The Epithelial-Mesenchymal Transition, E-cadherin and Tumor Progression in Ovarian Serous Tumors

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**Background:** The epithelial-mesenchymal transition (EMT), which is a change in the cell phenotype from epithelial to mesenchymal morphology, is an important step in the invasion process and metastasis of ovarian carcinomas. It is known that the suppression of cell adhesion molecules such as E-cadherin and the expression of mesenchymal markers such as Vimentin are key processes in EMT. There is controversy in the literature about the EMT status of ovarian carcinomas.

**Aim:** To investigate EMT status using immunohistochemical expression of E-cadherin in benign, primary malignant serous ovarian tumors and metastases from them in order to assess their significance in tumor progression.

**Materials and methods:** The study included a retrospective investigation of 217 ovarian epithelial tumors. Ninety-two cases of serous ovarian tumors and metastases were examined for expression of E-cadherin.

**Results:** In our study, the predominant histological subtype in benign ovarian tumors and carcinomas was serous (73% and 61%, respectively). 65% of benign tumors demonstrated EMT negative status. The majority of carcinomas demonstrated EMT positive status (82%), whereas negative EMT status was only observed in 18% of cases. 89% of the metastases showed EMT positive status, whereas only 11% of them showed negative EMT status. In 6 selected cases with positive EMT status we found Vimentin expression in tumor cells.

**Conclusion:** Positive EMT status (reduced E-cadherin expression) is a characteristic of ovarian carcinomas and metastases, but not of benign serous ovarian tumors.

**BACKGROUND**

Epithelial-mesenchymal transition (EMT), which is a change in the epithelial cell phenotype in mesenchymal morphology, is an important step in the invasion process and metastasis of ovarian carcinomas. This process is associated with the suppression of apical and basolateral cell adhesion molecules such as E-cadherin and cytokeratins and the expression of mesenchymal markers such as Vimentin.

EMT and MET (mesenchymal-epithelial transition) in carcinogenesis are determined by 4 major changes in the cell phenotype. These changes correspond to the successive stages of the metastatic process and in particular the formation of peritoneal metastases in ovarian carcinomas. At the initial stage, cancerous epithelial cells undergo genetic alterations leading to EMT. It is noted with loss of expression of epithelial markers such as E-cadherin and cytokeratin 18 and increased expression of mesenchymal markers such as Vimentin and fibronectin. These changes cause severely reduced cell adhesion. Tumor epithelial cells acquire a spindle shape with loss of cellular polarity, increased mobility and increased proliferative potential. EMT allows cancer cells to survive hypoxic conditions in the tumor. In the next step, single cells or small groups of cells separated from the primary tumor are distributed over the peritoneum and the omentum carried by the physiological movements of the peritoneal fluid. Tumor cells invade the extracellular matrix in lymph and blood vessels. In the third stage, the tumor cells reach the site of implantation. At the same time, loss of E-cadherin leads to increased fibronectin receptor production, which allows cancer cells to adhere to the site of future metastasis. At the time of reaching the new implant site, the cancer cells undergo a mesenchymal-epithelial transition (fourth stage) and their phenotype is transformed from mesenchymal to epithelial. This allows them to respond to paracrine growth factors and provides...
rapid metastatic growth.

E-cadherin-mediated cell adhesion is inactivated by various mechanisms in malignant tumors. It is known that the suppression of the E-cadherin / β-catenin complex as well as the enhancement of Vimentin are key processes in the EMT.

Decreased expression of E-cadherin is frequently observed in carcinomas. Similarly, some authors observed decreased expression of E-cadherin in ovarian carcinomas.

In most studies, E-cadherin membrane expression was observed in benign and malignant ovarian tumors but is absent in normal ovarian tissues. In another study, membrane E-cadherin expression was strongly positive in 84.8% of ovarian cancer patients. Other authors reported a small number of ovarian carcinomas with strong E-cadherin expression.

In several studies in primary ovarian carcinomas E-cadherin membrane expression was strong, whereas in advanced cases it is moderate, although complete loss of E-cadherin expression was rarely observed. Reduced expression of E-cadherin was described in a higher percentage in borderline and malignant ovarian tumors compared to benign ovarian tumors.

The initial process that causes EMT is a disruption of E-cadherin-mediated intercellular interaction. Reduced E-cadherin expression was observed in a very advanced stage of ovarian carcinoma. On the other hand, another study showed that E-cadherin expression is significantly elevated in ovarian carcinoma metastases compared to primary carcinomas, suggesting that E-cadherin expression changes periodically during ovarian carcinoma development and is required for the growth of primary and metastatic tumors. Over-regulation of E-cadherin in peritoneal metastases of ovarian carcinomas can be an event in tumor progression and is possibly linked to mediation of survival of tumor cells at the new site by inhibition of anoikis and apoptosis or can be considered as an element of MET as demonstrated in other types of carcinomas.

AIM
To investigate EMT status using immunohistochemical (IHC) expression of E-cadherin in benign, primary malignant serous ovarian tumors and metastases from them in order to assess their importance in tumor progression.

MATERIALS AND METHODS
The study included a retrospective investigation of 217 ovarian epithelial tumors over a period of 3 years (2012-2014). Of these, we selected 92 cases of serous histological subtype tumors, which were immunohistochemically investigated: a total of 31 benign ovarian serous tumors, 33 serous ovarian carcinomas and 28 metastases from serous ovarian carcinomas. Inclusion criteria were: 1) cases of histologically confirmed clinical diagnosis of ovarian serous tumor; 2) cases of metastases in the peritoneum and other sites with histologically proven origin of primary ovarian serous carcinoma.

The histological and immunohistochemical investigation of the biopsy materials was performed in the laboratory of the Department of Clinical Pathology of St George University Hospital, Plovdiv.

IMMUNOHISTOCHEMICAL ANALYSIS
Immunohistochemistry was performed with an antibody of Leica Biosystems Newcastle Ltd: Bond E-Cadherin, clone 36B5, ready to use, 7 mL, cat. No. PA0387. 4 mkm slides were deparaffinized and rehydrated in descending alcohols. Washing was done with BondTM wash solution. An antigenic recovery was performed by incubation in Epitope Retrieval Solution 2, pH9. Novolink Polymer Detection System for visualization was used. For the positive control of E-cadherin, prostate tissue was selected according to the manufacturer’s instructions. For positivity of E-cadherin we accepted clear membrane or cytoplasmic staining of tumor cells.

To assess IHC expression of E-cadherin, we used a modified system of Takai M et al. : 0 - negative and with a positivity of less than 10% of tumor cells in order to eliminate false positive results; 1+ - weak expression of more than 10% of tumor cells; 2+ - moderate expression of more than 10% of tumor cells; 3+ - strong expression of more than 10% of tumor cells.

For the staining pattern, we used the system of Giurgea L et al., which they applied to evaluate the expression of p53 and Ki-67, but we considered it suitable for evaluation of E-cadherin in order to detect areas of the tumor with different intensity of IHC expression: 1) a focus pattern with small number of tumor expressing cells; 2) a heterogeneous pattern with islands of strong or moderate positive expression alternating with regions of weak positive expression; 3) a diffuse pattern representing diffuse positivity.

In our study, we also detected areas with nega-
tive IHC expression for E-cadherin. Similar to Takai M et al., for EMT-negative status (preserved E-cadherin expression), we adopted 3+ evaluation of E-cadherin expression, and for EMT-positive status (reduced E-cadherin expression) - 0, 1+, 2+ and the presence of negative expression regions, regardless of the evaluation.

**STATISTICAL ANALYSIS**

Statistical hypotheses with accurate p-value calculation were applied. For comparisons with variables of discrete meaning, Fisher’s exact test was applied. Calculations were made using R-statistics and MS Excel 2016.

**RESULTS**

The predominant histological subtype of benign ovarian tumors in our study was serous subtype - 58/80 (73%), followed by mucinous - 22/80 (27%). In all examined ovarian carcinomas predominated the serous histological subtype - 42/69 (61%). Mucinous ovarian carcinomas were the second most frequent subtype - 18/69 (26%). The endometrioid histological subtype of carcinomas was 12% (8/69). The most uncommon was clear cell carcinoma subtype - 1%.

**ASSESSMENT OF IMMUNOHISTOCHEMICAL EXPRESSION OF E-CADHERIN.**

From immunohistochemically tested benign tumors, all cases showed positive E-cadherin expression. A total of 20/31 cases (65%) had strong E-cadherin expression in over 10% of tumor cells (Fig. 1), 9/31 cases (29%) showed moderate expression and in only 2/31 cases we observed weak expression (Table 1). The majority of benign tumors demonstrated EMT negative status - 20/31 (65%) (Table 1). Regarding the localization of expression, we observed membrane staining in all examined benign ovarian tumors.

Of the immunohistochemically examined ovarian carcinomas, strong E-cadherin expression showed 6/33 cases (18%), moderate - 11/33 cases (33%), weak expression - 13/33 cases (39%) and the remaining 3 cases were E-cadherin negative. The majority of carcinomas demonstrated a positive EMT status of - 27/33 (82%) (Fig. 2), whereas negative EMT status was only reported in 18% of cases (6/33) (Table 1). In all of the carcinomas examined, the localization of the expression was membranous.

In the metastatic group of serous ovarian carcinomas, only 5/28 cases (18%) showed strong E-cadherin expression, in 2 of them we observed areas with negative expression (1 case of metastasis in omentum and 1 case of metastasis in the wall of small intestine), 13/28 cases showed moderate expression (46%), 5/28 - weak expression and 5/28 were negative (Table 1). Similar to the carcinoma group, the majority of the tested metastases had a positive EMT status - 25/28 (89%) (Fig. 3), whereas only 3/28 cases (11%) demonstrated negative EMT status (Table 1). In all investigated metastases, the localization of the expression was membranous.

We found a statistically significant difference between the study groups and their EMT status (Fisher’s exact test, p<0.001) (Table 1).

In 6 selected cases of positive EMT status (3 high-grade serous carcinomas and 3 metastases from high-grade serous carcinomas) we performed investigation of IHC expression of Vimentin. In these cases we found positive membrane expression of Vimentin in tumor cells (Fig. 4).

In the comparative study on patterns of immunohistochemical staining for E-cadherin we found that the dominant pattern in benign tumors was diffuse while in carcinomas and metastases - heterogeneous. There was a statistically significant difference between the pattern and the investigated groups (Fisher’s exact test, p<0.001) (Table 2).

In the evaluation of E-cadherin expression we found the following immunohistochemical characteristic of each of the studied groups. Benign serous ovarian tumors were characterised by strong membrane expression of E-cadherin (negative EMT status), diffuse pattern of staining. Serous ovarian carcinomas were characterized by weak membrane expression of E-cadherin (positive EMT status), heterogeneous pattern of staining. Metastases from serous ovarian carcinomas were characterized by moderate membrane expression of E-cadherin (positive EMT status), heterogeneous pattern of staining.

**DISCUSSION**

EMT is a fundamental process in tumor pathogenesis. EMT and MET help tumor cells to avoid programmed cell death and to adapt to unfavorable conditions (such as hypoxia) without losing vital receptors that will allow them to benefit from the growth factors of the macro-organism at the new metastatic site. Thus, tumor cells use basic mechanisms in embryogenesis and morphogenesis for their survival, providing invasion and metastasis of the tumor.

Since EMT is a transition from epithelium to mesenchymal morphology of tumor cells, decreased
immunohistochemical expression (negative, weak and moderate expression) of epithelial markers such as E-cadherin is reported as positive EMT status of the investigated tumors.\(^6\) The reduction of E-cadherin expression may occur only in clusters of cells that are specific clones in the tumor with different adhesive properties.\(^8\) In order to be more precise in respect to the results for EMT positive status, in our study E-cadherin positive cases with presence of negative expression regions (heterogeneous pattern of staining) were assigned to the group of tumors with EMT positive status.

Relatively few studies on EMT include benign ovarian tumors. In the present study, all immunohistochemically tested benign tumors showed positive E-cadherin expression. The majority of them - 20/31 cases (65%) had a strong E-cadherin expression in over 10% of the tumor cells. Our results are similar to the results of other studies.\(^8,9,17\)

Concerning the EMT status of ovarian carcinomas, the data in the literature are contradictory. Some studies showed a strong expression of E-cadherin (negative EMT status) in serous ovarian carcinomas.\(^8,10\) In other studies, strong E-cadherin expression was not characteristic of ovarian carcinomas.\(^11,12,14,18,19\) Most of the carcinomas in our study demonstrated EMT positive status - 27/33 cases (82%), while negative EMT status was only
observed in 18% - 6/33 cases. Furthermore, in the subgroup of positive EMT status carcinomas in our study, cases of weak E-cadherin expression were predominant - 13/33 cases (39%). The results of our study support the above-mentioned group of studies, in which ovarian carcinomas were characterized by positive EMT status. In all the examined carcinomas in the presented study, the localization of E-cadherin expression was membranous, similar to the localization described by other authors.8,10

In our study, the results regarding the EMT status of carcinomas and metastases were similar. Similar to the group of carcinomas, the majority of the examined metastases had a positive EMT status (89%), whereas only 11% demonstrated negative EMT status, although in the subgroup of metastases with EMT positive status predominated cases of moderate E-cadherin expression (48%). In all investigated metastases, the localization of the expression was membranous. According to the literature, in several studies metastases of ovarian carcinomas showed moderate E-cadherin expression and have a positive EMT status.12,13,17 Our results supported these studies. The predominance of metastases with moderate E-cadherin expression, considering that low E-cadherin expression was predominant in the carcinoma group, can be explained as an element of MET that arises in order to provide metastatic growth at the new site.

According to some authors, a complete loss of E-cadherin expression in ovarian carcinomas and metastases is rarely observed.20 Our results support this claim, as only 3 of the 33 studied carcinomas and 5 of the 27 metastases were E-cadherin negative.

In order to support the data on the positive EMT status of carcinomas and metastases, we examined ICH expression of Vimentin in 6 selected cases (3 high-grade serous carcinomas and 3 metastases of high-grade serous carcinomas). In these cases, we found membrane expression of Vimentin in tumor cells, which confirmed that in decreased expression of epithelial markers (E-cadherin) there was a parallelly increased expression of mesenchymal markers (Vimentin) in tumor cells and demonstrated the presence of EMT in the investigated tumors. Similar results have

|                          | N          | Benign serous ovarian tumors | Serous ovarian carcinomas | Metastases from serous ovarian carcinomas |
|--------------------------|------------|-----------------------------|--------------------------|------------------------------------------|
| Strong E-cadherin expression | 20 (65%)  | 6 (18%)                     | 5 (18%)                  |
| Moderate E-cadherin expression | 9 (29%)  | 11 (33%)                    | 13 (46%)                 |
| Weak E-cadherin expression | 2 (6%)    | 13 (39%)                    | 5 (18%)                  |
| Negative E-cadherin expression | 0        | 3 (9%)                      | 5 (18%)                  |
| EMT positive status       | 11 (35%)  | 27 (82%)                    | 25 (89%)                 |
| EMT negative status       | 20 (65%)  | 6 (18%)                     | 3 (11%)                  |
| Total                     | 31 (100%) | 33 (100%)                   | 28 (100%)                |

|                          | N          | Benign serous ovarian tumors | Serous ovarian carcinomas | Metastases from serous ovarian carcinomas |
|--------------------------|------------|-----------------------------|--------------------------|------------------------------------------|
| Diffuse pattern          | 29 (94%)  | 7 (23%)                     | 7 (30%)                  |
| Heterogeneous pattern    | 2 (6%)    | 23 (77%)                    | 16 (70%)                 |
| Total                    | 31 (100%) | 30 (100%)                   | 23 (100%)                |
been reported by other authors. Since clusters of cells that are specific clones in the tumor are thought to exhibit different adhesive properties and to show a different EMT status, we considered it necessary, according to the purposes of our study, to take into account not only the level of E-cadherin expression but also the pattern of staining. The predominant pattern for E-cadherin in carcinomas and metastases was heterogeneous. Similar results have been observed by other authors.

CONCLUSION

Our study showed that the reduction of E-cadherin expression plays an important role in tumor progression of serous ovarian tumors. Positive EMT status is a characteristic of ovarian serous carcinomas and metastases, but not of benign serous ovarian tumors.

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Эпителиально-мезенхимальный переход, E-кадгерин и развитие опухолей при серозных опухолях яичников

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Введение: Эпителиально-мезенхимальный переход (ЭМП), который представляет собой изменение клеточного фенотипа от эпителиальной к мезенхимальной морфологии, является важным этапом инвазии и метастазирования рака яичников. Было подтверждено, что подавление молекул клеточной адгезии, таких как E-кадгерин, и экспрессия мезенхимальных маркеров, таких как Виметин, являются ключевыми процессами в ЭМП. В литературе существует противоречие относительно ЭМП-статуса опухолей яичников.

Цель: Изучить состояние ЭМП с помощью иммуногистохимической экспрессии Е-кадгерина в доброкачественных, первичных злокачественных серозных опухолях яичников и их метастазов, чтобы оценить их значение в развитии опухоли.

Материалы и методы: Исследование включало ретроспективное исследование 217 опухолей эпителия. 92 случая серозных опухолей яичников были исследованы на предмет экспрессии Е-кадгерина.

Результаты: В нашем исследовании преобладающим гистологическим подтипом при доброкачественных опухолях яичников и карциномах был серозный (73% и 61% соответственно). 65% доброкачественных опухолей показали отрицательный статус ЭМП. Большинство карцином показали положительный статус (82%), в то время как отрицательный статус наблюдался только в 18% случаев. 89% метастазов показали ЭМП-положительный статус, тогда как только 11% из них имели отрицательный ЭМП-статус. В 6 из отобранных случаев с положительным статусом ЭМП мы обнаружили экспрессию Вимтеина в опухолевых клетках.

Заключение: Положительный ЭМП-статус (пониженная экспрессия Е-кадгерина) характерен для рака яичников и метастазов, но не для доброкачественных серозных опухолей яичников.