RELATIONSHIP BETWEEN THE PROANGIOGENIC ROLE OF EG-VEGF, CLINICOPATHOLOGICAL CHARACTERISTICS AND SURVIVAL IN TUMORAL OVARY

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RELATIONSHIP BETWEEN THE PROANGIOGENIC ROLE OF EG-VEGF, CLINICOPATHOLOGICAL CHARACTERISTICS AND SURVIVAL IN TUMORAL OVARY (Abstract) Aim: To prove the presence of EG-VEGF in tumor ovary and to analyze its involvement in the ovarian carcinogenesis, as promoter of angiogenesis, in relationship with the clinicopathological prognostic factors and survival. Methods: The study group comprises tumor tissue specimens from 50 cases of surgically treated ovarian cancer that were immunohistochemically investigated. A scoring system based on the percentage of positive cells and the intensity of staining was applied for the semiquantitative assessment of EG-VEGF, as negative or positive. Statistics involved $\chi^2$ test, and Kaplan-Meier and log-rank test. Results: EG-VEGF was positive in 35 cases (70%) and negative in 15 cases (30%). Our data confirmed the predominance of EG-VEGF positivity in the serous subtype as compared to endometrioid and clear cell subtypes, and its absence in mucinous subtype. Moreover, we demonstrated that EG-VEGF is overexpressed mainly in high-grade ovarian carcinomas (type II) than in low-grade ones. Significant differences were registered between the EG-VEGF positive or negative expression and tumor stage and histological subtypes, respectively. Survival analysis showed no differences in patient’s survival and EG-VEGF positive and negative cases. Conclusions: The analysis of EG-VEGF expression in ovarian tumors points out the relationship between the enhanced potential for tumor angiogenesis and the tumor aggressivity. Keywords: OVARIAN CANCER, EG-VEGF, TUMOR ANGIOGENESIS, IMMUNOHISTOCHEMISTRY.

Although the existence of organ-specific angiogenic factors was hypothesized in the early ‘80s (1), the presence of such a factor has been later confirmed (2). Its specificity results from the expression characteristics, concretely by its identification and isolation in endocrine organs, as: testis, adrenal gland, ovary, and placenta (2). Consequently, it has been named endocrine gland - derived vascular endothelial growth factor (EG-VEGF). EG-VEGF action is restricted to endothelial cells be-
longing to the steroidal endocrine organs, without known potential on other types of endothelial cells. EG-VEGF has a complex role as regulator of the angiogenic processes including cell proliferation, migration, and survival, pseudovascular and spheroidal organization, permeability and paracellular transport (3).

Besides the above mentioned organs, a low expression of EG-VEGF has been noticed in brain, colon, skeletal muscle, small intestine, spleen, thymus, uterus, and liver, whereas it is absent in non-lactating breast and skin (2). The EG-VEGF overexpression is reported in colorectal (4), pancreatic (5) and ovarian carcinoma (6-9).

The ovarian site offers a generous model for angiogenesis research, both in physiological status characterized by a cyclic evolution and in pathological conditions that includes a large heterogeneity of morphological changes. However, data concerning the EG-VEGF involvement in the ovarian carcinogenesis are limited.

Within this context, our study aimed to analyze the relationship between the EG-VEGF ovarian expression and clinicopathological prognostic factors and survival.

**MATERIAL AND METHODS**

The study group consisted of 50 cases with epithelial ovarian carcinoma, aged 41 to 77 years, diagnosed in “Cuza Voda” Obstetrics and Gynecology University Hospital from Iasi, Romania, during 2009-2012 intervals.

According to tumor stage, 15 cases (30%) have been classified as stage I, 12 cases (24%) as stage II, and 23 cases (46%) as stage III. Five cases (10%) have been graded as G1, 21 cases (42%) as G2, and 24 cases (48%) as G3, respectively. Epithelial ovarian carcinomas have been included in the following histological subtypes: 33 cases (66%) as serous, 10 cases (20%) as endometrioid, 4 cases (8%) as mucinous, and 3 cases (6%) as clear cell subtype; 14 cases (28%) have been classified as type I category (low-grade) and 36 cases (72%) as type II category (high-grade).

Serial sections have been cut at 4 μm thickness from the tumor specimens, in order to perform routine histological technique, followed by immunohistochemical (IHC) staining with anti-EG-VEGF antibody (Santa Cruz, Biotechnology Inc., Santa Cruz, USA) using the automated system Dako Autostainer Plus (Dako Cytomation, Glostrup, Denmark). A semiquantitative method has been used (8) to quantify the results, each slide receiving a score for the percent of immunoreactive tumoral cells and a score for the tumor tissue staining intensity from the same microscopic field. The sum of the two scores determined the final score, ranging between 0 and 6. A final score less than 2 has been considered negative, while ≥3 score has been considered as positive for the evaluated marker.

Statistical analysis has been performed using the commercially available GraphPad Prism software. We have applied χ² test for correlation with clinicopathological characteristics, and Kaplan-Meier curves and log-rank test for survival analysis.

**RESULTS**

Our study had shown a positive expression of EG-VEGF in 35 cases (70%) and a negative expression in 15 cases (30%). The distribution of EG-VEGF expression within tumor stages has shown the following pattern: 9 cases were positive and 6 cases were negative in stage I, 10 cases were positive and 2 cases were negative in stage II, and 7 cases were positive and 16 cases
were negative in stage III. The correlation of EG-VEGF expression with the tumor grade has been the following: 2 positive cases and 3 negative cases in G1, 14 positive cases and 7 negative cases in G2, and 19 positive cases and 5 negative cases in G3. Expression of EG-VEGF has been more common among the serous subtype (fig. 1), represented by 26 cases followed by endometrioid subtypes, represented by 6 positive cases (fig. 2), and clear cell subtype – 3 cases (fig. 3). EG-VEGF has been undetected in 15 cases (7 case of serous, 4 cases of endometrioid, and 4 cases of mucinous type, respectively). Regarding the tumor type, EG-VEGF expression has been overexpressed in 28 cases of type II, comparative to 7 cases of type I tumors.

The statistical analysis had revealed significant differences between the EG-VEGF expression (positive versus negative) and tumor stage (p=0.009) and histological subtypes (p=0.017), respectively (tab. I). No differences in patient’s survival and EG-VEGF positive and negative cases had been registered (p=0.218) (fig. 4).

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**Fig. 1.** EG-VEGF positive reaction in serous ovarian carcinoma (IHC, anti-EG-VEGF, x 40).

**Fig. 2.** EG-VEGF positive reaction in endometrioid ovarian carcinoma (IHC, anti-EG-VEGF, x 40).

**Fig. 3.** EG-VEGF positive reaction in clear cell carcinoma (IHC, anti-EG-VEGF, x 40).

**Fig. 4.** Kaplan-Meier curves showing no differences in overall survival (p=0.2186).
Relationship between the proangiogenic role of EG-VEGF, clinicopathological characteristics and survival in tumoral ovary

### TABLE I.
**EG-VEGF expression in ovarian epithelial carcinoma**

<table>
<thead>
<tr>
<th>Clinicopathological characteristics</th>
<th>Cases</th>
<th>EG-VEGF positive cases</th>
<th>EG-VEGF negative cases</th>
<th>p value</th>
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<tr>
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<td>#</td>
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<tr>
<td>Tumor stage</td>
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<tr>
<td>I</td>
<td>15</td>
<td>9</td>
<td>60</td>
<td>6</td>
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<td>II</td>
<td>12</td>
<td>10</td>
<td>83.33</td>
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<tr>
<td>III</td>
<td>23</td>
<td>7</td>
<td>30.43</td>
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<td>Tumor grade</td>
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<td>1</td>
<td>5</td>
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<td>40</td>
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<td>21</td>
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<td>7</td>
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<td>Low-grade serous carcinoma</td>
<td>4</td>
<td>3</td>
<td>75</td>
<td>1</td>
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<tr>
<td>Low-grade endometrioid carcinoma</td>
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<td>1</td>
<td>33.33</td>
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<td>Mucinous carcinoma</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>4</td>
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<tr>
<td>Clear cell carcinoma</td>
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<td>3</td>
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<td>0</td>
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<tr>
<td>High-grade serous carcinoma</td>
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<td>79.31</td>
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<tr>
<td>High-grade endometrioid carcinoma</td>
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<td>71.43</td>
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<tr>
<td>Tumor Type</td>
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<tr>
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<td>Type II</td>
<td>36</td>
<td>28</td>
<td>77.78</td>
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**DISCUSSION**

The first data concerning EG-VEGF involvement in the angiogenic processes in steroidal organs have been published 3 decades ago (2). EG-VEGF belongs to a class of proteins that includes Bv8 (7, 10). Although EG-VEGF accomplishes comparable functions as VEGF, its structure is completely different (10).

As a difference from its physiological functions, the EG-VEGF role in tumor environment is to induce endothelial cells proliferation and fenestration in tumor vascular capillaries, by different growth factors activation, as a consequence of hypoxia and action of proinflammatory cytokines (4). The binding of EG-VEGF to its specific receptors (PKR1 and PKR2) determines angiogenic effects (mediated by PKR1) and cellular permeability (mediated by PKR2) (7, 10). As a consequence, EG-VEGF/EG-VEGF-R system represents a paracrine system, the ligand being produced by non-endothelial cells, while the receptors are selectively expressed in vascular endothelium present in the steroidal tissues (7, 10). These facts justify its specificity for the endocrine organs characterized by steroid hormones secretion (8).

Few data concerning EG-VEGF in ovarian carcinoma have been reported in the mainstream publications (6, 8, 9) and its involvement in regulating tumoral cells growth and survival is yet unclear.
As reference in the research in this field, the study conducted by Zhang et al. (6) had analyzed EG-VEGF and VEGF comparative expression at mRNA level (using real-time quantitative RT-PCR) in 50 cases of human ovarian tumors, suggesting that EG-VEGF is not directly expressed by ovarian tumor cells, but more likely it is the result of leukocyte inflammatory activity or of stromal cell intervention. The authors have demonstrated EG-VEGF mRNA higher expression in benign and borderline ovarian tumors, and also in tumor stage I (early stage) compared with stage III. VEGF expression had been opposite to that of EG-VEGF, VEGF exhibiting a predominant expression in advanced cases, compared to early stages. Moreover, the investigation of the inflammatory extracellular matrix role related to EG-VEGF expression had identified a higher EG-VEGF mRNA in peritumoral stroma compared to tumoral one; reported to inflammatory peritumoral cells (lymphocytes CD3, CD19, CD45, and NK), EG-VEGF had been overexpressed in CD3 lymphocytes supporting the inflammation role in EG-VEGF liberation.

Later on, an attempt to determine the potential prognostic value of EG-VEGF in ovarian carcinoma had been accomplished by Bălu et al. (9) who analyzed EG-VEGF expression in 30 patients with ovarian tumors, aiming the correlation of EG-VEGF expression related to classical parameters. The obtained results have shown statistically significant differences between EG-VEGF and tumor stage, tumor grade and histological subtypes.

Therefore we consider our research is worthwhile in supplementing data on the EG-VEGF characterization in relationship with the behavior of ovarian tumors. The novelty of our study, based on our previous interest on the role of this molecule in ovarian carcinogenesis (11), is sustained not only through the correlation with the clinicopathological characteristics, but also by those with patients’ survival – to the best of our knowledge not yet reported in the literature.

In accordance with the published papers (6, 9), our data revealed statistically significant differences related to tumor stage and histological subtypes. The EG-VEGF decline in advanced stages of ovarian carcinoma may be attributed to a negative action of the steroidal stroma on tumor growth. This observation opens perspectives for considering EG-VEGF as a therapeutic agent in early phases of ovarian carcinogenesis. Within the framework of the histological heterogeneity of ovarian carcinoma, we confirmed the predominance of EG-VEGF positivity in the serous subtype as compared to endometrioid and clear cell subtypes, and its absence in mucinous subtype. Moreover, we demonstrated that EG-VEGF is overexpressed mainly in high-grade ovarian carcinomas (type II) than in low-grade ones. These statements can be interpreted as evidences for a direct relationship between the enhanced potential for tumor angiogenesis and the tumor aggressivity.

The EG-VEGF prognostic role for survival was not proved, but this negative result may be attributed to the relatively reduced number of cases included in the study – a more extensive research being necessary to clarify this issue.

CONCLUSIONS

The analysis of EG-VEGF expression in ovarian tumors, founded on the peculiar
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morbidity of endocrine organs characterized by steroid hormones secretion, offers complementary data for a better understanding of the angiogenesis role in carcinogenic mechanisms.

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