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# Mig-7 expression and vasculogenic mimicry in malignant ovarian tumors

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## ABSTRACT

**Objectives:** To investigate the possible association of vasculogenic mimicry (VM), VE-cadherin and MIG-7 expression with clinicopathological features of women with malignant ovarian masses.

**Material and methods:** VM was studied with the PAS reaction and VE-cadherin was assessed with immunohistochemistry in 108 women with malignant ovarian tumors. Additionally, quantitative expression of *MIG-7* mRNA was performed in 52 ovarian cancers with qRT-PCR.

**Results:** VM was found in 48/108 cases (44%), more often in higher FIGO stage tumors (83% cases; 40 vs. 8; $p = 0.01$). High expression of VE-cadherin was present in 37% of all ovarian masses. Ovarian tumors without VM more often expressed low levels of VE-cadherin than tumors where VM was found (37.6% vs.14.6%). No expression or very low expression of *MIG-7* mRNA was found in all normal ovarian tissues and in 32 cancer samples. Median RQ of *MIG-7* mRNA in tumor samples was higher than in normal ovarian tissue (RQ = 0.29 vs. RQ = 0.05, respectively; $p < 0.005$) and higher than in non-malignant ovarian masses (0.98 vs. 0.05 respectively; $p = 0.03$). Expression of *MIG-7* mRNA was significantly correlated with VM ($p = 0.039$). In tumors with PAS-positive structures median RQ *MIG-7* mRNA was higher than in tumors with PAS-negative findings (1.89 vs. 0.13 respectively). VE-cadherin expression was more frequently found in tumors where *MIG-7* mRNA was present ($p = 0.004$).

**Conclusions:** Vasculogenic mimicry exists in malignant ovarian tumors and advanced clinical stages of malignancy are accompanied by a high incidence of VM formation. *MIG-7* mRNA and VE-cadherin expression may serve as additional molecular markers of VM in ovarian malignancies.

**Key words:** ovarian cancer, vasculogenic mimicry, MIG-7, VE-cadherin

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## INTRODUCTION

Ovarian cancer is the leading cause of gynecological malignancies related death in women [1]. The most common and aggressive cancer forms are characterized by early extensive tumor invasion and peritoneal metastases which is the reason why the majority of women present in the advanced stage of the disease. Despite constant scientific progress and initial success of aggressive surgery and chemotherapy in most cases treatments failures are observed [2]. The main responsible factors are drug resistance and cancer metastasis, and because of this, discovering new molecular factors controlling cancer growth and inventing more effective precisely targeted treatments is imperative [3]. The growth and spread of various solid malignant tumors is at least partially dependent on the formation of sufficient vascular support [4]. So far the most promising antiangiogenic strategies in women with ovarian cancer include anti-angiogenesis factors like VEGF antibodies and angiopoietin antagonists but other molecules targeting cancer angiogenesis with the use of different mechanisms have been tested, including Cediranib, a potent inhibitor of VEGF Tyrosine Kinase Inhibitor. This drug when administered oral-

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ly has already demonstrated potent activity in both phase II and phase III studies [5].

Microvascular networks can be formed by several different pathways that include the adaptation of the pre-existing tissue vessels, intususceptive microvascular growth, postnatal vasculogenesis, glomeruloid angiogenesis and vasculogenic mimicry (VM) [6]. Vasculogenic mimicry (VM) is the formation of fluid-conducting channels by highly invasive and genetically deregulated tumor cells [7] . VM is important for an alternative tumor pathway of blood supply and is frequently found in aggressive types of cancers. Tumors containing pseudovascular channels display higher malignancy potential, rapid proliferation, and high incidence of metastases that spread via a vascular system. The PAS-positive human uveal melanomas originally identified by Maniotis et al. demostrated various patterns including: linear, parallel linear, cruciform, half-moon, annuliform, and lattice forms [7]. Aggressive ovarian cancer cells are capable to generate matrix-rich, embryonic- like, patterned networks independent of endothelial cells [8]. These tumors are also typically characterized by high plasticity and ability to express some specific markers of vascular epithelial cells.

A remarkable example of plasticity is tumor cell vasculogenic mimicry, that was first detected in aggressive melanomas and later also in ovarian cancers where tumor malignant cells expressed endothelial-associated genes and formed new vasculogenic-like networks. To highlight matrix-associated vascular channels lined with cancer cells the periodic acid Schiff reagents staining (PAS) is typically used [9]. PAS-positive vascular channels appear to be an important alternative pathway of blood supply to the tumors that sustains their growth and supports metastasis formation. The presence of VM is in turn frequently associated with a high tumor grade, short survival, invasion and metastasis formation [9, 10].

Vascular endothelial cadherin (VE-cadherin) is an adhesive protein and a component of endothelial cell-to-cell junctions that plays a key role in the maintenance of vascular morphology and stability [11]. Endothelial cells (ECs) express various members of the cadherin superfamily, in particular vascular endothelial VE-cadherin, which is the main adhesion receptor of endothelial adherent junctions. High expression of VE-cadherin by aggressive melanoma tumor cells results in their ability to mimic endothelial cells and form embryonic-like, patterned, vasculogenic networks [12]. VE-cadherin may be detected in highly aggressive malignant tumors but not in less-aggressive ones. More recently, tyrosine phosphorylation of VE-cadherin has been implicated in the disruption of endothelial cell adherent junctions and in the diapedesis of metastatic cancer cells [13]. Aberrant extra-vascular expression of VE-cadherin has been observed in certain cancer types associated with VM [14].

Discovery and targeting of tumor cell-specific gene expressions could lead to more effective cancer managements with a reduced amount of toxic side effects. First described by Crouch et al. in 2004, who studied MIG-7 protein with an antibody to its first nine amino acids and demonstrated reproducible and significant inhibition of endometrial carcinoma cell invasion in vitro [15]. MIG-7 protein has been proposed as one of the essential factors that facilitate tumor cell dissemination by aggregate invasion and by promoting the process of vasculogenic mimicry [15]. Expression of *MIG-7* mRNA can be found at early stage of pregnancy during trophoblast development. Except from this site it is specific to malignant tumor cells and is not found in normal adult cells. Induction of MIG-7 mRNA expression is dependent on multiple factors, that include cytokines like epidermal growth factor (EGF) and hepatocyte growth factor (HGF). It has been suggested that MIG-7 could serve as promising marker for circulating cancer cells detection and metastasis [16]. MIG-7 protein induces invasion and vessel-like structure formation by cancer cells in three dimensional (3D) cultures in vitro and the knockdown of *MIG-7* gene in cancer cells line causes their reduced invasion in 3D cultures. The gene activation promotes lung cancer metastases formation by activating the cyclooxygenase-2 (COX-2)–prostaglandin E2 (PGE2) signaling cascade [17]. Immunohistochemical (IHC) studies performed with MIG-7 antibodies revealed expression of this marker in circulating tumor cells, proposing its potential as an early marker for metastatic carcinomas [18, 19]. The use of peptides specific to MIG-7 treatment has induced increased monocyte expression of tumor necrosis factor (TNF) and killing of breast carcinoma cells in vitro [20]. Another hypothesis is that MIG-7 stimulates vascular mimicry prior to tumor angiogenesis, thus contributing to early growth and metastasis of ovarian cancer [21]. The aim of the present study was to investigate the possible association of vasculogenic mimicry, VE-cadherin and *MIG-7* mRNA expression with selected clinicopathological features of women with malignant ovarian masses.

## MATERIAL AND METHODS

The studied group included 108 women with ovarian tumors who were operated between years 2009–2011 in the I$^{st}$ Department of Gynecologic Oncology and Gynecology of the Medical University of Lublin. All participants were informed of the nature of the study and gave their informed consent. The average age of patients was 52.3 years (range: 18 to 86 years). Among them there were 47 premenopausal (44%) and 61 postmenopausal women (56%). Examined group included 6 cases of benign ovarian masses, 9 borderline ovarian tumors and 93 malignant ovarian cancers. Malignant tumors included 17 metastatic tumors to

the ovary and 5 non-epithelial tumors: 3 dysgerminomas and 2 granulosa cell tumors. All specimens were examined by an experienced pathologist to confirm the diagnosis of ovarian malignancy. The proportion of malignant cells in selected tumor samples used in this study was always more than 50%. The histological type and grade of the tumors were classified according to the criteria of the World Health Organization (WHO). The clinical stage of each cancer was established according to International Federation of Gynecology and Obstetrics (FIGO) criteria.

### Immunohistochemical studies

Immunohistochemical staining for PAS and VE cadherin was performed in all overian masses. Four-micrometer sections from formalin-fixed, paraffin-embedded tissue were mounted on silanized slides (DAKO Cytomation, Denmark). The slides were air-dried and the tissue was deparaffinized and rehydrated. For PAS staining, the sections were incubated with periodic acid for 5 min, rinsed with distilled water and then incubated with Schiff reagent for 15 min (PAS staining kit, Sigma, USA), next they were dehydrated, mounted and cover slipped. PAS-positive channels with red blood cells, as wells as walls composed of tumor cells, were verified by hematoxylin-eosin staining (HE staining) and assessed and 100× and 200× magnifications (Fig. 1). For the purpose of this study all our malignant tumor cases were divided into two groups: VM-positive and VM-negative groups.

VE-cadherin expression assessment was performed with VE-cadherin (H-72) rabbit polyclonal antibody (Santa Cruz Biotechnology, USA). Following deparafinization, rehydration and antigen retrieval with the Target Retrieval Solution at pH = 6.0 (Dako Cytomation,Denmark), three cycles of heating in a microwave oven (each for 5 mins, at 750 W) were performed. Tissue sections were incubated with the primary antibody for 1 hour in room temperature (dilution: 1:200). The slides were incubated with the secondary antibody conjugated with streptavidin-biotin-peroxidase complex (rabbit/mouse EnVision kit; DAKO Cytomation) and color reaction was developed using DAB (3′-3-diaminobenzidine tetrahydrochloride, DAKO) according to the manufacturer's protocol. The sections were counterstained with Mayer's hematoxylin. For each case, negative control was applied by replacing the antibody with PBS or nonimmune serum.

VE-cadherin expression was assessed semi-quantitatively and both the intensity and percentage of positive cells were measured (Fig. 2). As a rule at least 10 microscopic fields in one representative tumor section were observed under 400× magnification. The VE-cadherin intensity of staining was graded 0 for absent immunoreactivity, 1 for weak, 2 for moderate, and 3 for intense positivity. The number of

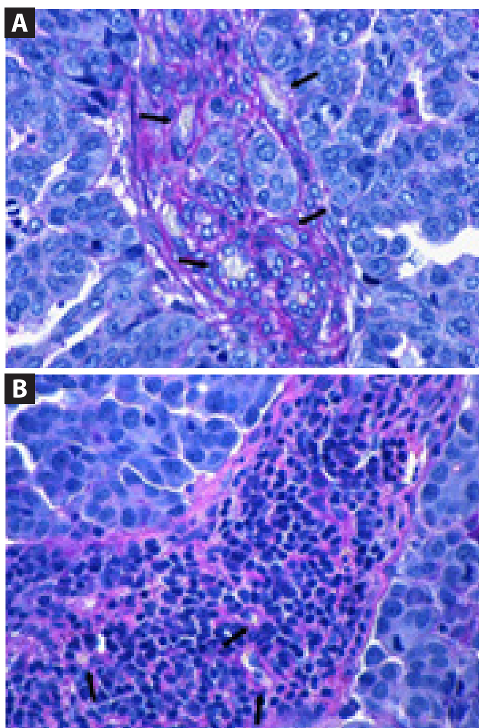


**Figure 1.** Example of PAS-positive structures in ovarian cancer tissues (colored in pink). Arrows indicate pseudovascular channels where erythrocytes can be found 100× (**A**). Channel walls surrounded by the cancer cells but not endotheliocytes (cell nuclei are circular rather than flattened) 200× (**B**)

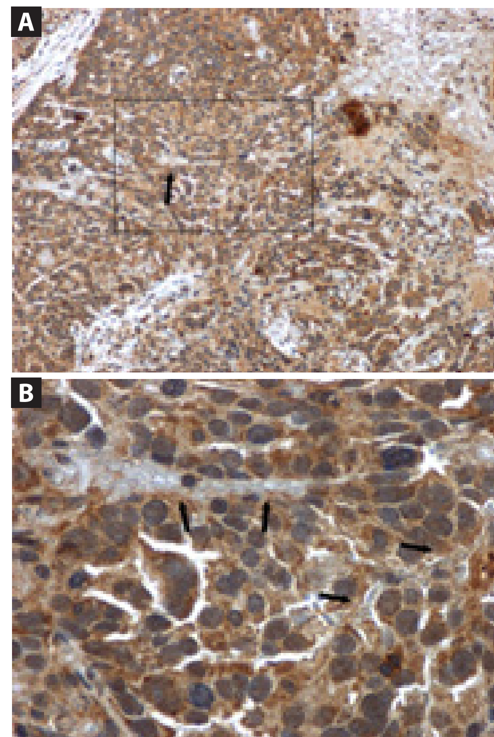


**Figure 2.** An example of VE-cadherin expression in ovarian cancer tissue 100× (**A**). Brown color is apparent in the cytoplasm of tumor cells but not in the stromal cells. Selected area in the box on the lower picture 400× (**B**). Arrows indicate pseudovascular channels in which some red blood cells can be seen

positive cells was visually evaluated and cell expression was stratified as follows: negative; for < 10% positive cells (weak or absent reaction), low; for 10–30% positive cells (weak or moderate); medium for 31–65% positive cells (moderate or strong reaction) and high for > 65% positive cells (strong reaction).

### Quantitative real-time -PCR (qRT-PCR) for *MIG-7* mRNA

Quantitative expression of *MIG-7* mRNA was performed in 52 ovarian tumors tissue that were collected from consenting patients immediately after tumor removal. The 3–4 mm representative tumor samples were snap-frozen in liquid nitrogen and stored in –80° C until processing. Ten nonmalignant tissue samples were taken from ovaries of women who had bilateral oophorectomies for suspected malignancy, but were found to have benign histology. The samples were collected from apparently normal contralateral ovaries and were used as a reference group for qRT-PCR analysis. Total RNA was extracted from the ovarian tissues using Trizol Reagent (Life Technologies; 1 mL/35–45 mg of tissue) according to the manufacturer's protocol. Reverse transcription of total RNA was performed with the use of the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems) according to the supplier's instructions. Each target was amplified in triplicate with a MIG-7— specific primer probe set as described by Petty et al. [20] and designed by "Primer 3" v. 0.2 software. The following primer sequences were used in this part of the study: forward, 5'-CACCTGCCTCTGGTCGTTAGG-3'; reverse 5'-TACTGGATTCCTCTAGCTTTGGTGTT-3'; probe 5'-AAACTCTCAGTGATCTCT-3'. For endogenous control a GAPDH primer probe set was used (TaqMan® Gene Expression Assay, Hs00182176; Applied Biosystems, USA). The expression of GAPDH was used for normalization of Real-Time reaction. Real Time PCR was performed with the use of 7900HT Fast Real-Time PCR System (Applied Biosystems, USA) at 50°C for 2 min and at 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min. The *MIG-7* mRNA relative quantity (RQ) was calculated using comparative CT Method (ΔΔ CT Method) (ABI User Bulletin no. 2). ΔCT values were generated from the housekeeping gene multiplexed in each reaction as the endogenous control. The ΔΔCT values were generated by comparing the reference gene (GAPDH) to the tested gene (MIG-7) in all samples.

### Statistical analysis

Statistical analysis was performed with the use of Statistica v.6.0 software (Statsoft, Poland). The correlation between selected clinical and pathological features and the expression of VE-cadherin, *MIG-7* mRNA, PAS+ structures in tumor specimens was analyzed using Pearson's $\chi^2$ test or nonparametrical U Mann Whitney and Kruskall-Wallis ANOVA tests, where appropriate. The criterion of statistical significance applied in all calculations was $p < 0.05$.

**Table 1.** Immunohistochemical staining and qRT-PCR results compared with tumor type, FIGO stage and histological grading of studied tumors

| | IHC analysis (PAS staining and VE-cadherin expression) n = 108 | Real time-qPCR MIG-7mRNA expression n = 52 |
|---|---|---|
| **Histological grade** | | |
| G1 | 26 (24%) | 5 (9.6%) |
| G2 | 37 (34.3%) | 18 (34.6%) |
| G3 | 45 (41.7%) | 29 (55.7%) |
| **FIGO stage** | | |
| I | 25 (23.1%) | 7 (13.4%) |
| II | 6 (5.5%) | 1 (1.9%) |
| III | 72 (69.4%) | 39 (75%); |
| IV | 5 (4.6%) | 2 (3.8%) |
| **Histological subtype** | | |
| Serous | 29 (26.8%) | 16 (30%) |
| Mucinous | 9 (8.3%) | 3 (5.7%) |
| Endometroid | 21 (19.4%) | 16 (30.7%) |
| Undifferentiated | 26 (24%) | 10 (19.2%) |
| Metastatic | 17 (15.7%) | 6 (11.5%) |
| germinal* | 5 (4.6%) | 1 (1.9%) |

*three dysgerminomas and two granulosa cell tumors

## RESULTS

Median age of all studied women was 52.3 years (range: 18–86 years). The selected clinical and pathologic variables of these patients are listed in Table 1. Based on FIGO grade and stage, patients were divided into two groups, low stage (FIGO stage I and II; n = 31; grade 1 or 2; n = 63) and high stage (FIGO stage III or IV, n = 77; grade 3, n = 45). All of the forms described by Maniotis et al. [7] were found in our group and most of them was parallel, linear, cruciform, half-moon and back to back-loop patterns. Besides, red blood cells were also found in the pseudovascular channels (Fig. 1). There were no PAS-positive structures in both normal ovarian tissues and in benign tumors as well as in borderline tumors. Vasculogenic mimicry, as confirmed by PAS staining was found in 48/108 cases (44%), most commonly in serous and endometrioid ovarian cancer subtypes (Table 2). Half of metastatic tumors and almost half of the ovarian cancers demonstrated PAS-positive structures. In the case of ovarian serous tumors, nearly half (13 of 29; 45%) were characterized by the presence of pseudovascular PAS positive VM channels. For endometriod type of cancer, a much as 76% (16 of 21 cases) had PAS-positive structures present. In the case of clear cell type, no cases of vasculogenic mimicry as stained by PAS reaction were found (0 of 4). The differences between these groups were statistically significant (p = 0.02). VM occurred more often in high FIGO stage tumors (III and IV) (83% cases; 40 vs. 8), than in women with low FIGO stage cancers (p = 0.01). Age and menopausal status were not significantly correlated with the presence of vasculogenic mimicry in studied ovarian malignant tumors tissues.

High expression of VE-cadherin (staining intensity 2 or 3 in over 65% of tumor cells) was present in 37% of all cases. Figure 2 shows representative staining patterns for VE-cadherin. Low or no expression of VE-cadherin (intensity 1 or 2 in 40% tumor cells) was found in 27% of tumors (Table 3). There was no correlation with histological type of malignant tumor, FIGO stage or grade (Table 4). The expression of VE-cadherin was substantially associated with the presence of pseudovascular channels (p = 0.002). Ovarian tumors without VM more often expressed low levels of VE-cadherin than tumors where VM was found (37.6% vs.14.6%).

Quantitative RT-PCR analysis of total RNA detected *MIG-7 mRNA* expression in 48% (n = 20) of tumor tissues. Median relative quantity of MIG-7 mRNA in tumor tissue was RQ = 0.29 (range 0.04–3.11) (Table 5). In all controls (normal ovarian tissue samples) and in 32 cancer samples no expression or very low expression of MIG-7 mRNA was found (RQ = 0.05 range 0.01–0.19). In EOC samples median RQ of MIG-7 MRNA was significantly higher than in non-EOC tissue probes (where RQ = 0.98 vs. 0,05 respectively; p = 0.03) (Table 6). No correlation of RQ MIG-7 mRNA expression with FIGO stage or tumor histological grade was found. Interestingly, high expression of MIG-7 mRNA was frequently found in tumors with VM (RQ = 1.89 range 0.05–17.3) and in cases without VM median RQ was significantly lower (RQ = 0.13 range 0.03–1.57) and the differences between

**Table 2.** Association of selected clinicopathological variables with tumor vasculogenic mimicry

| | Vasculogenic mimicry | | |
|---|---|---|---|
| | **PAS-negative n = 60 (56%)** | **PAS-positive n = 48 (44%)** | **p ($Chi^2$ test)** |
| **Histological grading** | | | |
| Low grade (G1 or G2) n = 63 | 39 (61.9%) | 24 (38.1%) | **0.08** |
| High grade (G3) n = 45 | 21 (46.6%) | 24 (53.3%) | |
| **FIGO stage** | | | |
| Low (I or II) n = 31 | 23 (74.1%) | 8 (25.8%) | **0.01** |
| High (III or IV) n = 77 | 37 (48.1%) | 40 (51.9%) | |
| **Histologic subtype** | | | |
| EOC n = 59 (serous or mucinous or endometrioides) | 28 (47.4%) | 31 (52.5%) | **0.06** |
| Non EOC n = 49 (undifferentiated or metastatic or germinal) | 32 (65.3%) | 17 (34,69%) | |

**Table 3.** Association of malignant ovarian tumors FIGO stage and histological grade with VE-cadherin expression in tumor tissue

| | VE-cadherin expression | | | |
|---|---|---|---|---|
| | Low n = 29 (26.8%) | Medium n = 39 (36.2%) | High n = 40 (37%) | p (Chi[2] test) |
| **Histological grading** | | | | |
| Low grade (G1 or G2) n = 63 | 17 (26.9%) | 23 (36.5%) | 23 (36.5%) | 0.9 |
| High grade (G3) n = 45 | 12 (26.6%) | 16 (35.5%) | 17 (37.7%) | |
| **FIGO stage** | | | | |
| Low (I or II) n = 31 | 8 (25.8%) | 11 (35.5%) | 12 (38.7%) | 0.9 |
| High (III or IV) n = 77 | 21 (27.3%) | 28 (36.3%) | 28 (36.3%) | |
| **Histologic subtype** | | | | |
| EOC n = 59 (serous or mucinous or endometrioid) | 12 (20.3%) | 23 (38.9%) | 24 (40.6%) | 0.2 |
| Other n = 49 (undifferentiated or metastatic or germinal) | 17 (34.6%) | 16 (32.6%) | 16 (32.6%) | |

**Table 4.** Association of VE-cadherin expression with tumor vasculogenic mimicry

| VE-cadherin expression | Low n = 29 | Medium n = 39 | High n = 40 | p (Chi[2] test) |
|---|---|---|---|---|
| **Vasculogenic mimicry, n = 108** | | | | |
| PAS–(n = 60) | 22 (36.7%) | 24 (40%) | 14 (23.3%) | **0.002** |
| PAS+(n = 48) | 7 (14.6%) | 15 (31.2%) | 26 (54.2%) | |

**Table 5.** Relative Quantity (RQ) of MIG-7 mRNA assessed by real-time qPCR in ovarian tumors and in the control group

| RQ-MIG-7 mRNA expression | | |
|---|---|---|
| **Median** | **Range** | **Min-max** |
| Control (normal ovarian tissue) (n = 10) | | |
| 0.05 | 0.01–0.19 | 0.001–0.27 |
| Tumor tissue (n = 52) | | |
| 0.29 | 0.04–3.11 | 0.001–4711 |

these groups were statistically significant (p = 0.039). Also, a significant relationship between VE-cadherin expression and *MIG-7*mRNA presence (p = 0.002) was found. In 12 malignant tumor samples where strong VE-cadherin staining signal was found median RQ of MIG-7mRNA was higher than in subgroup with medium or low expression of VE-cadherin ( where RQ = 44.3 vs. RQ = 1.04; and RQ = 0.05 for high medium and low VE-cadherin expression, respectively) (Table 6). This correlation was statistically significant (p = 0.004).

## DISCUSSION

Development of the most appropriate combination of anticancer and antivascular therapy in a given ovarian malignant tumor is a new and extremely important challenge. Since its discovery in 1999, vasculogenic mimicry has been recognized as alternative blood and nutrient supply network that is induced in several types of highly malignant tumor types including ovarian high grade cancers. Therefore, this study was undertaken to evaluate and correlate VM phenomenon with VE-cadherin and *MIG-7* gene expression, both of which may play an important role in malignant ovarian tumors adequate blood supply that is independent from VEGF-driven angiogenesis. Various vasculogenic signaling pathways that are normally restricted become active in highly aggressive cancers. Despite many published studies to date and significant new insights that they have brought to the ovarian cancer angiogenesis the precise mechanisms controlling the multipotent vasculogenic mimicry phenotype of ovarian cancer are still largely unknown.

Taking into account histological and clinical characteristics, we have found that vascular mimicry was present primarily in a subset of ovarian malignant tumors that most

**Table 6.** Association of selected clinical and pathological variables with RQ of *MIG-7* mRNA, VE-cadherin expression and vasculogenic mimicry in ovarian cancer tissue

| *MIG-7* mRNA expression (RQ) | | | | |
|---|---|---|---|---|
| | Median | Range | Min–max | p –value U Mann-Whitney test |
| **Histological grading** | | | | |
| Well differentiated (G1 or G2) n = 23 | 0.13 | 0.02–6.76 | 0.002–4711 | Z = –0.45; p = 0.65 |
| Low differentiated (G3) n = 29 | 0.98 | 0.05–0.98 | 0.001–202 | |
| **FIGO stage** | | | | |
| Low (FIGO I\II) n = 11 | 0.57 | 0.08–2.91 | 0.02–385 | Z = 0.7; p = 0.48 |
| High (FIGO III\IV) n = 41 | 0.17 | 0.03–3.24 | 0.001–4711 | |
| **Histologic subtype** | | | | |
| EOC n = 35 (serous or mucinous or endometrioides) | 0.98 | 0.06–7.38 | 0.009–4711 | Z = 2.1; p = 0.03 |
| Non EOC n = 17 (undifferentiated or metastatic or germinal) | 0.05 | 0.01–1.89 | 0.001–312 | |

**Table 7.** Association of *MIG-7* mRNA expression (RQ) with vasculogenic mimicry and VE-cadherin expression in ovarian cancer tissue

| *MIG-7 mRNA* (RQ) | Median | Range | Min–max | p-value (U Mann-Whitney; Kruskal-Wallis ANOVA tests) |
|---|---|---|---|---|
| **Vasculogenic mimicry** | | | | |
| PAS– (n = 25) | 0.13 | 0.03–1.57 | 0.001–444 | **Z = –2.1; 0.039** |
| PAS+ (n = 27) | 1.89 | 0.05–17.3 | 0.009–4711 | |
| **VE-cadherin expression** | | | | |
| Low (n = 16) | 0.05 | 0.01–0,15 | 0.001–2.9 | **H = 10.8; p = 0.004** |
| Medium (n = 24) | 1.04 | 0.08–3.11 | 0.001–444 | |
| High (n = 12) | 44.3 | 0.14–257 | 0.009–4711 | |

often were also highly aggressive. Pseudovascular channels were detected in 44% of malignant tumors cases, most commonly in serous and endometrioid ovarian cancer subtypes. Patterned matrix VM was distinct from angiogenic vessels on light microscopy because endothelial cell lined vessels do not form back-to-back loops in 2-dimensional histologic sections. With increasing clinical stage of studied tumors, the proportion of VM tumors increased gradually showing significant correlation with the presence of VM. We hypothesize that in more aggressive ovarian tumors, cancer cells initiate VM signaling cascade and proliferate more rapidly. The plasticity of these cancer cells is usually higher than in slow growing tumors and because of this feature, the process of their differentiation is more immature [4, 10]. The alternative network of blood supply to the tumor is provided even though the rapid cancer growth may not be precisely synchronized with tumor angiogenesis. Some tumors cells may form abnormal vessel-like vascular channels by excretion of extracellular matrix which helps to form blood cells transporting system [9, 20].

Identification of specific genes that are expressed in a cancer cell and which are capable of VM formation may be further used to find specific molecular markers that can be used clinically in the detection and disease progression assessment. Since VM activity is associated with the expression of VE-cadherin, together with the Mig-7 gene activation may initiate aggressive tumor behavior [21]. Hendrix et al. studied in vitro a mouse VM model and have found that if VE-cadherin expression was blocked, the formation of new pseudovascular channels was not further observed [22]. Our

study provides another evidence in support of the hypothesis that expression of VE-cadherin by ovarian cancer cells results in their facility to mimic endothelial cells and form embryonic-like, patterned vasculogenic networks. In a study by Alvero et al. the expression of VE-cadherin was correlated with the occurrence of tumor pseudovascular channels [23]. They have also found that ovarian cancer cells have the capacity to gain an endothelial phenotype and could form vessel-like structures in vitro. Authors showed that CD44+/ /VE-cadherin–/CD34– Type I EOC cells can differentiate into a CD44+/VE-cadherin+/CD34+ phenotype when cultured in high-density Matrigel and mimic the behavior of normal endothelial cells. This process was complemented by the achievement of endothelial marker CD34 and VE-cadherin [23]. Wang et al. have found that treatment with VEGF-A increase expression of VE-cadherin in ovarian cancer cells in vitro and stimulates vasculogenic mimicry trough stimulation signaling cascade of EphA2 and MMP2 and MMP9 [24].

To our knowledge, this is the first report on the expression of *MIG-7* mRNA in ovarian cancer tissues assessed with the use of quantitative real-time PCR. Our results indicate that high *MIG-7* mRNA expression was detected only in a subset of EOC cells but not in cells from normal ovarian tissue samples and not in benign tumor samples. PAS staining revealed that in these tumors pseudovascular structures were also present. Moreover, we have found a significant relationship between high expression of *MIG-7* mRNA and VE-cadherin. Taken together, these observations suggest that high expressions of MIG-7 in tumor tissues concurrent with high expression of VE-cadherin may have a synergistic effect that promotes VM formation. Similar results were reported by Petty et al. [17] when these investigators analyzed MIG-7 expression in breast cancer. They have demonstrated the specificity of MIG-7 expression to breast cancer tissue as MIG-7 was not detected in normal breast tissues nor it was found in blood from normal subjects. In this study immunohistochemical analyses with specific MIG-7 protein antibody showed that staining reaction was present in 53% of total breast cancer tissues. It is possible that this specificity of MIG-7 expression may be activated by multiple tumor microenvironment factors. To date, MIG-7 protein was found to be localized on normal embryonic cytotrophoblast cells and on cancer cells as well as within the lumen of vessel-like structures. Immunohistochemistry demonstrated that MIG-7 protein co-localizes with endothelial marker factor VIII, VE-cadherin and laminin 5 γ2 domain fragment III [17].

More recently, Liao and Gao have studied the immunohistochemical expressions of MIG-7-7 and MMP-2 related to VM in gastric cancer patients [25]. Vasculogenic mimicry was found in 32% of samples and its presence was significantly correlated with the tumor grade and lymph nodes metastases. Expression of MIG-7 was found in 95% of gastric cancer cases and was also associated with lymph node metastasis. The expression rates of MIG-7 and MMP-2 were higher in the VM-positive group than in the VM-negative group. In this study detection of vasculogenic mimicry in tumor samples was closely associated with the invasion, metastasis and poor prognosis of gastric cancer patients [25].

Currently, anti-VM therapies were proposed by several groups of investigators. Most of them aimed to remodel the extracellular matrix and tumor microenvironment or to block biochemical and molecular signaling pathways of VM or to inhibit tumor cell plasticity [26]. With the challenge of finding new drugs which could inhibit VM, the soybeans isoflavone called genistein was proposed. This molecule was found to be able to inhibit VM formation of uveal melanoma through down-regulation of VE-cadherin *in vitro* [27, 28]. In another study Itzhaki et al. tested the ability of nicotinamide to inhibit VM activity in melanoma cells [29]. They have found that nicotinamide acted as an epigenetic gene regulator and downregulated VE-cadherin expression. VE-cadherin was downregulated significantly on both RNA and protein levels. Clinical use of novel, targeted therapies including angiogenesis inhibitors has revealed new features of malignant ovarian tumors and important cancer molecular pathways checkpoints like MIG-7 activation that could be blocked with monoclonal antibodies [30].

Several lines of evidence indicate that ovarian cancer is a "moving target" and various treatment regimens may change its signaling features as well as metabolic characteristics [3, 4]. Phenotype-switching and plasticity comparable to that of embryonic cells is the main reason of complexity of the metastatic phenotype and should be taken into consideration when designing new therapies. Moreover, the significant heterogeneity within the cancer masses may enable escape from conventional therapy and in such cases new concurrent and targeted treatment regimens might lead to improved tumor responses and cures. Examples of other promising new approaches against ovarian cancer include targeting aberrant OC signaling such as the PI3K/Akt/mTOR network, the epidermal growth factor receptor, the tyrosine kinase and the folate receptor alpha [5]. Targeting only endothelial cells has no effect on tumor cells that are involved in VM network formation. Therefore, there is a strong clinical need to develop new ways of targeted antivascular modalities which would be based not only on cancer stage but also on a type of its vascularization [31, 32]. Moreover, specific inhibitors to these molecular mechanisms that control specific microvascular network need to be developed. The in-depth knowledge on the specific VE-cadherin specific signaling pathways and the association with the stem cell-like phenotype may be a key component of VM regulation [33].

## CONCLUSIONS

1. Vasculogenic mimicry exists in malignant ovarian tumors and clinical advanced stages of malignancy are accompanied by a high incidence of VM formation.
2. MIG-7 mRNA expression and VE-cadherin assessment may serve as additional molecular markers of VM in ovarian malignancies.

### *Authors' contributions*

AC, SC,NS,TŁ and GG had full access to all the data in the study and take responsibility for the integrity of the data, the accuracy of the data analysis, and the decision to submit the manuscript for publication. AC,NS,TŁ and GG conceived, designed and obtained partial funding for the study. SC, NS and AC collected the samples and patient's data. SC performed immunohistochemical and qRT-PCR studies. SA, NS, TŁ and GG analyzed the data and drafted the manuscript. AC supervised the study and revised the manuscript. All authors interpreted the data, critically revised the draft for important intellectual content, and gave final approval of the manuscript to be published.

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