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Prognostic significance of VEGF and its receptors in endometrioid endometrial cancer

Znaczenie prognostyczne VEGF i jego receptorów w raku *endometrium* typu endometrioidalnego

Dobrzycka Bożena[1], Terlikowski Sławomir Jerzy[1], Kwiatkowski Marcin[1], Garbowicz Magdalena[2], Kinalski Maciej[3], Chyczewski Lech[2]

[1] Department of Obstetrics, Gynecology and Obstetrics/Gynecological Care, Medical University of Bialystok, Poland
[2] Department of Clinical Molecular Biology, Medical University of Bialystok, Poland
[3] Department of Gynecology, District Hospital in Bialystok, Poland

Abstract

Background: *Angiogenesis is of crucial importance for endometrial tumor growth and Vascular Endothelial Growth Factor (VEGF) is the key mediator of angiogenesis.*

Objective: *The purpose of our study was to assess the prognostic value of VEGF and its receptors in relation to endometrioid endometrial carcinomas.*

Material and methods: *In this study, we conducted an immunohistochemical evaluation of VEGF and VEGFRs expression in 84 tissue samples obtained from endometrioid endometrial cancer patients undergoing curative surgical treatment.*

Results: *Out of 84 cancers, strong positive expression of VEGF was seen in 35 (42%) tumors. The overall strong positive rates were 33% for VEGFR-1 and for 15% for VEGFR-2. There was a significant correlation between clinical stage and VEGF and VEGFR-1 overexpression (p=0.027 and p=0.004, respectively). Additionally, there was a significant correlation between histological grade and VEGF and VEGFR-1 overexpression (p<0.001 and p<0.001, respectively). The 5-year DFS of patients with VEGF and VEGFR-1 overexpression was significantly lower than that of those with a weakly positive or negative tumor (p<0.001).*

Conclusion: *Immunohistochemical evaluation of VEGF and VEGFR-1 overexpression may be a useful marker for predicting 5-year DFS in endometrioid endometrial cancer.*

Key words: **VEGF** / **VEGFR-1** / **VEGFR-2** / **endometrial cancer** / / **disease free survival** /

Correspondence:
SJ Terlikowski
Department of Obstetrics, Gynecology and Obstetrics/Gynecological Care,
Medical University of Bialystok
Warszawska 15, 15-062 Bialystok, Poland
tel.: +48857488863, fax.: +48857488860
e-mail: sterlikowski@gmail.com

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Streszczenie

Wstęp: *Angiogeneza ma istotne znaczenie we wzroście raka endometrium. Naczyniowo-śródbłonkowy czynnik wzrostu (VEGF) jest kluczowym mediatorem angiogenezy.*

Cel pracy: *W pracy oceniono wartość prognostyczną VEGF i jego receptorów w endometrioidalnym raku endometrium.*

Materiał i metody: *Przeprowadzono immunohistochemiczną ocenę ekspresji VEGF i VEGFR w 84 preparatach uzyskanych od chorych leczonych operacyjnie.*

Wyniki: *Wśród 84 raków intensywnie dodatnią ekspresję VEGF obserwowano w 35 (42%) przypadków. Intensywnie dodatnią ekspresję VEGFR-1 stwierdzono w 33% a VEGFR-2 w 15% przypadków. Wykazano istotny statystycznie związek pomiędzy stopniem zaawansowania klinicznego a nadekspresją VEGF i VEGFR-1 (p=0,027 i p=0,004). Ponadto, stwierdzono znamienny statystycznie związek pomiędzy nadekspresją VEGF i VEGFR-1 a zróżnicowaniem histologicznym (p<0,001 i p<0,001). Pięcioletni czas przeżycia wolny od choroby (DFS) pacjentek, u których wykazano nadekspresję VEGF i VEGFR-1 był statystycznie znamiennie krótszy niż tych, u których ekspresji nie wykazano lub była ona słaba (p<0,001).*

Wnioski: *Immunohistochemiczna ocena ekspresji VEGF i VEGFR-1 może być przydatna w prognozowaniu pięcioletniego czasu przeżycia wolnego od choroby w raku endometrioidalnym.*

Key words: **VEGF** / **VEGFR-1** / **VEGFR-2** / **rak *endometrium*** / / **czas wolny od choroby** /

Introduction

Angiogenesis has been shown to be a critical aspect of endometrial cancer growth and metastasis [1]. The induction of angiogenesis by a tumor is a controlled process, influenced by angiogenic and angiostatic factors which involves a complex interaction between tumor and endothelial cells. Among the many reported angiogenic factors, vascular endothelial growth factor (VEGF) is the most powerful endothelial-cell-specific mitogen that plays a key role in the complicated process of angiogenesis [2]. It has been shown to be significantly upregulated in various human malignant tumors and to be associated with tumor angiogenesis and disease outcome [3, 4].

The effects of VEGF are mediated through binding to two homologous VEGF receptors, VEGFR-1 (Flt-1) and VEGFR-2 (KDR), which are expressed on vascular endothelial cells. Like other growth factor transmembrane tyrosine kinase receptors, VEGF receptors undergo ligand-induced dimerization. This triggers signal transduction by promoting receptor phosphorylation and subsequent recruitment of specific downstream signal transduction mediators. VEGF binding to VEGF receptor-2 elicits an efficient endothelial cell response. Although VEGF receptor-1 binds VEGF with high affinity, it is believed to act primarily by modulating the availability of VEGF for binding to VEGF receptor-2 [5, 6].

Besides established prognostic factors in endometrial cancer, such as histologic grade, stage, depth of myometrial invasion, and pelvic lymph node metastasis, angiogenesis has also been associated with survival [7, 8].

The purpose of our study was to assess the prognostic value of VEGF and its receptors in relation to endometrioid endometrial carcinomas.

Material and methods

Patients and tumor specimens

A total of 84 patients with endometrioid endometrial cancers (aged 54–72 years; mean age – 62.8 years), who underwent a total abdominal hysterectomy with bilateral salpingoophorectomy at the Department of Gynecology of the District Hospital in Bialystok, were included in the study. All patients had primary cancers and were receiving first surgical treatment. Patients gave informed written consent for the study. All tumors were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). The histological grade was classified according to the criteria of the World Health Organization. The protocol had been previously approved by the Bioethical Committee of the Medical University of Bialystok (R-I-003/115/2006). There were 32 patients with stage I, 40 with stage II, and 12 stage III endometrioid cancer. The samples were grouped by histological grade: 44 grade 1, 29 grade 2 and 11 grade 3 cancers.

Immunohistochemical staining for VEGF and VEGFRs

Four-µm sections were cut from formalin-fixed paraffin embedded tissue with a microtome and dried overnight at 37°C on a silanized-slide (DakoCytomation, Carpinteria, CA, USA). Samples were deparaffinized in xylene at room temperature for 80 min. and washed with a graded ethanol / water mixture and then with distilled water and stained for VEGF, VEGFR-1 (Flt-1) and VEGFR-2 (KDR) by the labeled streptavidin biotin method using the LSAB2 Kit (DakoCytomation, Carpinteria, CA, USA).

Rabbit primary polyclonal antibodies to VEGF and VEGFRs were used at a 1:100 dilution (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA). All primary antibodies were incubated for 12h at 4°C in a moist chamber. The binding sites of peroxidase were determined using 3,39-diaminobenzidine as the substrate. The sections were then counterstained with hematoxylin for microscopic examination. A positive control section was included in each staining run. Positive controls for reaction to the anti-VEGF antibody were represented by paraffin-embedded sections from ovarian carcinomas, and those for the three receptors included paraffin-embedded sections from human placenta. As negative controls, preimmune rabbit serum or antibodies absorbed with respective antigens were used instead of the primary antibodies.

Immunohistochemical staining was evaluated according to Volm et al. scoring method [9]. Briefly, a score was established

corresponding to the sum of the percentage of positive cells (0 - 0% immunopositive cells; 1 - 25% positive cells; 2 - 26–50% positive cells; and 3 - 50% positive cells) and staining intensity (0 - negative; 1 - weak; 2 - moderate; and 3 - high). The sum for the assigned values of the positive cell percentage and the staining intensity was less than 6. Scores between 0 and 2 were regarded as negative, scores of 3 and 4 as weakly positive, and scores of 5 and 6 as strongly positive. The staining scores of 5 and 6 were considered to indicate overexpression.

Statistical analysis

Statistical analysis was performed using Statistica software version 8.0 (StatSoft, Inc., StatSoft Polska Sp. z o.o., Poland). Data are expressed as mean ± SD. Fisher's exact test was used to determine significance between the two groups. A chi-square test was used to evaluate the relationship between categorical variables. A p-value of < 0.05 was considered as statistically significant.

Results

VEGF immunoexpression was localized in the cytoplasm of endometrial carcinoma cells with more diffuse staining within the stroma and stromal cells. The positive staining of VEGFR-1 and VEGFR-2 was localized in the cytoplasm.

The VEGF overexpression group consisted of 35 patients (42%) out of 84 endometrioid adenocarcinomas. Prognosis for patients with VEGF tumor overexpression was significantly poorer than for those with a VEGF weakly positive or negative tumor. 31% of the stage I endometrioid endometrial carcinoma samples were VEGF-strong positive, while 38% and 83% represented stage II and III, respectively. (Table I).

Table I. The relationship between VEGF and VEGFRs overexpression and tumor stage.

Stage	No. of cases	VEGF	VEGFR-1	VEGFR-2
		n (%)	n (%)	n (%)
I	32	10 (31)	8 (25)	4 (13)
II	40	15 (38)	11 (28)	5 (13)
III	12	10 (83)	9 (75)	4 (33)
p value		0.027	0.004	0.18

There was a significant correlation between clinical stage and VEGF and VEGFR-1 overexpression (p=0.027 and p=0.004, respectively). Additionally, there was a significant correlation between histological grade and VEGF and VEGFR-1 overexpression ($p<0.001$ and $p<0.001$, respectively). VEGFR-2 overexpression did not correlate with tumor stage or grade of differentiation. (Table I and II).

Out of all examined samples, VEGFR-1 and VEGFR-2 overexpression were 33% and 15%, respectively. Patients with VEGFR-1 overexpression had a significantly worse prognosis than those with VEGFR-1 weakly positive or negative tumor. (Table III).

Table II. The relationship between VEGF and VEGFRs overexpression and tumor histologic grade.

Grade	No. of cases	VEGF	VEGFR-1	VEGFR-2
		n (%)	n (%)	n (%)
G_1	44	8 (18)	9 (20)	8 (18)
G_2	29	18 (62)	9 (31)	3 (10)
G_3	11	9 (82)	10 (91)	2 (18)
p value		<0.001	<0.001	0.64

Table III. The correlation of VEGF and VEGFRs immunoexpression with 5-year DFS.

IHC	No. of cases	5-year DFS	p value
VEGF			
negative	30	30	<0.001
weakly	19	13	
strong	35	5	
VEGFR-1			
negative	34	34	<0.001
weakly	22	12	
strong	28	4	
VEGFR-2			
negative	45	45	0.18
weakly	26	19	
strong	13	5	

IHC - immunohistochemistry

The 5-year DFS of patients with VEGF and VEGFR-1 overexpression was significantly lower than of those with a weakly positive or negative tumor ($p<0.001$). On the other hand, there was no apparent statistical significance in the 5-year DFS for patients with VEGFR-2 overexpression. (Table III).

Discussion

Cancer of the uterus is the seventh most commonly diagnosed cancer that occurs in women, with 189,000 new cases and 45,000 deaths occurring worldwide each year [10]. In Poland, the age-adjusted incidence was 13.7/100,000 women annually [11].

Angiogenesis is crucial for normal growth and development and in protective responses, such as wound healing and inflammation [12]. However, aberrant angiogenesis can occur in a variety of pathological settings including growth and dissemination of tumors [13].

VEGF has been identified as an important regulator of tumor angiogenesis in the endometrium, but its role as a predictor of metastases and survival is yet to be defined. The expression of VEGF is 4 to 10 times higher at the invading tumor front than in the central tumor areas. Indeed, it has been shown that overexpression of VEGF and its receptors are related to poor prognosis in patients with endometrial carcinomas [1]. In various human cancers, VEGF expression was correlated with tumor angiogenesis and prognosis [14, 15, 16, 17].

Also, in our study, VEGF strong positive tumors showed a poorer prognosis than VEGF negative tumors. There was a trend towards an association between the strong positive expression of VEGF and 5-year DFS. These results suggest that VEGF may be an important, clinically relevant, inducer of angiogenesis in endometrioid endometrial cancer.

However, in our study, the overexpression of VEGFR-2 did not correlate with VEGF expression. Our results suggest that tumor angiogenesis, through the regulation of VEGF in endometrioid endometrial cancer, may be independent of VEGFR-2 status. In our study, strong positive expression of VEGFR-1 was significantly associated with poor survival. These results suggest that tumor angiogenesis may be a useful marker for predicting 5-year DFS in endometrioid cancer and, furthermore, that VEGF and VEGFR-1 overexpression are significant, independent, prognostic factors.

In conclusion, VEGF seems to be an important, clinically relevant inducer of angiogenesis, and tumor angiogenesis assessed by the immunohistochemistry of VEGF and VEGFR-1 may be a useful marker for predicting 5-year DFS.

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