Expression and Localization of E-Cadherin in Epithelial Ovarian Cancer

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Abstract. *Background: Findings for the role of E-cadherin in ovarian cancer (OC) are controversial. The aim of this study was to analyze the expression and prognostic role of E-cadherin in OC. Materials and Methods: Expression analysis of E-cadherin was performed by immunohistochemistry in 36 patients (12 primary OC, 15 recurrent OC, 9 benign ovarian lesions). Tumor specimens were collected within OC. Correlation analysis with clinicopathological factors and survival was performed. Results: E-Cadherin was significantly reduced in OC compared to benign ovarian lesions (p=0.024). In primary OC, E-cadherin was comparable in ovarian tumor and corresponding metastatic tumor tissue. E-Cadherin showed no association with clinicopathological factors. A significant correlation between increased volume of ascites and higher E-cadherin immunoexpression was found in primary OC (p=0.029). E-Cadherin expression showed no statistically prognostic significance for survival (p=0.856). Conclusion: The function of E-cadherin in OC remains controversial and needs to be elucidated further in larger studies.*

Epithelial ovarian cancer (OC) is the eigth leading cancer in women and the leading cause of death from all gynaecological malignancies. The majority of patients present with advanced disease at the time of diagnosis. Despite radical surgery and adjuvant platinum-based systemic treatment, relapse will occur in more than half of the patients. The 5-year survival rate remains poor (1).

Current therapeutic management is based on few conventional prognostic factors, such as tumor stage and postoperative tumor residual mass (2). Identification of new molecular markers could potentially lead to significant modification of clinical management improving clinical outcome.

In solid tumors, the process of tumor progression entails the invasion of tumor cells into the surrounding tissue and the dissemination of tumor cells, with the clinical consequence of metastasis to distant organs. During tumor progression, cell-to-cell and cell-to-substrate interactions seem be crucially altered (3). Various families of surface glycoproteins and glycoconjugates are responsible for mediating cell-to-cell adhesion, such as the immunoglobulin cell–cell adhesion molecule superfamily (Ig-CAMs) and the cadherin superfamily, and cell-to-extracellular matrix (ECM) interactions, such as the integrin family and other cell-surface receptors such as CD44 (4).

Cadherins represent the major component of adherens junctions and mediate cell–cell adhesion through the calcium-dependent homophylic interaction with the ECM (5). Epithelial cadherins, with E-cadherin as the prototype family member, play a crucial role in the formation and maintenance of epithelial structures. Furthermore, loss of cell- cell adhesion may play a relevant role in malignant transformation and the invasive behavior of malignant tumors. Loss of E-cadherin function during the development of most types of human epithelial cancer, including of the breast, colon, prostate and lung, has been reported in different clinical and experimental studies (6). The loss of E-cadherin function is correlated with de-differentiation, infiltrative tumor growth and metastasis, suggesting its role as a tumor suppressor. Different mechanisms seem to be responsible for the loss of E-cadherin

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function and expression, including deletion and mutational inactivation of the E-cadherin gene, changes in the expression of proteins that are part of the E-cadherin adhesion complex, such as α-catenin and β-catenin, as well as chromatin rearrangement, hypermethylation and loss of transcription factor binding (7, 8).

In OC, the expression of E-cadherin has been analyzed in different studies with heterogenous patient cohorts. A switch from N-cadherin expression to E-cadherin expression in malignant transformation of the ovarian surface epithelium and in early-stage epithelial OC was reported (9). Regarding the expression of E-cadherin in advanced-stage epithelial OC, controversial results exist: some studies have reported a loss of E-cadherin expression (10, 11), while others have demonstrated an increase (12). Furthermore, the prognostic significance still remains unclear (13).

Therefore, in the present study we analyzed the expression and localization of E-cadherin in primary and recurrent epithelial OC. Furthermore, the clinical and prognostic role of E-cadherin was evaluated.

Materials and Methods

Patients. All patients included in this mono-institutional study were treated surgically between 2000 and 2003 at the Department of Gynecology and Obstetrics, Charité, Campus Virchow Clinic, Berlin, Germany. Written informed consent was provided by each patient. Histological diagnosis was confirmed by the Department of Pathology, Charité, Berlin, Germany. The study protocol was approved by the Institutional Review Board of the Medical Faculty of the Humboldt University, Charité, Berlin. Overall, 36 patients were recruited into the study, including 12 patients with primary OC, 15 with recurrent OC and a control group of 9 patients with benign ovarian lesions. Within the control group, normal ovarian surface epithelium (NOSE) from 2 cystic ovarian lesions, 3 serous cystadenofibroma, 1 mucinous cystadenofibroma, 2 cystoma and 1 normal ovary were included.

The majority of patients with a diagnosis of primary ovarian cancer had undergone radical surgery according to standard operating procedures with the primary objective of maximal tumor reduction. Most of the patients with primary ovarian cancer (92%) received standard adjuvant chemotherapy with carboplatin and paclitaxel. Surgical treatment in patients with recurrent ovarian cancer involved maximal tumor debulking. Patients with recurrent disease had previously received from 1 to 4 chemotherapy lines.

The median follow-up was 41 months (range, 1-70 months). The median recurrence-free survival was 21 months (range, 1-70 months) in primary OC and 14 months (range, 1-38 months) in recurrent OC. The median OC was 45 months (range, 3-70 months) in primary OC and 24 months (range, 1-66 months) in recurrent ovarian cancer.

The clinicopathological characteristics of the patients are summarized in Table I.

Tissue collection. Tumor specimens were collected at surgical intervention immediatly after removal of the tumor, according to standard operating procedures used within the Tumorbank Ovarian

Table I. *Clinicopathological characteristics of the patients (n=36).*

	Median (range)	
Age, years	55 (26-86)	
Follow-up period, months	41 (1-70)	
	n	
Diagnosis		
Primary ovarian cancer (OC)	12	
Recurrent (OC)	15	
Benign ovarian lesion	9	
Total OC, n=27		
	Primary OC n (%)	Recurrent OC n (%)
Tumor stage (FIGO)		
II	1 (8.3%)	0
III	7 (58.3%)	0
IV	4 (33.3%)	0
Histological grade		
1	2 (16.7%)	3 (20%)
2	4 (33.3%)	4 (26.7%)
3	6 (50%)	8 (53.3%)
Tumor spread pattern (IMO) (15)		
1-3	8 (66.7%)	2 (13.3%)
4-6	3 (25%)	13 (86.7%)
7-9	1 (8.3%)	0
Lymph node status		
NX	3 (25%)	1 (6.7%)
N0	4 (33.3%)	5 (33.3%)
N1	5 (41.7%)	9 (60%)
Peritoneal carcinomatosis		
Yes	8 (80%)	9 (60%)
No	2 (20%)	6 (40%)
Postoperative residual tumor mass		
Macroscopically tumor-free	8 (66.7%)	7 (46.7%)
≤2 cm	3 (25%)	6 (40%)
>2 cm	1 (8.3%)	2 (13.3%)
Volume of ascites		
No ascites	3 (25%)	8 (53.3%)
≤500 ml	5 (41.7%)	5 (33.3%)
>500 ml	4 (33.3%)	2 (13.3%)

Table II. *Correlation of E-cadherin expression with clinicopathological factors.*

	p-Value
Age	0.069
Tumor stage (FIGO)	0.298
Histological grade	0.515
Tumor spread pattern	0.199
Peritoneal carcinomatosis	0.540
Lymph node status	0.737
Postoperative tumor residual mass	0.893
Volume of ascites	0.486

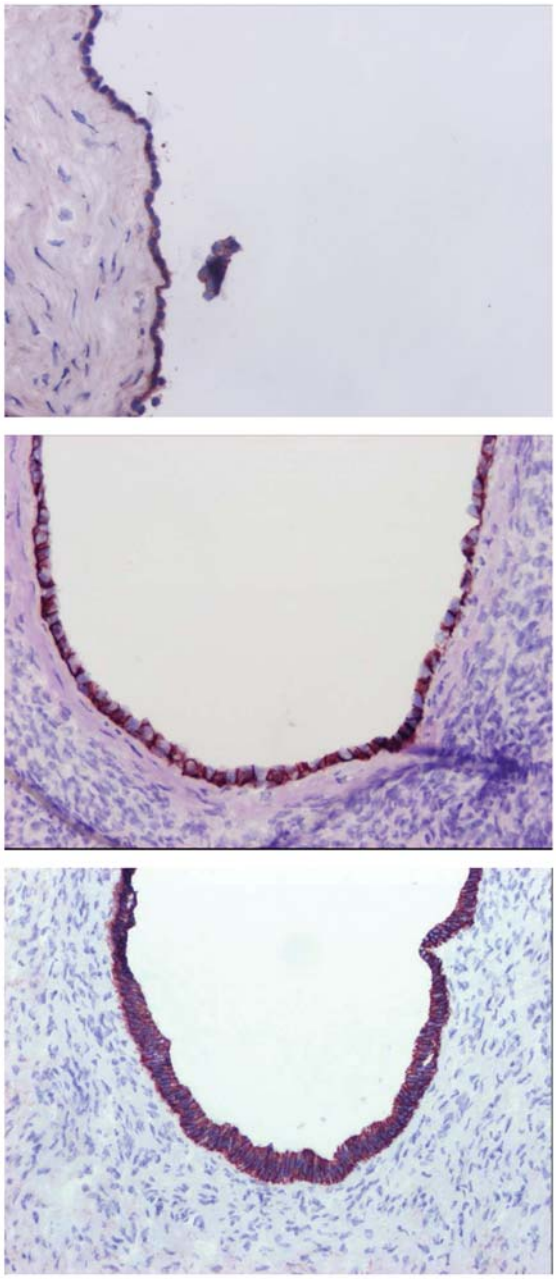

Figure 1. *E-Cadherin expression in normal ovarian surface epithelium (×10).*

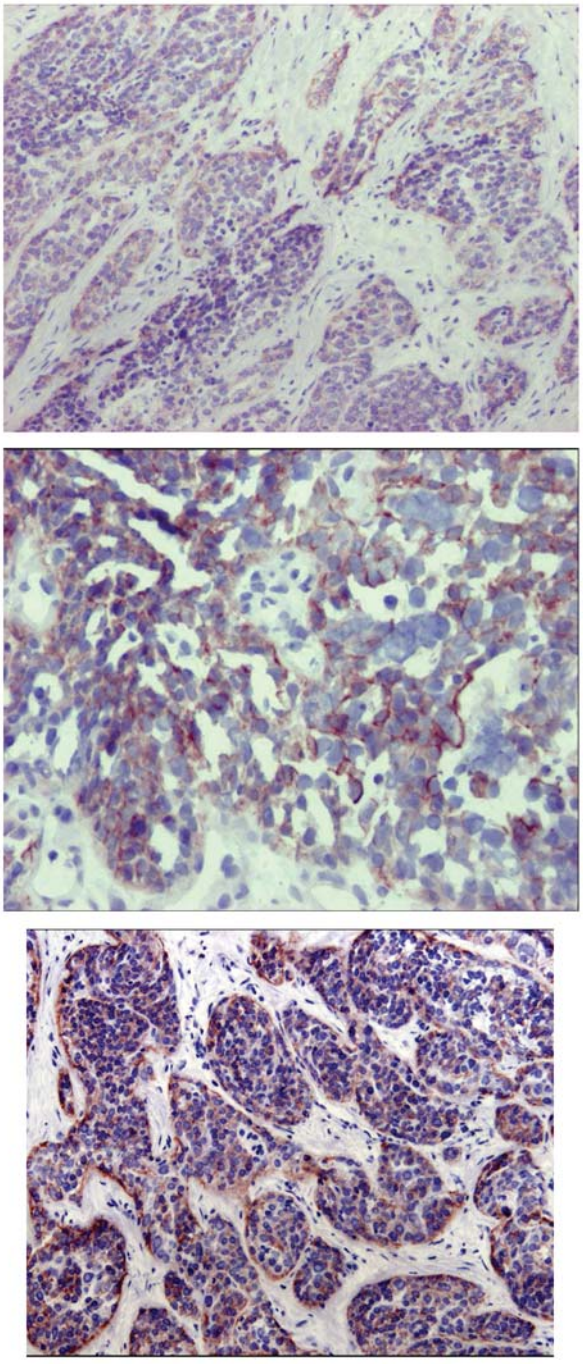

Figure 2. *E-Cadherin expression in FIGO stage III ovarian cancer (×10, ×20).*

Cancer (TOC) Network (www.toc-network.de). Fresh tumor samples were immediately after removal, frozen in liquid nitrogen and stored at –180˚C until use. The tumor samples were histologically classified by a pathologist. In primary OC, ovarian tumor tissue and corresponding metastatic tumor tissue were collected to allow comparative expression analysis.

Immunhistochemistry. An immunohistochemical approach was used to analyze the expression of E-cadherin. Serial tissue sections of 8 μm thickness were cut in a cryostat, mounted on glass slides (Star Frost, Roth, Karlsruhe, Germany), air-dryed for 30 min and then fixed in 4% paraformaldehyde (formaldehyde 37%; Merck, Darmstadt, Germany) for 10 min. Immunohistochemistry (indirect method) was performed according to a standard protocol using a mouse anti-human E-cadherin monoclonal IgG (Clone 4A2C7; Zymed Laboratories, San Francisco, CA) in a 1:100 dilution (Antibody Diluent Solution, Zymed Laboratories), a biotinylated anti-mouse antibody (Universal

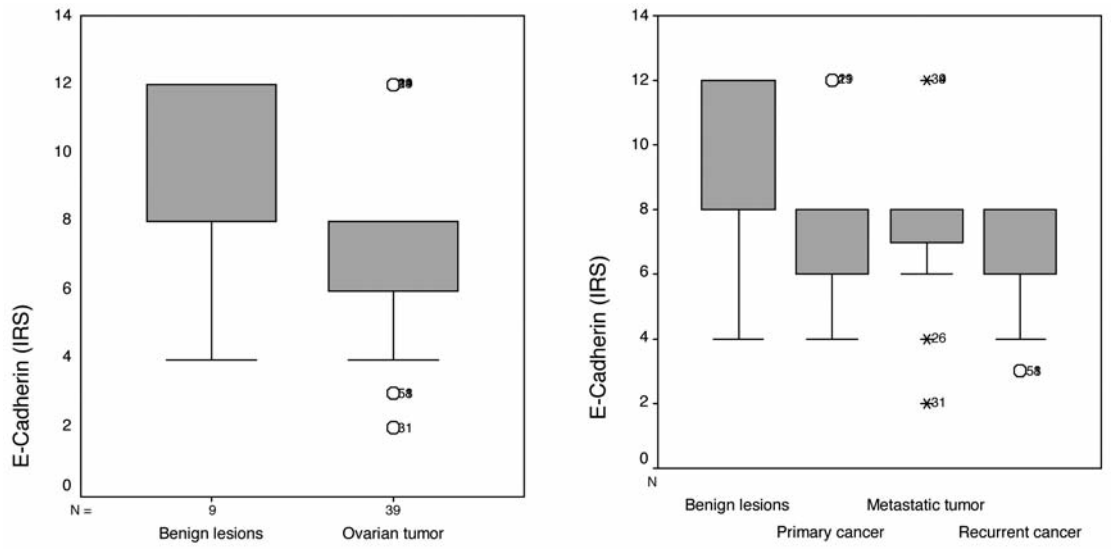

Figure 3. *Comparison of E-cadherin expression in benign ovarian lesions and ovarian cancer. IRS, Immunoreactive score.*

Link, Biotinylated Secondary Antibody, Biocarta, Hamburg, Germany) and a streptavidin-horseradish peroxidise complex solution (Streptavidin-Enzyme Conjugates; Biocarta). For visualization Romulin AEC Chromogen (AEC; Biocarta) was used, the slides were counterstained with hematoxylin (Hämatoxylin krist.; Merck), dehydrated in a series of graded ethanols (70, 80, 95, 95, 100 and 100%) and cleared in xylene and mounted.

Analysis of expression. The expression of E-cadherin was evaluated by two independent observers (C.F. and I.K.) who were blinded to the clinical data. Histology and the proportion of tumor tissue in each section were evaluated with conventional hematoxylin/eosin staining by an independent pathologist. A semi-quantitative immunoreactive scoring system (IRS) was used for evaluation of the level of expression of E-cadherin (14). This scoring system considers the percentage of positively stained cells (0%=0, <10%=1, 10-50%=2, 51-80%=3, >80%=4) and the intensity of the staining (negative=0, low=1, moderate=2, strong=3). The level of expression was classified into four groups: 0 (no staining), 1-4 (low), 5-8 (moderate) and 9-12 (strong staining).

Data collection. All clinical data, including age, FIGO tumor stage, tumor spread pattern, surgical procedures, postoperative residual tumor and histopathological data were collected using a standardized and validated documentation tool, IMO (15). Follow-up visits were performed every 3 months by standard follow-up examination, by checking the regional tumor registry or by telephone interview.

Statistical analysis. The statistical analysis was performed using SPSS 11.5 software (SPSS Inc., Chicago, IL, USA). The Mann-Whitney, Wilcoxon and Kruskall-Wallis tests were used to analyze the differences between groups. Two-tailed *p*-values and 95% confidence intervals (CI) were calculated. Two-tailed *p*-values <0.05 were considered statistically significant. The Kaplan-Meier method was applied to estimate survival, and values were compared using the log-rank test.

Results

Expression of E-cadherin. E-Cadherin expression was detected in NOSE of benign ovarian lesions and epithelial ovarian cancer cells in a varying pattern.

E-Cadherin was not expressed in stromal cells.

Benign ovarian lesions. The expression of E-cadherin in benign ovarian lesions was localized in the intercellular border and pericellular region of NOSE. E-Cadherin was expressed over the whole surface of polarized epithelial cells. No nuclear or cytoplasmic expression was detected (Figure 1). E-Cadherin immunoexpression showed a median IRS of 12 (range, 4-12) in benign ovarian lesions (moderate in 67%, strong in 33%).

Primary and recurrent OC. E-Cadherin showed reduced expression in epithelial OC and was localized irregularly in the intercellular border and pericellular region of epithelial OC cells. Polarization of epithelial cells was not detectable (Figure 2). E-Cadherin immunoexpression showed a median IRS of 8 (range, 4-12) in primary OC (moderate in 83%, strong in 17%) and a median IRS of 6 (range, 3-8) in recurrent OC (low in 13%, moderate in 87%). The expression of E-cadherin was significantly lower in primary (median IRS=8) and recurrent (median IRS=6) OC in comparison to that in benign ovarian lesions (median IRS=12) (p=0.024).

Primary OC and corresponding metastatic tumor tissue. In primary OC, the expression of E-cadherin was analyzed in ovarian tumor and in its corresponding metastatic tumor tissue, showing comparable results (p=0.850). In primary OC, strong expression was found in 17% and moderate in

83%, whereas in the corresponding metastatic tumor, strong expression was found in 17%, moderate in 75% and low expression in 8%. No statistically significant correlation between E-cadherin expression and advancing tumor progression was found (p=0.064) (Figure 3).

Correlation of the E-cadherin expression with clinicopathological factors. In correlation analysis, E-cadherin expression showed no association with clinicopathological factors (Table II). Only in subgroup analysis was a statistically significant correlation between increased volume of ascites and higher E-cadherin immunoexpression found within the primary OC group (p=0.029).

Prognostic significance of E-cadherin. The E-cadherin expression showed no statistical prognostic significance for recurrence-free survival (p=0.856) nor for overall survival (p=0.877). Only tumor spread pattern (p=0.003), volume of ascites (p=0.043) and postoperative residual tumor mass (p=0.022) showed prognostic significance for recurrence-free survival. Furthermore, the volume of ascites (p=0.012) and postoperative residual tumor mass (p=0.013) remained prognostically significant for overall survival.

Discussion

In the present study, E-cadherin expression was analyzed using immunohistochemistry in a clinically well-described cohort of patients with primary and recurrent OC and a control group of normal ovarian tissue and benign ovarian tumors.

We demonstrated that the expression of E-cadherin is localized in the intercellular border and pericellular region of NOSE and surface epithelium of benign ovarian lesions. While some studies showed no expression of E-cadherin in NOSE (16), others found immunohistochemical expression of E-cadherin in invaginations of NOSE (17) and benign cystadenomas of the ovary (18). Moreover, Maines-Bandiera and Auersperg observed an association between the expression of E-cadherin in ovarian surface epithelium and the morphology of cells, with expression of E-cadherin mainly predominant in cuboid and columnar cells (16). It can be postulated that the expression of E-cadherin might be associated with metaplastic and dysplastic changes of the ovarian surface epithelium.

In our OC patients, an irregular pattern was detected in epithelial cancer cells. The expression of E-cadherin was significantly lower in primary and recurrent OC in comparison to that of NOSE and benign ovarian lesions, but there were no significant differences in expression between primary and recurrent OC. No significant correlation of E-cadherin expression and tumor progression was found. These results are in accordance with different studies showing a decreased expression of E-cadherin in invasive ovarian tumors in comparison to that in benign ovarian lesions and well-differentiated ovarian tumors (6, 18). These findings support the hypothesis that E-cadherin might play a crucial role as a tumor suppressor (7, 8).

In our study, E-cadherin showed a comparable expression in primary OC and its corresponding metastatic tumor tissue. Reports in the literature with regard to the expression of E-cadherin in metastases are inconclusive. Some authors described an overexpression of E-cadherin in dissemated lesions in comparison to the primary tumor, suggesting that the presence of E-cadherin might promote metastasis and invasion (19), while others found a decreased expression of E-cadherin in metastatic lesions (11). Our findings are in accord with these of Voutilainen *et al*. (20) reported a comparable expression of E-cadherin in primary OC tumor tissue and its corresponding metastatic tumor tissue. These controversial findings might reflect the effects of different mechanisms of regulation of E-cadherin expression during tumor progression and metastasis. Furthermore, the different methods used by different groups and the heterogeneous clinicopathological characteristics of the patient collectives, *e.g*. tumor stage, histological type, postoperative residual tumor, might explain these differing results.

Our correlation analysis showed no association of E-cadherin with classical clinicopathological factors. A statistically significant correlation between increased volume of ascites and higher E-cadherin immunoexpression was, however, found within the primary OC group. Furthermore, a trend towards a decreased E-cadherin expression with increasing residual tumor was found. A correlation between residual tumor and E-cadherin expression has been reported previously (13), suggesting a loss of E-cadherin expression with increasing tumor aggressiveness.

Previous studies on the prognostic role of E-cadherin in ovarian cancer are very limited and inconclusive. A few studies reported its prognostic significance (18, 20), while others could not attest to any prognostic role (11). In our analysis, E-cadherin expression showed no statistical prognostic significance for recurrence-free and overall survival in OC. However, these controversial results might be explained by the different evaluation methods, the low number of patient samples analyzed and the heterogeneous patient collectives used by different working groups. The prognostic value of E-cadherin needs to be evaluated further.

In our study, we did confirm a reduced expression of E-cadherin in OC. In primary OC, the expression of E-cadherin was comparable in ovarian tumor and in its corresponding metastatic tumor tissue. Furthermore, a statistically significant correlation between increased volume of ascites and higher E-cadherin immunoexpression was found within the group of patients with primary OC. However, no further correlation with clinicopathological factors was found. Nor

was any prognostic significance found. Thus, the function of E-cadherin in tumor development remains controversial and needs to be elucidated further in larger studies.

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