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Immunoexpression of E-, P- and N-cadherins in ovarian serous malignant tumors

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Abstract

Alteration of cadherin immunophenotype is associated with the epithelial–mesenchymal transition, a complex biomolecular mechanism involved in carcinomas progression. The study investigated the immunoexpression of E-, P- and N-cadherins in 50 serous malignant tumors of ovary related to the histopathological prognostic parameters of the lesions, using a quantification based on scores that took into account the number of marked cells and the intensity of the reactions. The E-cadherin and P-cadherin immunostainings were significantly superior in serous borderline tumors (SBTs) compared to carcinomas, as well as in advanced carcinomas compared to early stages. Although the immunoreactions indicated higher scores in high-grade serous carcinomas (HGSCs) *versus* low-grade ones (LGSCs), the aspect was without statistical significance. Immunoreactions of N-cadherin were present only in HGSC, being significantly superior in the advanced stages of tumors. Ovarian serous malignant tumors expressed E-, P- and N-cadherins in different proportions, the altered cadherin phenotype being associated with progression of the disease. The results can be used to identify tumors with progression potential and to better stratify patients for specific therapy.

Keywords: ovarian serous tumors, epithelial–mesenchymal transition, cadherins.

Introduction

The epithelial–mesenchymal transition (EMT) is a complicated and reverse process characterized by the modification of the cell phenotype, function and expression of a large number of molecules [1, 2]. It has been reported not only in relation to tumor invasive and metastatic phenotype but also to the incipient stages of tumorigenesis [3, 4]. The classical EMT is characterized by the decreasing of epithelial markers expression [E-cadherin, cytokeratin (CK), claudins] and the increasing of mesenchymal markers expression (vimentin, N-cadherin, fibronectin). Among the markers of epithelia, the lost of E-cadherin expression represents the EMT mark, because the protein is a basic component of the cell adhesion system and alteration of expression can initiate EMT [5].

It is recognized that most malignant epithelial tumors progress initially by EMT, which causes the adherence decrease of epithelial cells and facilitates the attachment to the basal membrane [6, 7]. The cellular characteristic changes of this process occur as a result of disturbance in the expression of some genes and the corresponding proteins, including CK and vimentin low levels, along with changes in cell–cell and cell–extracellular matrix adhesion molecules [8]. Another key feature of the EMT process is the "cadherinic switch", in which the decreasing expression of E-cadherin is associated with the acquisition of N-cadherin expression [6, 7], a change that allows for increased motility of the cancer cells and the increase of their invasiveness [6, 9].

As a result, lately, the interest in investigating the expression of adhesion molecules and transcription factors has increased, with an emphasis on their role in tumor progression and the response to therapy.

Aim

We proposed to evaluate the involvement of EMT in the progression of malignant serous ovarian tumors by evaluating the expression of some intercellular adhesion molecules. Thus, for all the cases investigated, we followed the immunohistochemical expression of E-cadherin, N-cadherin and P-cadherin.

Materials and Methods

We investigated a number of 50 malignant serous ovarian tumors from the Clinics of Surgery and Gynecology, Emergency County Hospital of Craiova, Romania. For the fixation of surgical specimens, 10% neutral buffered formalin was used, and then embedded in paraffin and stained in routine Hematoxylin–Eosin (HE). The assessment of lesions was done in compliance with the *World Health Organization* (WHO) 2014 criteria [10].

Next, we obtained subsequent sections that were processed by immunohistochemistry, with an amplification polymer – Horseradish Peroxidase (HRP) system (Histofine–Nichirei Bioscience Inc., Tokyo, Japan). Also, we used Heat-Induced Epitope Retrieval (HIER) (citrate, pH 6), endogenous Peroxidase and unspecific sites blocking. The chromogen 3,3'-Diaminobenzidine (DAB) tetrahydrochloride was used to visualize the reactions (Dako, Glostrup, Denmark). To certify the reactions were used the primary antibody omission and normal positive tissues for the analyzed markers (Table 1).

Table 1 – *Protocols for the used antibodies*

Antibody	Clone	Manufacturer	Dilution	External control
E-cadherin	NCH-38	DAKO	1/50	Mammary gland
P-cadherin	Polyclonal	Atlas Antibodies	1/75	Placenta
N-cadherin	IAR06	Novocastra	1/100	Tonsil

Two pathologists examined the semiquantitative expression of E-, P- and N-cadherins by an adjusted system, considering the reactions intensity and the average number of labeled cells on each case 10 microscopic fields (MFs) ×400, reported as percentages [11]. The intensity score was considered 1 (weak), 2 (moderate) or 3 (strong) and the cutoff for the reactions positivity was 5% labeled cells. The score of labeled cells was considered 1 (6–25%), 2 (26–50%), 3 (51–75%) or 4 (≥76%). The intensity and percentage scores were multiplied resulting the final staining scores (FSS). For the statistical analysis, the FSS were classified as low or high for 1–4 and 6–12 values, respectively.

In this study were used mean values and comparison tests [one-way Analysis of Variance (ANOVA), *chi*-squared (χ^2), Pearson] in Statistical Package for the Social Sciences (SPSS) 10 automated software.

Results

The histopathological analysis of the 50 selected tumors revealed 13 cases of serous borderline tumors (SBTs) in the stage I of disease, five cases of low-grade serous carcinoma (LGSC) corresponding to stage I, II and III of disease, as well as 32 high-grade serous carcinomas (HGSCs) classified in all four *International Federation of Gynecology and Obstetrics* (FIGO) stages.

The statistical analysis of E-, P- and N-cadherins expression in relation to the type, degree and stage of tumors revealed some significant differences (Table 2).

Table 2 – *Cadherin scores distribution depending on the type, grade and tumor stage*

Cadherin type / FSS		E-cadherin	P-cadherin	N-cadherin
Tumor type	SBT	9.4	8.7	–
	SC	6.1	6.6	–
	*p (χ^2 test)	0.006	0.013	–
Tumor grade	LGSC	7.4	7.7	–
	HGSC	5.7	6.4	–
	*p (χ^2 test)	0.269	0.601	–
Tumor stage	I	9.3	8.8	–
	II	8.2	10.5	4.0
	III	2.1	4.2	9.2
	IV	2.6	2.6	9.5
	*p (χ^2 test)	<0.001	<0.001	<0.001

FSS: Final staining score (average values); SBT: Serous borderline tumor; SC: Serous carcinoma; LGSC: Low-grade serous carcinoma; HGSC: High-grade serous carcinoma.

E-cadherin immunoreaction

Immunoreaction for E-cadherin was identified in 37 (74%) of the investigated cases in the epithelial tumor component in both SBTs and serous carcinomas, regardless of tumor grade and stage. The immunostaining was membranous for SBT and LGSC, or membranous and apical cytoplasmic in HGSC (Figure 1, A–C).

High E-cadherin FSS were identified in all analyzed tumor types, both in SBTs and in serous carcinomas, their mean value being 9.4 and 6.1, respectively, with moderate/strong intensity for the first category, and variable for the second one and a mean number of labeled cells of 69.4± 15.7 and 55.7±28.9, respectively, for the two types of lesions.

In relation with the carcinomas differentiation, the average number of labeled cells was 65±25.5 for LGSC and 53±29.8 for HGSC, the moderate/strong intensity for the first category and the variable for the second, the average FSS values being 7.4 and 5.7, respectively.

Related to tumor stage, E-cadherin immunoexpression presented high FSS mean values for early stages and low values for the advanced stages. Thus, for stages I/II, the average number of labeled cells was 74.5±12.1, the intensity was moderate/strong, with a mean FSS value of 9.1, while for stage III/IV, the mean number of E-cadherin-positive cells was 22.7±6.7, the intensity moderate/weak and the average FSS value 2.3.

The statistical analysis indicated significant differences in E-cadherin expression related to the tumor type (p=0.006, χ^2 test) and tumor stage (p<0.001, χ^2 test), as well as relation without significance depending on tumor grade (p=0.269, χ^2 test) (Figure 2, A and B).

P-cadherin immunoreaction

Immunoreaction for P-cadherin was identified in 35 (70%) of the investigated cases, also in the epithelial tumor component, both in SBT as well as in LGSC and HGSC. Immunorexpression was membranous for the stage I, II and III tumors, regardless of the histological grade, while the membranous and cytoplasmic pattern was identified in stages III and IV (Figure 3, A–C).

For SBT, the average number of labeled cells was 67.9±18.4, with moderate/strong intensity, and mean FSS value of 8.7, while for carcinomas the values were 66.4± 16.8 and 6.6, respectively, the intensity of the reactions being variable. Related to carcinomas differentiation, the average value of P-cadherin-positive cells was 65±19.1 for LGSC and 66.7±16.9 for HGSC, the mean FSS values being 7.7 and 6.4, respectively, while it was observed various reaction intensity for both categories.

Related to the stage, the average number of labeled cells for stages I/II was 71.7±14.8, and for stages III/IV was 56.5 ± 18.1, with predominantly strong reactions in early stages and predominantly weak/moderate intensity in the case of advanced stages. The average FSS values of P-cadherin were in case of stage I – 8.8, stage II – 10.5, stage III – 4.2, and stage IV – 2.6.

The statistical study revealed differences for P-cadherin expression depending on the tumor type (p=0.013, χ^2 test) and tumor stage (p<0.001, χ^2 test), as well as a relation without significance depending on tumor grade (p=0.601, χ^2 test) (Figure 4, A and B).

N-cadherin immunoreaction

Immunoreactivity for N-cadherin was present only membranous in 16 (32%) of the investigated cases, in the epithelial component of tumors (Figure 5A). We noticed the absence of positivity for all tumors in stage I. The immunostaining was identified only in HGSC, with low FSS values in stage II and higher in the tumors stages III and IV. The statistical analysis indicated significant differences in N-cadherin expression related to the tumor stage (p<0.001, χ^2 test) (Figure 5B).

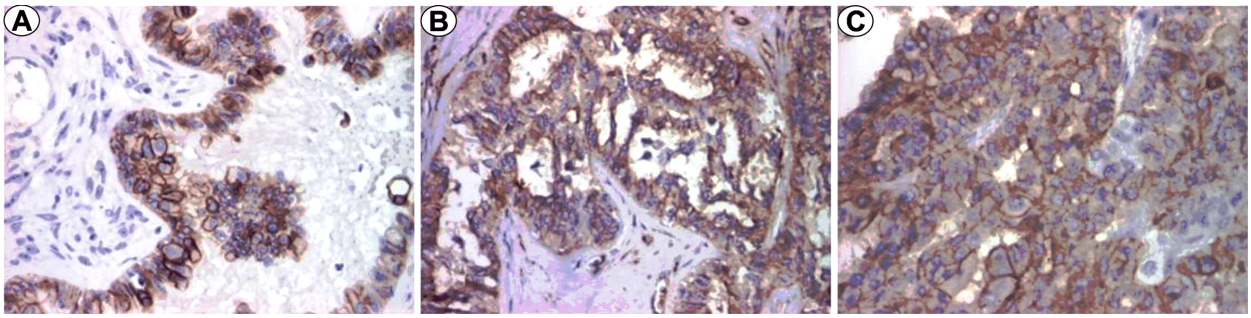

Figure 1 – *(A) Serous borderline tumor (SBT); (B) Low-grade serous carcinoma (LGSC); (C) High-grade serous carcinoma (HGSC). E-cadherin immunostaining: (A–C) ×200.*

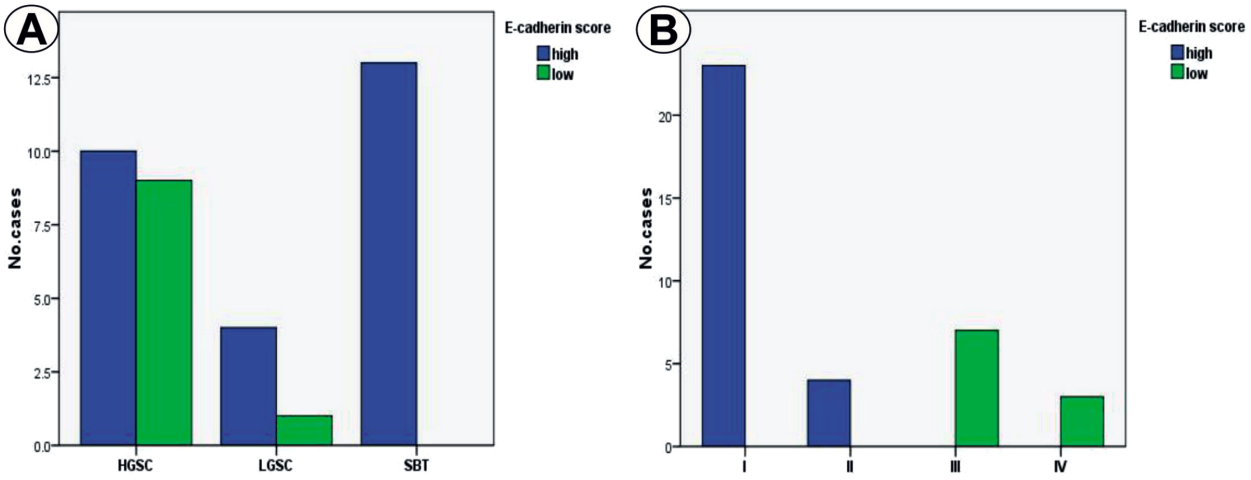

Figure 2 – *E-cadherin scores depending on tumor type (A) and tumor stage (B).*

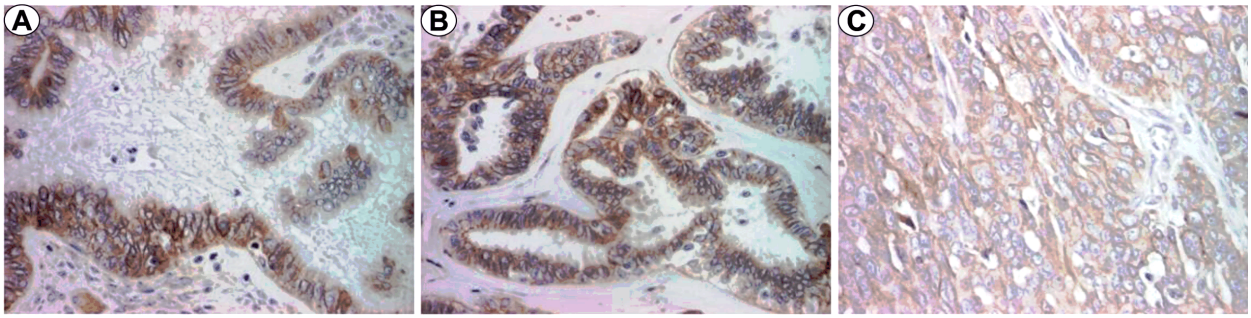

Figure 3 – *(A) Serous borderline tumor (SBT); (B) Low-grade serous carcinoma (LGSC); (C) High-grade serous carcinoma (HGSC). P-cadherin immunostaining: (A–C) ×200.*

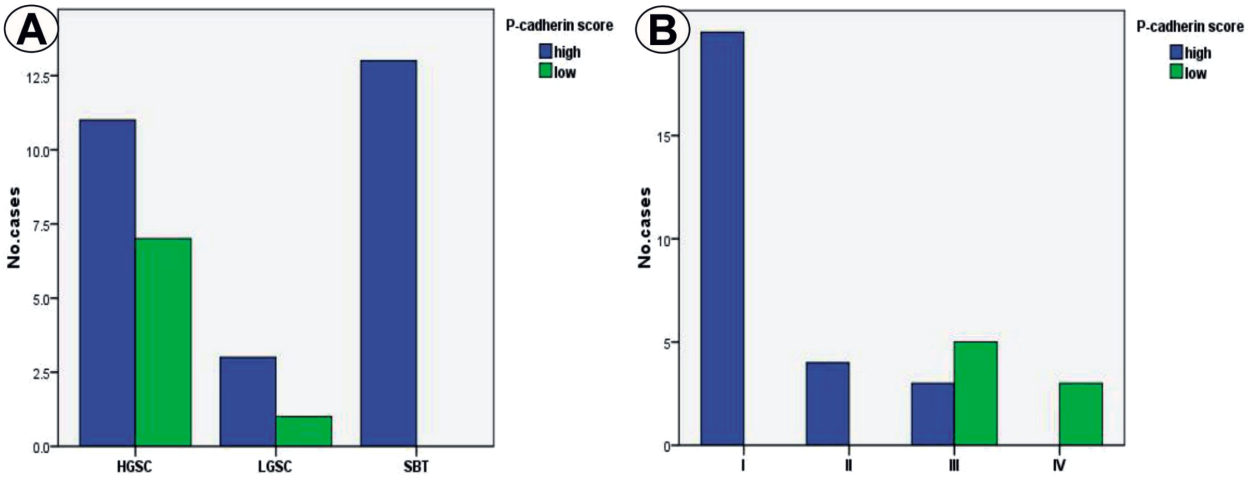

Figure 4 – *P-cadherin scores depending on tumor type (A) and tumor stage (B).*

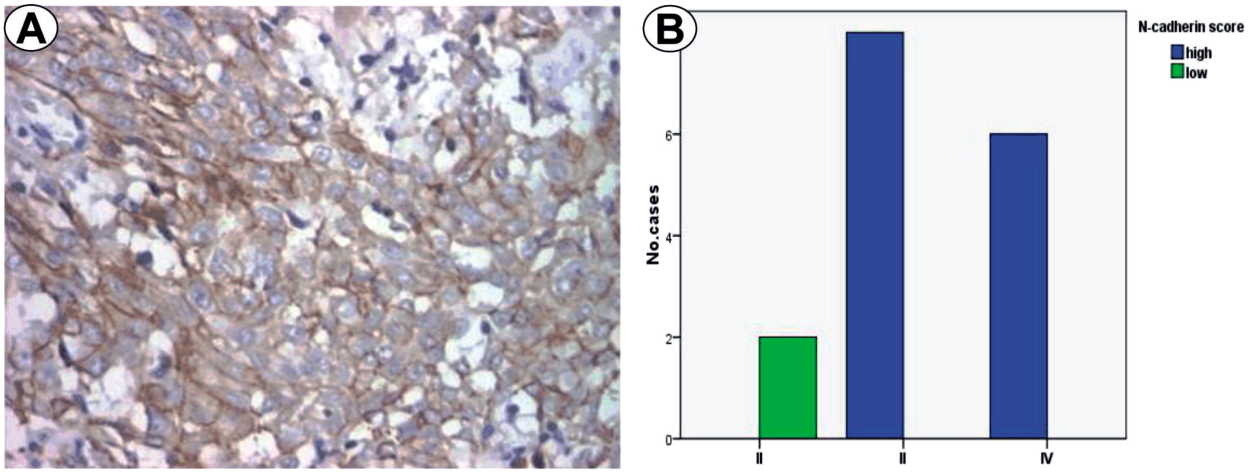

Figure 5 – ***(A) High-grade serous carcinoma (HGSC) (N-cadherin immunostaining, ×200); (B) N-cadherin scores depending on tumor stage.***

The distribution of percentage values for the investigated cadherins indicated a positive linear correlation between the E-/P-cadherin (p<0.001, Pearson's test), a negative linear correlation between E-/N-cadherin (p<0.001, Pearson's test), as well as a negative linear relation between P-/N-cadherin (p=0.246, Pearson's test).

Discussions

The development of ovarian cancer was associated with the modification of cadherin expression [12, 13]. In the majority of carcinomas, E-cadherin, which is involved in the intercellular adhesion maintenance, presents a decrease in expression during malignant shift and progression [5, 14], the aspect being observed in classical EMT together with the presence of N-cadherin expression.

In this study, E-cadherin was expressed in 74% of the cases, the pattern of the expression being membranar only in SBT and LGSC of stages I and II, in advanced stages, respectively III and IV, the immunostaining was membranous and cytoplasmic apical. P-cadherin had expression in 70% of cases, with membranous pattern for stage tumors I, II and III, regardless of histological grade, while the membranous and cytoplasmic pattern was identified in stages III and IV. The expression of N-cadherin was identified in only 32% of cases, only in HGSC and in stages II, III and IV of the disease. We noticed that the aberrant pattern of E- and P-cadherin expression was present in the advanced stages of the disease, in which we noticed the presence of N-cadherin expression.

Similar aspects have been reported in other studies that have observed that the patterns of E-cadherin immunoexpression in normal and tumoral ovaries are complicated and do not completely respect the classical EMT model [15]. The ovarian surface epithelium, believed to originate at least for a group of ovarian epithelial tumors, has morphogenesis and anatomical continuity with the epithelium of peritoneum, expressing N-cadherin and is negative for E-cadherin, the last presenting expression just in cysts of inclusion, suspected as precursors of ovarian epithelial tumors [16]. Moreover, while the immunoexpression of E-cadherin is diminished in some primitive ovarian epithelial tumors, it is expressed again later in peritoneal metastases, the levels of expression being higher then the primitive carcinomas, suggesting that in ovarian carcinoma the tumor cells undergoes an incomplete EMT [17]. Another aspect in this direction is the capacity of ovarian tumor cells to coexpress E- and N-cadherins [18]. In addition, it has been reported that P-cadherin, which is frequently expressed in ovarian carcinomas, is the major cadherin involved in progression of stage I and II tumors [19].

A relationship between low levels of total and membranous E-cadherin and unfavorable prognosis was observed [20–22], E-cadherin messenger ribonucleic acid (mRNA) expression being a marker for differentiation between advanced stage ovarian tumors and the early stage lesions [22]. Unlike the total and membranous expression of E-cadherin, no correlation with clinical-pathological parameters was found for cytoplasmic expression of E-cadherin mRNA, and nuclear expression of E-cadherin was associated only with tumor grade, a greater proportion of low-grade tumors presenting this signal when it was compared to high-grade ones [22].

Hudson *et al.* have been observed that the ovarian epithelial tumors present significant heterogeneity of cadherin expression ranging from "pure" positive E-cadherin or N-cadherin neoplasms to "mixed cadherin" and "hybrid cadherin" phenotypes [16]. It has been suggested that the development of a partial EMT allows the creation of a new group of hybrid cells, as well as completely differentiated cells [23], followed by the *de novo* gain of adaptation and resistance to radiation and drugs [24–26].

The statistical analysis of percentages of E-, P- and N-cadherins immunoreactions allowed us to observe a significant positive linear correlation between E-/P-cadherin, a significant negative linear correlation between E-/N-cadherin, and a negative linear but statistically not significant relationship between P-/N-cadherin. These aspects confirm the involvement of the "cadherinic switch" in the progression of malignant serous ovarian tumors, the decrease in E-cadherin expression (but not P-cadherin) being associated with the acquisition of N-cadherin expression.

Adham *et al.* observed that, unlike E-cadherin, N-cadherin was expressed in all cases, both in the stroma and in the epithelium of normal ovary and benign serous tumors, as well as in 61.5% of ovarian serous carcinomas, significantly related to the tumor stage [27]. In an extensive study on HGSC, overexpression of E-cadherin was associated with better survival, whereas overexpression of N-cadherin and P-cadherin had opposite significance [28]. The increased

of P-cadherin immunoexpression was related with a reduced survival and was statistically significant higher in stage II *versus* stage I and III of the disease, in contrast to the loss of E-cadherin expression in stage III compared to the rest of the stages, indicating the that cadherin switching influences the progression of HGSC [28].

Conclusions

The ovarian serous malignant tumors express E-, P- and N-cadherins in varying proportions, cadherin switching being associated with disease progression, which supports the involvement of EMT in ovarian carcinogenesis. Establishing cadherinic phenotype in ovarian serous tumors may improve the patient stratification criteria for antitumor therapy. Specific therapies can bring superior survival to these tumors, but the cadherin profile in ovarian carcinomas and their association with prognosis require further studies.

Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper. All authors read and approved the final manuscript.

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