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Immunoexpression of Beta-catenin – E-cadherin Complex in Primary Serous Ovarian Tumors

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Ovarian cancer takes a fourth place as cause of death from all cancers and a first place from gynecologic malignancies in Poland. Up to now, relatively little is known about immunohistochemical markers accepted as prognostic indicators for ovarian cancers. Recent investigations took a note of prognostic significance of catenin-cadherin adhesion complex and Ki-67 proliferation protein in serous ovarian cancers. The aim of the study was to evaluate the immunoexpression of beta-catenin and E-cadherin in metastatic and non-metastatic serous ovarian tumors, as well as to find possible relationships between this immunoexpression and tumor proliferation activity. The analysis comprised of 66 women diagnosed and treated for epithelial ovarian tumors. On immunohistochemical examinations it was found a significantly lower immunoexpression of beta-catenin and E-cadherin in invasive serous ovarian cancers than in cystadenomas. Additionally, in metastatic group the immunoexpression of both beta-catenin and E-cadherin was significantly decreased as compared with patients without metastases. Moreover, the significant inverse correlations have been shown between immunoexpression of Ki-67 and beta-catenin as well as Ki-67 and E-cadherin. In conclusion, our data suggest that decreased immunoexpression of beta-catenin and E-cadherin in serous ovarian tumors may be helpful in identifying the cases of higher metastatic potential and infiltration ability.

Introduction

Ovarian cancer is the sixth most common form of women malignancy in Poland, and accounts for 4% of all women cancers. It has a high mortality as it takes a fourth place as cause of death from all cancers and a first place from gynecologic malignancies in Poland. The 5-year survival rate depends on time of diagnosis and on the stage of disease. For patients with early-stage disease, the 5-years survival is about 80 - 90% compared to about 25% for patients with advanced-stage disease [3]. In western countries the survival rate is higher in advanced-stage disease, and it takes about 52% [2]. The classic prognostic factors for ovarian cancer are: the clinical stage (FIGO classification), degree of differentiation, size of tumor, presence (or absence) of peritoneal implants and their invasiveness, volume of residual disease and the patient's age. Up to now, relatively little is known about immunohistochemical markers accepted as prognostic indicators for ovarian cancers. Last years investigations took a note of prognostic significance of adhesion and cell proliferation proteins in serous ovarian cancers [5, 11, 21, 22]. The significance of beta-catenin and E-cadherin adhesion proteins as well as Ki-67 (MIB-1) proliferation protein is especially emphasized but literature is rather scant yet.

Therefore, the aim of the present study was to evaluate the immunoexpression of beta-catenin and E-cadherin in metastatic and non-metastatic serous ovarian tumors, as well as to find the possible relationships between this im-

munoexpression and tumor proliferation activity expressed by Ki-67 immunoexpression.

Materials and Methods

Patients

The analysis comprised of 66 women diagnosed and treated for epithelial ovarian tumors at Gynecology and Obstetrics Institute of Medical University of Lodz between 1997 and 2002. Women were aged from 19 to 83 years (mean ± SD = 54.8 ± 14.4). Fifteen women aged from 19 to 75 years (mean ± SD = 52.5 ± 17.7) had benign tumors – cystadenomas of the ovary. Eight women aged from 36 to 68 years (mean ± SD = 55.4 ± 10.5) were treated from borderline serous ovarian tumors. Eight women aged from 24 to 73 years (mean ± SD = 48.6 ± 14.0) had G1 serous cancer. In this group there were no women with cancer in FIGO IV and III stage, 3 women (37.5%) had tumor in FIGO stage II and 5 patients (62.5%) – in stage I. Fourteen women aged from 31 to 83 years (mean ± SD = 56.6 ± 16.7) were treated from G2 serous cancer. In this group 2 women (14.29%) had cancer in FIGO IV stage, 7 women (50.0%) – in stage III, 3 women (21.42%) had tumor in FIGO stage II and 2 patients (14.29%) – in stage I. Twenty one women aged from 38 to 80 years (mean ± SD = 57.2 ± 11.6) had G3 serous cancer. In this group 5 women (23.81%) had cancer in FIGO IV stage, 13 women (61.91%) – in stage III, one woman (4.76%) had tumor in FIGO stage II and 2 women (9.52%) – in stage I.

For statistical purposes all patients were also divided into metastatic and non-metastatic groups. Seventeen of patients (80.95%) with G3 cancers had metastatic lesions. In group of patients with G2 cancer, metastases were found in 9 cases (64.29%) and in group of patients with G1 cancer, metastatic lesions were stated in 3 women (37.5%). All metastatic patients stated 29 cases (67.44%), out of 43 malignant cases. All borderline cases were included into the non-metastatic group of patients. The mean age in metastatic group was 56.59 years with SD = ± 13.01 years, whereas in non-metastatic group this value was formed at the level of 53.91 years with SD = ± 13.99 years. In part of malignant cases (4 women with G3 tumors, 4 with G2 tumors and 1 with G1 tumor) the material comes up both from the operation of primary tumor and from re-operation of residual tumor.

Light microscopy

Paraffin embedded tissue sections taken from postoperative material were diagnosed using a standard haematoxylin and eosin staining.

Immunohistochemistry

Paraffin sections were mounted on Superfrost slides, deparaffinised, then treated in a microwave oven in a solution of citrate buffer, pH 6.0 for 2 x 5 minutes and transferred to distilled water. Endogenous peroxidase activity was blocked by 3% hydrogen peroxide in distilled water for 5 minutes, and then sections were rinsed with Tris-buffered saline (TBS, DakoCytomation, Denmark) and incubated overnight at 4°C with the primary antibody for E-cadherin (mouse monoclonal anti-human E-cadherin, Dako; dilution 1:100), for beta-catenin (mouse monoclonal anti-human beta-catenin, Novocastra; dilution 1:200), and for Ki-67 (mouse monoclonal anti-human Ki-67; Novocastra; dilution 1:100). Afterwards LSAB+/HRP Universal kit (DakoCytomation, Denmark) prepared according to the instructions of the manufacturer was used. Visualisation was performed by incubating the sections in a solution of 0.5 mg 3.3'-diaminobenzidine (DakoCytomation, Denmark), per ml Tris-HCl buffer, pH 7.6, containing 0.02% hydrogen peroxide, for 10 minutes. After washing, the sections were counter-stained with haematoxylin and coverslipped. For each antibody and for each sample a negative control was processed. Negative controls were carried out by incubation in the absence of the primary antibody and always yielded negative results. The immunoexpression of beta-catenin and E-cadherin was graded semiquntitavely by two independent observers and graded from 0 (negative), 1 (weakly positive), 2 (moderately positive) and 3 (strongly positive). The mean grade was calculated by averaging grades assigned by the two authors and approximating the arithmetical mean to the nearest unity.

Morphometry

The Ki-67 immunoexpression was assessed by means of image analysis system consisting of a PC computer equipped with a Pentagram graphical tablet, Indeo Fast card (frame grabber, true-color, real-time), produced by Indeo (Taiwan), and color TV camera Panasonic (Japan) linked to a Carl Zeiss microscope (Germany). This system was controlled by MultiScan 8.08 software, produced by Computer Scanning Systems, Poland, working under macroinstructions written specially for this analysis. A percentage of immunopositve cells in a 1000 tumor cells for each slide was measured.

Statistical analysis

Differences between groups were tested using one-way ANOVA with post-hoc LSD test, preceded by evaluation of normality and Levene's test. The Mann-Whitney U

test was used where appropriate. Correlation coefficients were calculated using Spearman's method. Results were considered statistically significant if $p<0.05$.

Results

On immunohistochemical examinations (Fig. 1-4) it was found a significantly lower immunoexpression of beta-catenin and E-cadherin in invasive serous ovarian cancers than in cystadenomas. In the borderline tumors, the significantly higher immunoexpression of investigated complex was observed in comparison with a group of G2 and G3 cancers. There was significant difference between cystadenocarcinomas of G1 and G2 group for beta-catenin, while we did not observed the similar relationship for E-cadherin immunoexpression.

In metastatic group the immunoexpression of both E-cadherin and beta-catenin proteins was significantly decreased as compared with patients without metastases of ovarian cancer. Conversely, it was found a statistical higher immunoexpression of Ki-67 in serous adenocarcinomas (G1, G2 and G3) than in serous cystadenomas and cystadenomas of borderline malignancy (Table 1). Also it was stated, that there were statistically significant differences among immunoexpression of Ki-67 in particular groups of cancer-increasing with a histological grade of cancer. There was no statistical difference between Ki-67 immunoexpression in serous cystadenomas and in borderline tumors. On the other hand, Ki-67 immunopositive cells were significantly more numerous in metastatic group in comparison with non-metastatic patients (Table 2). Moreover, in analyzed group of patients with serous ovarian tumors, significant inverse correlations have been shown between immunoexpression of Ki-67 and beta-catenin ($r = - 0.79$, $p<0.001$) (Fig. 5) as well as Ki-67 and E-cadherin ($r = - 0.80$, $p<0.001$) (Fig. 6).

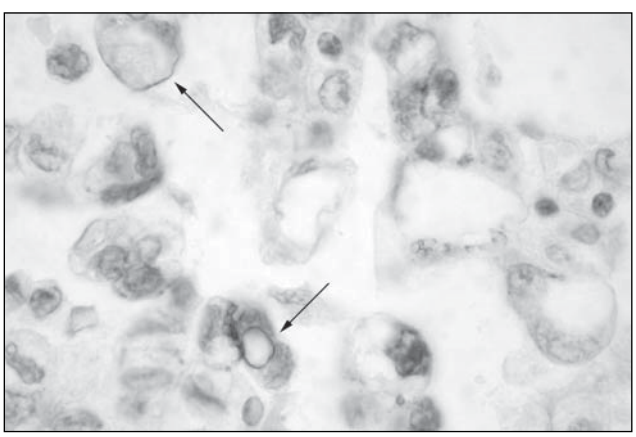

Fig. 1. The immunoexpression of beta-catenin in G3 ovarian cancer (arrows). Magn. 400 x.

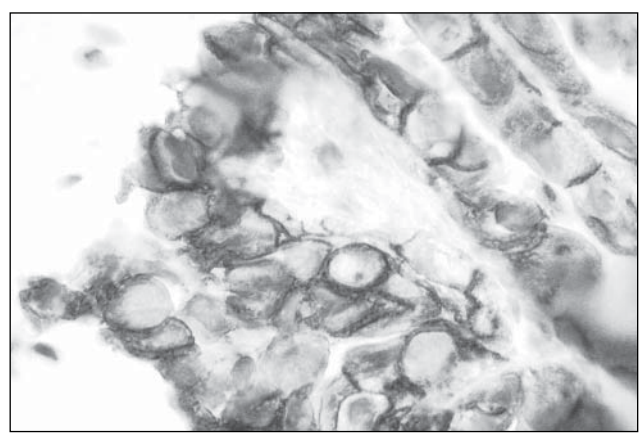

Fig. 2. The immunoexpression of beta-catenin in borderline ovarian cystadenoma. Magn. 400 x.

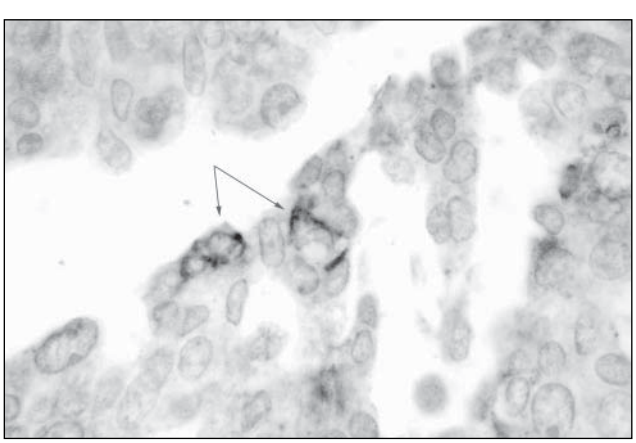

Fig. 3. The immunoexpression of E-cadherin in G3 ovarian cancer (arrows). Magn. 400 x.

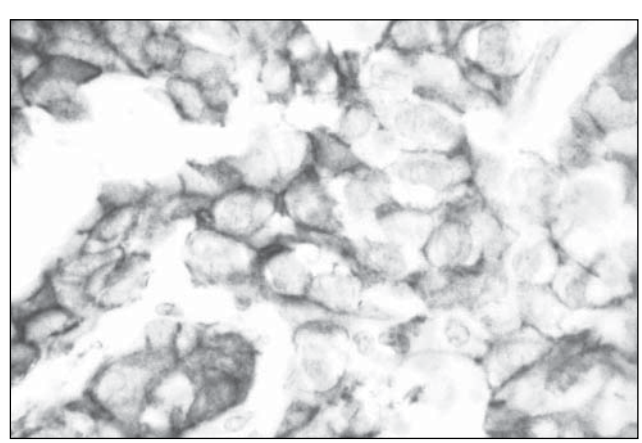

Fig. 4. The immunoexpression of E-cadherin in borderline ovarian cystadenoma. Magn. 400 x.

TABLE 1
The immunoexpression of beta-catenin, E-cadherin and Ki-67 in serous primary ovarian tumors

Group	Beta-catenin	E-cadherin	Ki-67 (percent)
Serous cystadenoma (n=15)	2.16±0.54[3,4,5]	1.92±0.44[3,4,5]	1.49±1.53[3,4,5]
Cystadenoma of borderline malignancy (n=8)	2.13±0.50[4,5]	1.95±0.54[4,5]	6.27±4.58[3,4,5]
Cystadenocarcinoma G1 (n=8)	1.98±0.41[4,5]	1.65±0.55[5]	14.07±7.33[1,2,4,5]
Cystadenocarcinoma G2 (n=14)	1.52±0.45[1,2,3,5]	1.29±0.51[1,2,5]	31.59±8.94[1,2,3,5]
Cystadenocarcinoma G3 (n=21)	1.06±0.49[1,2,3,4]	0.77±0.43[1,2,3,4]	39.86±9.34[1,2,3,4]

Statistically significant ($p<0.05$) versus:
[1)] serous cystadenoma
[2)] cystadenoma of borderline malignancy
[3)] cystadenocarcinoma G1
[4)] cystadenocarcinoma G2
[5)] cystadenocarcinoma G3

TABLE 2
The immunoexpression of beta-catenin, E-cadherin and Ki-67 in patients with and without metastases of serous primary ovarian tumors

Group	Beta-catenin	E-cadherin	Ki-67 percent
Patients with metastases (n=29)	1.27±0.65	1.04±0.64	33.65±12.89
Patients without metastases[1] (n=22)	1.76±0.49	1.45±0.60	22.23±15.37
P value	<0.004	<0.03	<0.006

[1)] This group consists of cancer patients without metastases and patients with borderline tumors.

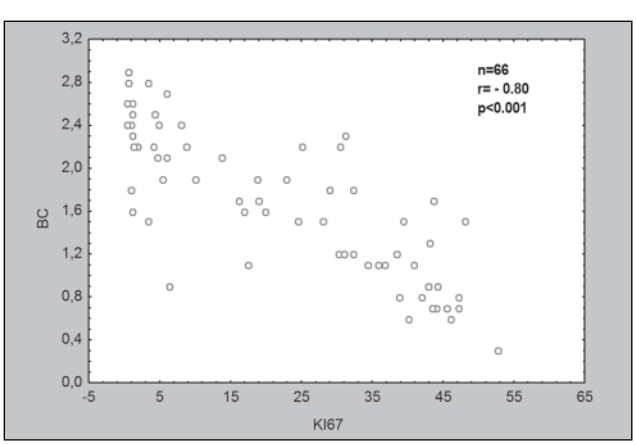

Fig. 5. The correlation between immunoexpression of beta-catenin and Ki-67 in patients with serous ovarian tumors.

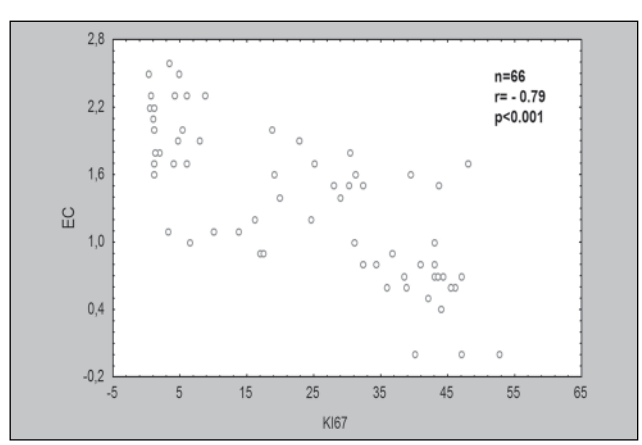

Fig. 6. The correlation between immunoexpression of E-cadherin and Ki-67 in patients with serous ovarian tumors.

Discussion

The beta-catenin is the multifunctional cytoplasmatic protein, with gene localised at 3p21 chromosome. Beta-catenin plays together with E-cadherin an important role in cytoskeleton structure. Loss of the beta-catenin expression has been reported in advanced stages of serous and clear-cell ovarian cancers, associated with poor tumor differentiation and a presence of metastases [5]. Beta-catenin not only acts as "intercellular glue" in complex with E-cadherin but also takes a part of the Wnt signaling pathway [14]. The Wnt signaling pathway regulates cellular proliferation and differentiation by stabilizing beta-catenin, and plays an important role in tumorgenesis. Wnt protein, acting at cell membrane via Frizzled and lipoprotein receptor-related protein, inhibits the phosphorylation and cytoplasmatic degradation of beta-catenin [7]. This degradation depends of a complex of proteins that includes Axin, GSK-3beta, and the APC protein. Inhibition of Axin complex results in intracytoplasmatic accumulation of beta-catenin. Accumulated beta-catenin is translocated into the nucleus, where it binds to the transcription factors T-cell factor (Tcf) and lymphoid enhancer factor (Lef) [8]. It triggers the expression of various genes, including among others the APC gene (responsible for polyposis coli and colorectal cancer), LRP5 gene (responsible for osteoporosis and osteosarcoma), cyclin D1 gene (responsible for breast cancer, colorectal cancer and others) or Axin2 gene (causes tooth agenesis and predisposes to colorectal cancer) [1, 17]. Literature for Wnt signaling pathway in ovarian epithelial cancer is yet short. Lin Xiao et al. revealed the role beta-catenin-APC-Tcf pathway in ovarian epithelial carcinogenesis by indicating the higher abnormal cytoplasmatic expression of beta-catenin in malignant and borderline ovarian tumors, than in benign ones, and inverse expression for APC protein, which may be caused by mutation in APC gene [23]. Although the Wnt signaling pathway is an important way of carcinogenesis, and should be further investigated, in our study we have focused at adhesive role of beta-catenin, which it plays together with E-cadherin. E-cadherin is an adhesion molecule; the gene for it is localised at 16q22.1 chromosome. It contains two domains: intracellular domain, responsible - together with catenins - for cytoskeleton forming, and extracellular domain, connected with intercellular adhesion processes and polarisation of the epithelial cells [11]. This is noteworthy that the literature for prognostic value of beta-catenin – E-cadherin complex immunoexpression in serous ovarian tumors is quite limited. The beta-catenin – E-cadherin complex plays significant role in intercellular adhesion processes, important for restraining of metastasing and infiltration of malignant neoplasms. It was suggested that the reducing of beta-catenin – E-cadherin complex immunoexpression is related to the intensification of malignancy progression and its metastasing potential in serous ovarian cancer [6]. In the present study we stated that the immunoexpression of beta-catenin and E-cadherin was significantly higher in benign tumors and tumors of low malignant potential than in malignant and aggressive ovarian tumors. In some investigations this association was questionable, but majority of the literature is similar to our results [5, 10, 15]. A major finding in this study, however, was the demonstration that there were significant differences between beta-catenin and E-cadherin immunoexpression in metastatic and non-metastatic groups. As in metastatic group the immunoexpression of beta-catenin – E-cadherin complex was significantly decreased, our results support suggestions that these adhesion molecules play a crucial role in metastasing potential of serous ovarian cancer [6].

Interestingly, in the present study we revealed a significant negative correlation between immunoexpression of Ki-67 proliferation molecule and immunoexpression of both adhesion molecules beta-catenin as well as E-cadherin in serous ovarian tumors. The Ki-67 protein expression is strictly connected with cellular cycle. The gene for Ki-67 protein is located at chromosome 10q25. This antigen appears in G1, S, G2 and M cellular cycle phase, remaining in hide in G0 and early G1 phase [16]. Therefore, it can be regarded as crucial proliferation marker at a given cell population. Although the majority of authors point the Ki-67 immunoexpression as an indicator of degree of proliferation for serous ovarian cancers [12, 18, 19, 20], some of them does not accept this molecule immunoexpression as a prognostic factor for those tumors [9, 20]. Our study revealed statistically significant differences between Ki-67 immunoexpression in metastatic group of patients, as compared to non-metastatic group. We have also stated an increase of Ki-67 immunoexpression along with increasing of tumor's histological grade. Thus, these findings confirmed the prognostic value of investigated marker in serous ovarian cancers. In our material we have not stated statistically significant differences between Ki-67 immunoexpression between serous cystadenomas and tumors of borderline malignancy. Although some authors reveal those differences [13], our investigations do not confirm these reports. Similar results were stated by Darai and al.[4]. The data on the relationship of Ki-67 and beta-catenin – E-cadherin complex are rather scant in the literature. Up to now, the histological grade of tumor is one of major, classic prognostic factors for epithelial ovarian cancers. It depends, among the others, on proliferating ability of tumor cells (which is related with expression of Ki-67 protein), and on its metastasing ability in which the loss of beta-catenin

– E-cadherin complex takes an important part by decreasing of cells adhesion. It leads to expansion of a tumor and increases its metastasing potential. Our findings of distinct negative correlation between immunoexpression of those proteins seem to be in concordance with these prognostic observations.

In conclusion, our data suggest that decreased immunoexpression of beta-catenin and E-cadherin proteins in serous ovarian tumors may be helpful in identifying the cases of higher metastatic potential and infiltration ability.

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