Expression of E-cadherin in squamous cell carcinomas of the cervix with correlations to clinicopathological features

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Summary

Objective: To evaluate the expression of E-cadherin, a calcium-dependent cell adhesion molecule, in a retrospective analysis of paraffin-embedded tissue specimens of cervical squamous carcinoma and the relationship with histopathological differentiation and lymph node status.

Methods: In this study, we investigated by immunohistochemistry E-cadherin expression in ten normal cervical epithelia and 24 cervical invasive squamous carcinomas.

Results: Normal cervical squamous epithelium showed strong expression of E-cadherin at the membrane of the cell and intercellular junctions. In 24 tumors immunnostained by E-cadherin antibody, 11 (46%) showed preserved expression and 13 (54%) reduced expression. There was no significant correlation between E-cadherin expression and histological differentiation ($p = 0.650$, $p = 0.294$). In the status of lymph node metastasis, reduced expression of E-cadherin was seen in 11/15 (73%) with lymph node metastasis versus 2/9 (22%) without lymph node metastasis. There was a significant inverse correlation between E-cadherin expression and lymph node metastasis ($p = 0.032$).

Conclusion: Reduced E-cadherin expression may be an important factor among a variety of biologic events that occur during the process of metastasis. However, this should be explored by a large scale study.

Key words: Cervix; Squamous cell carcinoma; E-cadherin.

Introduction

Recent advances in molecular biology have led to the identification and characterization of a series of specific cell-to-cell adhesion molecules as general and important determinants of morphogenesis during embryonic development [1, 2]. Epithelial cadherin (E-cadherin) is a cell-cell adhesion molecule that connects epithelial cells via homotypic calcium-dependent interactions. The E-cadherin cell adhesion molecule is important in the maintenance of normal epithelial structures, and its altered expression may be important in epithelial tumor carcinogenesis, particularly in the process of invasion and metastasis. Many studies documenting loss or reduction of E-cadherin protein expression have been reported in various human cancers: in neoplastic thyroid tissue [3], in esophageal cancer [4], in breast cancer [5], in gastric and pancreatic carcinoma [6, 7], in bladder and prostatic cancer [8, 9], in melanoma [10], and in meningioma [11]. In gynecologic cancer, decreased and altered expression of E-cadherin has been described in endometrial [12], cervical [13, 14], and ovarian tumors [15]. Generally, E-cadherin expression was found to be strong in well-differentiated cancers, which maintain their cell-cell adhesiveness and are less invasive, but reduced in undifferentiated cancers, which have lost their cell-cell adhesion and show a strong invasive tendency. Therefore, inactivation of the E-cadherin-mediated invasion suppressor system was considered to result from reduced expression of E-cadherin. Abnormalities of E-cadherin expression have also been described in cervical dysplasia and in invasive squamous cervical carcinomas [13].

In the present study, we have investigated, by immunohistochemical analysis, the tissue distribution of human E-cadherin in normal epithelial tissue and squamous cell carcinoma of the cervix using the monoclonal antibody specific for human E-cadherin and have assessed the results with histopathological differentiation and lymph node metastasis.

Material and Methods

The specimens of tissues were obtained from 24 patients with cervical carcinoma who had undergone hysterectomies from January 1999 to December 2003 at the Department of Pathology, Dicle University Hospital, Diyarbakir, Turkey. They did not receive irradiation and anticancer chemotherapy before surgery. Ten positive control samples of normal cervical tissue from hysterectomies for nonneoplastic reasons were used simultaneously to establish the normal pattern of E-cadherin expression. The clinical data were obtained from clinical files and pathological reports.

For microscopic examination, tissue was routinely fixed with formalin before being embedded in paraffin. A 4-μm section from each specimen block was stained with H & E for histological evaluation, and representative blocks were chosen for immunohistochemical study .

Histologically, all cervical carcinomas were squamous cell carcinomas. The differentiation of squamous cell carcinomas was keratinizing in 12, nonkeratinizing large cell in seven and small cell in five cases.

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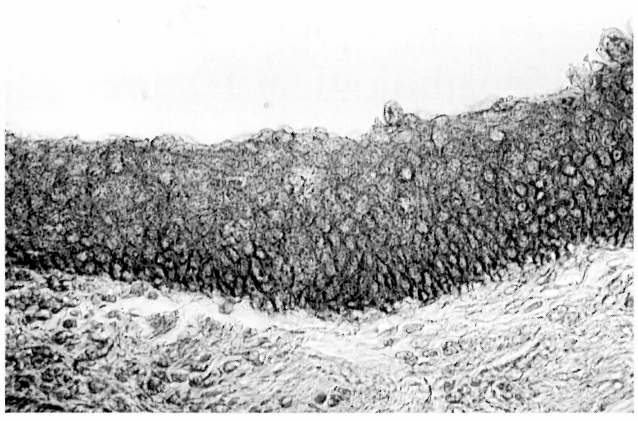
Fig. 1



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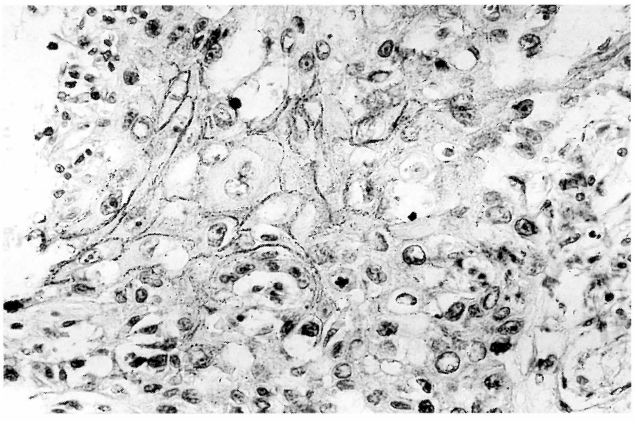

Fig. 3

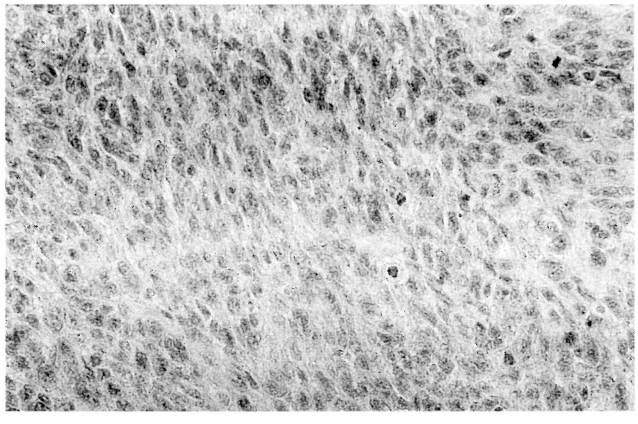

Figure 1. — Positive expression of E-cadherin in normal cervical squamous epithelium, (original magnification x 350).

Figure 2. — Reduced heterogeneous expression of E-cadherin in cervical carcinoma cells (original magnification x 350).

Figure 3. — Negative expression of E-cadherin in cervical carcinoma cells (original magnification x 350).

Immunohistochemical study

One or two blocks from each tumor and normal tissue were stained for immunohistochemical analysis using the Avidin-Biotin and immunoperoxidase methods. The formalin-fixed paraffin-embedded tissues were cut into 4 μm sections and dried on capillary-cap glass slides. The sections were deparaffinized with Standard xylene and hydrated through graded alcohol into water. An antigen retrieval procedure was performed using citrate buffer and heating for 10 min in a pressure cooker. Slides were placed for 15 min into a 3% hydrogen peroxide blocking medium and then allowed to react with the primer antibody, the anti-E-cadherin antibody (clone-NCH-38, Dako, Carpinteria, CA, USA). Immunoperoxidase detection was employed using the diaminobenzidine substrate (DAB). Counter staining was performed with hematoxylin.

Evaluation of immunohistochemical staining

All immunostained sections were analyzed by two different pathologists who had no knowledge of the patient's clinical and pathological status. Based on the previous immunohistochemical studies [16], the staining was localized mainly on the membranes of the tumor cells. The rate of stainings of the tumor cells was estimated as a percentage of > 500 tumor cells in five fields selected at random (x 400) and scored in one of the following categories: (a) preserved expression: > 70% of tumor cells were stained; and (b) reduced expression: < 70% of tumor cells were stained.

Distribution of the ratio of stained cells showed bipolarity, and we separated these into two groups, at the level of 70%. Necrotic areas were not taken into consideration. Heterogeneous staining was classified as reduced expression when < 70% of the tumor cells were stained.

For statistical analysis, Fisher's exact test was used to assess the statistical significance of E-cadherin expression in relation to histopathological differentiation and lymph node metastasis ($p < 0.05$ was accepted as statistically significant).

Results

In this study, we have investigated E-cadherin expression and distribution in normal cervical epithelium and cervical squamous cell carcinoma (Table 1).

Normal cervical squamous epithelium showed strong expression for E-cadherin at the membrane of the cell and the intercellular junctions. All of the specimens from all ten normal cervical epithelium showed the same E-cadherin expression (Figure 1). The expression of E-cadherin was more marked in the prickle cell layer and para-basal cell layers.

Table 1. — *Relationship between expression of E-cadherin and histopathological differentiation and lymph node status in patients with cervical carcinoma.*

Group	Total	E-cadherin Preserved (%)	E-cadherin Reduced (%)	Significance
Tumor differentiation				
Keratinizing	12	7 (58)	5 (42)	
Non-keratinizing large cell	7	3 (43)	4 (57)	p = 0.650
Small cell	5	1 (20)	4 (80)	p = 0.294
Lymph node metastasis				
Yes	15	4 (27)	11 (73)	
No	9	7 (78)	2 (22)	p = 0.032
Normal squamous epithelium	10	10 (100)	0 (0)	

In 24 tumors immunostained by E-cadherin antibody, 11 (46%) showed preserved expression and 13 (54%) reduced expression. Of the 12 keratinizing carcinomas examined, seven tumors (58%) preserved E-cadherin expression. However five tumors (42%) had reduced E-cadherin expression (Figure 2). Of the seven nonkeratinizing large cell carcinomas examined, three tumors (43%) preserved E-cadherin expression. However four tumors (57%) had reduced E-cadherin expression. Of the five small cell carcinomas examined, one tumor (20%) preserved E-cadherin expression. However four (80%) tumors had reduced E-cadherin expression (Figure 3). There was no significant correlation between E-cadherin expression and histological differentiation ($p = 0.650$, $p = 0.294$).

In 15 cases with lymph node metastasis, four (27%) showed preserved expression and 11 (73%) reduced expression. In nine cases without lymph node metastasis, seven (78%) showed preserved expression, and two (22%) showed reduced expression. There was a significant inverse correlation between E-cadherin expression and lymph node metastasis ($p = 0.032$).

Discussion

Abnormalities of cell adhesion molecule expression occur in various neoplastic diseases, and there is some evidence to suggest that these abnormalities are significant in tumor progression and may be associated with an adverse prognosis in certain tumor types [17]. Loss or dysfunction of E-cadherin may be associated with or result in the acquisition of invasive capacity and altered E-cadherin expression may be associated with higher tumor grade and with a poor prognosis [18, 19].

Abnormal expression of E-cadherin has been correlated in several human carcinomas with the pathological characteristics of tumor stage, grade of differentiation, invasiveness and lymph node involvement [20, 21]. Moreover, reduced expression of E-cadherin has been correlated with clinical variables such as disease relapse and disease-free survival [22-23].

Alterations of cell adhesion molecule expression have also been found in invasive cervical carcinoma. Generally, E-cadherin expression was found to be high in well differentiated cancers, but reduced in undifferentiated cancers.

We have immunohistochemically analyzed normal tissues and squamous cell carcinomas of the uterine cervix and investigated them clinicopathologically in relation to factors including histological type and lymph node metastasis. All samples of normal cervical epithelium were positive for E-cadherin expression. A homogeneous weak cytoplasmic staining was observed in normal squamous epithelium with intense reactivity observed at cell-to-cell borders.

Morphologically, the most important phenomenon associated with neoplastic transformation in stratified squamous epithelia is the loss of normal maturation. Honda found that six patients of 38 patients with cervical cancer exhibited homogeneous staining of E-cadherin, while 32 showed heterogeneous expression, suggesting that cell-to-cell adhesion is not uniform in most cases [24]. The immunohistochemical staining of E-cadherin in normal and malignant cervical epithelium was examined. The E-cadherin expression level for the 12 keratinizing squamous cell carcinomas was 58%. There was not a significant decrease in E-cadherin levels for the seven nonkeratinizing large cell carcinomas with a mean positive stain of 43% and that of the five small cell carcinomas with a mean positive stain of 20%. We found keratinizing carcinoma to be higher (58%) than nonkeratinizing large cell carcinoma (43%) and small cell carcinoma (20%). However, there were no statistically significant differences between E-cadherin expression and histopathological differentiation ($p = 0.650$, $p = 0.294$).

Our findings revealed that reduction of E-cadherin is not associated with the degree of differentiation. Several immunohistochemical studies have reported that decreased expression E-cadherin in the primary carcinoma is correlated with regional lymph node metastasis [25-27].

We found that E-cadherin was reduced or absent in two (22%) of nine cases without regional lymph node metastasis and 11 (73%) of 15 cases with regional lymph node node metastasis. There were significant differences in the expression of E-cadherin between those tumors with and without nodal metastases ($p = 0.032$).

In conclusion, we have shown that abnormal expression of E-cadherin is a common feature in cervical squamous cell carcinomas and correlates well with dedifferentiated carcinoma and lymph node metastasis. Reduction of E-cadherin expression in these carcinomas may be an independent predictor of lymph node metastases and their immunohistochemical determination might be useful in identifying patients with clinically negative lymph nodes. However it should be explored further by a large-scale study.

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