherins are divided into subgroups on the basis of their tissue distribution: E-cadherin (epithelial), P-cadherin (placental), N-cadherin (neural), and L-cell adhesion molecule (liver) [3]. E-cadherin has five extracellular domains, an intracellular tail, and connects to the actin cytoskeleton through a complex with the cytoplasmatic catenin [4]. E-cadherin has been shown to play a central role in cellular organization and to mediate the transmission of extracellular signals to cells [1, 5]. The expression of E-cadherin is also critical for the regulation of apoptosis of tumor cells [1]. Reduction and loss of E-cadherin expression in cancer cells seems to destruct the junctional cell structure, affecting therefore intracellular adhesion and promoting tumoral progression and metastasis [6]. Absent or decreased expression of E-cadherin is more likely to be associated with poorly differentiated or non-endometrioid endometrial carcinomas and with poorer outcomes [2, 7, 8].

The purpose of the present study was to examine the distribution of E-cadherin immunohistochemical expression in formalin-fixed, paraffin-embedded tissue specimens from primary endometrial carcinoma tissues of Greek patients and its relation with classical clinicopathological prognostic factors such as histological type, histological grade, depth of myometrial invasion, and extent to the cervix.

Revised manuscript accepted for publication December 22, 2011

Materials and Methods

Patients

Surgically-resected tissues of 30 patients with primary endometrial carcinomas who underwent surgery between 2006 and 2009 were randomly selected. The following histopathological parameters were determined: histological type and grade, depth of myometrial invasion, lymph-vascular space, and fallopian tube or ovarian invasion, presence of tumoral necrosis, and extent to the cervix. Pelvic and para-aortic lymph nodes were not dissected in all patients. Endometrial carcinomas were graded according to the World Health Organization (WHO) System.

Histological analysis

For histological examination, endometrial carcinomas were routinely fixed with formalin, embedded in paraffin, thin-sectioned, and stained with hematoxylin and eosin (H&E). Four-μm-thick sections including sufficient quantities of neoplasm mass were mounted on silane-coated glass slides.

Immunohistochemical analysis

Antibodies used for labelling paraffin-embedded tissue sections were fixed in formalin. Pre-treatment of tissues with heat-induced epitope retrieval was performed for each antigen. Staining procedure was performed on deparaffinized sections using the standard avidin-biotin-peroxidase complex method with automated immunostainer and 45 minutes incubation at room temperature with the primary antibody. Antibodies of immunohistochemistry were anti-human syndecan-1 (anti-human CD138, clone M115, mouse monoclonal, 1:50 dilution, and anti-human E-cadherin (clone 4A2C7, mouse monoclonal, 1:100 dilution. The final stage involved the dehydration and coverage of tile.

Evaluation of immunohistochemistry

The score of immunohistochemical expression of E-cadherin was classified into the following four categories: 0: < 5% immunopositive cells; 1: 5%-25% immunopositive cells; 2: 25%-75% immunopositive cells; 3: more than 75% immunopositive cells. Staining intensity was defined as weak, moderate, and strong.

Statistical analysis

Categorical variables are presented as frequencies and percentages, and continuous parameters are shown in terms of means and its corresponding standard deviations. Pearson chi-squared test was used to evaluate the potential association between categorical variables. The Kruskal-Wallis test, a non-parametric analogue of analysis of variance, was applied to test the hypothesis that samples were from the same population. In case of two samples, the Wilcoxon rank-sum test (also known as Mann-Whitney two sample statistic) was used instead. The results were considered significant if the corresponding p value was < 0.05.

Results

The mean age of patients was 65 years (± 11.41). The sample included 25 (83.33%) endometrioid carcinomas and five (16.67%) clear cell and papillary serous endometrial carcinomas. Using the WHO system, the cases were distributed as follows: grade G1 (well-differentiated adenocarcinomas) 11 cases (36.67%); grade G2 (moderately differentiated adenocarcinomas) 14 cases (46.87%); and grade G3 (poorly differentiated adenocarcinomas) five cases (16.67%). The mean myometrial invasion was 0.57 (± 0.24). Lymph-vascular space and fallopian tube invasion was found in seven (23.33%) and four cases (13.33%) respectively, while ovarian invasion was not observed. Necrosis was detected in nine cases (30%). Eight patients showed 5%-25% immunopositive cells, other eight cases had 25%-75% immunopositive cells, and 14 patients showed more than 75% immunopositive cells. The age of patients was not significantly different between these three groups (p = 0.40).

Endometrioid carcinomas included five cases with 5%-25% immunopositive cells, eight cases with 25%-75% immunopositive cells, and 12 cases with more than 75% immunopositive cells. In the clear cell and papillary serous endometrial carcinomas groups, E-cadherin was immunohistochemically expressed in 5%-25% of cells in three cases, and in more than 75% of cells in two cases. The score of immunohistochemical expression of E-cadherin was not statistically different between the above-mentioned histological types (p = 0.13).

Among those histologically classified as G1, three cases showed 5%-25% immunopositive cells, two cases had 25%-75% immunopositive cells, and six cases showed more than 75% immunopositive cells. In histological grade G2, immunopositivity for E-cadherin was detected in 5%-25% of cells in three cases, in 25%-75% of cells in five cases, and in more than 75% of cells in six cases. Finally, in histological grade G3, two cases had 5%-25% immunopositive cells, one case showed 25%-75% immunopositive cells, and two cases had more than 75% immunopositive cells. No statistically significant association was detected between histological grades and scores of immunohistochemical E-cadherin expression (p = 0.82).

Among eight cases that showed positive immunostaining for E-cadherin in 5%-25% of cells, the myometrial invasion was 0.65 (± 0.27). In eight cases with 25%-75% immunopositive cells and in 14 cases with more than 75% immunopositive cells, myometrial invasion was 0.60 (± 0.30) and 0.50 (± 0.17) respectively. These differences were statistically insignificant (p = 0.42) (Figures 1 and 2).

In case of lymph-vascular space invasion, immunopositivity for E-cadherin was detected in 5%-25% of cells in two patients, in 25%-75% of cells in two cases, and in more than 75% of cells in three patients. In the absence of lymph-vascular space invasion, 5%-25% immunopositive cells were found in six cases, 25%-75% in six patients, and more than 75% in 11 cases. There was no statistically significant association between lymph-vascular space invasion and scores of immunohistochemical E-cadherin expression (p = 0.97).

In the presence of tumoral necrosis, immunohistochemical expression of E-cadherin was found in 5%-25% of cells in two cases, in 25%-75% of cells in two cases, and in more than 75% of cells in five cases. If necrosis was absent, the corresponding frequencies for the three categories of E-cadherin expression were six, six, and nine, respectively. As evidenced by the chi-squared test, tumoral necrosis was not associated with the immunohistochemical E-cadherin expression (p = 0.82).

Out of 30 cases, 20 exhibited intense expression of E-cadherin and ten showed expression of moderate degree. The mean age of patients was not statistically different between these two groups (p = 0.27).

Strong positive expression was observed in nine cases of histological grade G1, in eight cases of grade G2, and in three cases of histological grade G3. The corresponding frequencies for moderate expression were two, six, and two, respectively. No statistically significant association was observed between the intense of E-cadherin staining and the histological grade (p = 0.41).

Concerning endometrioid carcinomas, strong positive expression was observed in 18 cases and moderate in seven patients. In the group of clear cell and papillary serous endometrial carcinomas, the E-cadherin staining was strong in two cases and moderate in three cases. The histological type of tumor and intensity of E-cadherin staining were not statistically related (p = 0.17).

The mean myometrial invasion was 0.55 (± 0.22) in the 20 cases with strong positive expression of E-cadherin and 0.60 (± 0.27) in the remaining patients with moderate staining. This difference was statistically insignificant (p = 0.63).

The intensity of staining was strong in 15 cases without lymph-vascular space invasion and moderate in the remaining eight cases. On the other hand, strong expression of E-cadherin was observed in five cases with

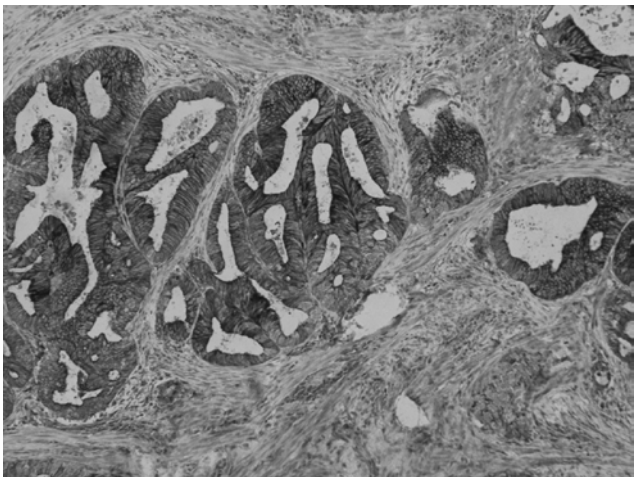

Figure 1. — Endometrial carcinoma: E-cadherin x 100.

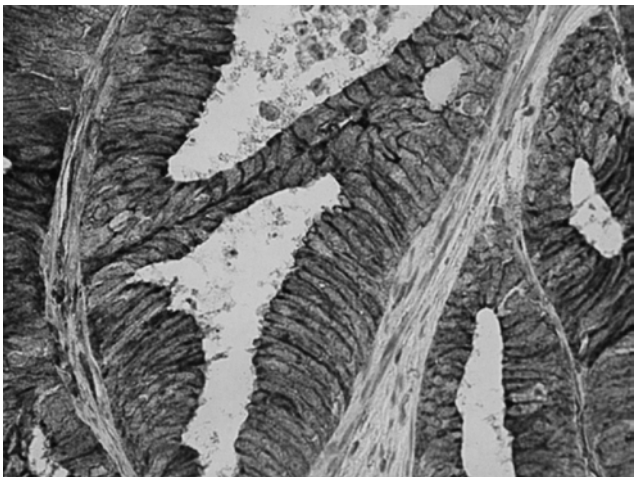

Figure 2. — Endometrial carcinoma: E-cadherin x 400.

lymph-vascular space invasion, while staining was moderate in the other two cases with tumor invasion in the lymph-vascular space. In statistical terms, the lymph-vascular space invasion and the intensity of E-cadherin staining were unrelated ($p = 0.76$).

In the presence of tumoral necrosis, the expression of E-cadherin was strong in five cases and moderate in four patients. Among cases without tumoral necrosis, 15 and six patients, respectively, showed strong and moderate E-cadherin staining. There was no statistically significant association between the presence of tumoral necrosis and the intensity of E-cadherin staining ($p = 0.40$).

Finally, in the presence of fallopian tube invasion, an equal number of cases (two) showed strong and moderate positive expression of E-cadherin. Once again, there was no statistically significant correlation between fallopian tube invasion and the intensity of E-cadherin staining ($p = 0.45$).

Discussion

Endometrial carcinoma is the most common malignancy of the female genital tract in developed countries with estimated 42,160 new cases diagnosed in the United States in 2009 [9]. In the majority of patients, the prognosis after primary therapy, typically total abdominal hysterectomy and bilateral salpingo-oophorectomy is excellent [10]. Patients with papillary serous or clear cell histology, tumor invasion depth to myometrium, poor tumor differentiation or extension of disease to other organs or lymph nodes within the pelvis, are at higher risk for disease recurrence [11]. However, better prognostic indicators are needed to identify which patients are most likely to develop extrapelvic metastases and thus, potential benefits from chemotherapy [8].

Cadherins represent an important class of the adhesion molecules, with an essential role in the homotypical cell-cell adhesion [12, 13]. The E-cadherin connects neighboring epithelial cells [12, 14]. The cellular adhesion depending on cadherins requires the formation of some compounds between E-cadherin and some cytoplasmatic proteins, known as catenins. The cytoplasmic part of E-cadherin interacts with the other components of adherens junctions, in particular the armadillo repeat proteins p120-catenin, γ-catenin/plakoglobin, and β-catenin. By binding of β-catenin to α-catenin the adherens junction complex is linked to the cortical actin cytoskeleton, thereby mediating mechanical stability. If the adherens junction components, in particular E-cadherin and β-catenin, are impaired, tumorigenesis is favored [12, 14-16]. E-cadherin has important roles not only in cell-cell adhesion, but also in tumor progression [6]. Decreased expression of E-cadherin is associated with a loss of cell-cell cohesive forces and has been shown to precede tumor cell motility [17]. Alterations of E-cadherin expression have been reported in many human tumors. In cholangiocarcinomas, E-cadherin expression was reduced in aggressive histological types [6, 18]. In the Rip-Tag mouse model of beta-cell tumors of the pancreas, E-cadherin loss was a prerequisite for progression from adenoma to invasive carcinomas, and in humans an inverse correlation between formation of entire adhesion complexes including E-cadherin/a-cetenin complexes, and survival of breast cancer patients has been demonstrated [19].

However, the expression of E-cadherin is the most important hallmark of epithelial differentiation. Its loss is a prerequisite for detachment, invasion, and finally dissemination and metastasis of neoplastic cells and can be linked directly to an activated Wnt signaling cascade, which triggers further malignant cell proliferation [20]. Loss of E-cadherin could be a trigger to induce expression of ZEB1, which was suggested to be important for maintenance of an invasive phenotype of epithelial cancer cells [21]. In primary colorectal carcinomas CHD1, gene repressors were found to be correlated significantly with the metastatic spread of the tumor [22]. Poor survival is

often the result of tumor's increased tendency for remote metastasis, as well-shown in non-endometrioid carcinomas which tend to a have an extra-uterine dissemination. The aggressive behavior of these carcinomas might be due to decreased intracellular cohesiveness in these tumors, as seen in the instance of E-cadherin loss [1].

Decreased E-cadherin expression has been associated with increased invasive and metastatic potential in endometrial carcinomas. The first report to show the relationship between E-cadherin expression and the grade of the tumor was Sakuragi *et al*. [5]. Myometrial invasion and lymph node metastasis were also taken into account. According to their findings, decreased E-cadherin expression in endometrial carcinoma was associated with deep myometrial invasion and higher grade of the tumor. E-cadherin expression patterns were also more frequently associated with para-aortic node metastasis than positive patterns. Their data suggested that decreased E-cadherin expression may be a risk factor for deep myometrial invasion, even when the histologic grade of the tumor is considered. However, in the event that E-cadherin is expressed, the possibility of a functionally abnormal molecule or local stimulants should be considered. Some other studies also showed a decreased E-cadherin expression in endometrial tumors. Scholten *et al*. investigated the expression of E-cadherin, alpha-catenin, and beta-catenin in endometrial carcinoma in order to determine the prognostic value of these factors [7]. Negative E-cadherin, alpha-catenin, and beta-catenin expression was observed in 44%, 47%, and 33%, respectively of endometrial carcinomas, and was correlated with histologic FIGO grade 3. Negative E-cadherin expression was more often observed in non-endometrioid endometrial carcinomas (NEECs) than in endometrioid carcinomas (75% vs 43%). Combined positive E-cadherin, alpha-catenin, and beta-catenin expression was an independent positive prognostic factor for survival in patients with grades 1 and 2 carcinomas, while negative E-cadherin expression was found to be associated with histologic grade 3 (7). Holcomb *et al*. found that tumor grade and histological type were identified as significant predictors of E-cadherin expression [3]. Nevertheless, when grade was controlled, endometrioid carcinoma remained 23 times more likely to express E-cadherin than papillary serous and clear cell carcinomas. According to the study of Singth *et al*., both human endometrial cancer specimens and cell lines, that when ZEB1 is inappropriately expressed in epithelial-derived tumor cells, E-cadherin expression is repressed, and that this inverse relationship correlates with increased migratory and invasive potential [23]. In another study, reduced E-cadherin immunoreactivity was seen in 44.8% of the endometrial carcinomas and 65.4% of the metastases with a statistical correlation to higher tumor grade only in metastatic lesions [24]. Mell *et al*. concluded that decreased E-cadherin expression is an independent prognostic factor for disease progression and mortality in pathological Stages I to III endometrial cancer and suggested that evaluation of E-cadherin expression may aid in the selection of patients for more aggressive adjuvant therapy [8]. In this study, the authors evaluated the immunohistochemical expression in formalin-fixed, paraffin-embedded tissue specimens from primary endometrial carcinomas tissue from Greek patients and clarified its role in relation to the standard clinicopathological factors. No statistical correlation was found between the score of intense of E-cadherin staining (strong or moderate positive expression) and histological grade and type, lymph-vascular space invasion (presence or absence), presence of tumoral necrosis, and when there was fallopian tube invasion. E-cadherin immunoreactivity did not associate with any of the standard clinicopathological factors tested. As already reported, experimental studies suggest that loss of E-cadherin expression in the tumor cells leads to cell detachment, as well as to malignant phenotype changes that are essential for the tumor cells to invade extracellular matrix [8]. E-cadherin may indeed be an invasion-inhibiting molecule. The question however, if E-cadherin in transformed cells is functionally normal, has not yet been answered and simply detecting E-cadherin may not be the appropriate way to assess tumor invasiveness.

Conclusions

More than 70% of E-cadherin expression was observed in endometrial carcinomas in Greek patients. The association of E-cadherin immunoreactivity with the standard clinicopathological factors seems to be contradictory. Therefore, at present, the classical clinicopathological factors remain the most important parameters for the evaluation of endometrial carcinoma prognosis.

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Address reprint requests to:
M. VARRAS, M.D., M.Sc., Ph.D.
Platonos 33
Politia (Kifisia)
14563 Athens (Greece)
e-mail: mnvarras@otenet.gr