ORIGINAL PAPER

Expression of vascular endothelial growth factor (VEGF) and assessment of microvascular density with CD34 as prognostic markers for endometrial carcinoma

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Abstract

Introduction: Angiogenesis plays an important role in the uncontrolled proliferation, invasion and metastasis of cancers. Increased microvessel density (MVD) is known to be associated with evolution and aggressiveness of the endometrial carcinoma (EC). The formation of new vessels depends on interactions between various hormones and growth factors. VEGF is one of the most known promoters of angiogenesis. *Material and Methods*: In this study, we intend to evaluate the relation between MVD, the VEGF expression, and the clinicopathologic factors in patients with endometrial carcinoma. Formalin-fixed, paraffin-embedded tissue from 54 patients with EC were included. MVD was assessed with anti-CD34 in most intense areas of neovascularization. A semiquantitative scoring system was used to asses the intensity and degree of staining of VEGF. *Results*: MVD counts of patients with G1 EC was lower than patients with G2 and G3 EC. MVD counts of patients with stage I EC was lower as compared with stage II + III patients. There was no statistically significant difference between MVD counts in lymph node-negative and positive EC patients. The positive immunoreactions for VEGF were significantly more frequent in G1 EC in comparison to the patients with G2 + G3 EC. *Conclusions*: MVD and VEGF are important indicators of a poor prognosis in patients with endometrial carcinoma.

***Keywords*:** endometrial adenocarcinoma, angiogenesis, VEGF, CD34.

Introduction

Angiogenesis consists in the formation of new blood vessels from proliferation of new capillary from pre-existing vessels, playing a great role in the uncontrolled proliferation of cells, surviving of the malign cells, local as well as distance tumor invade. Increased microvessel density (MVD), indirect marker of intense tumor vascularization, is known to be associated both with evolution of disease and surviving. The formation of new vessels depends on the interaction between different hormones and growth factors. The endometrium expresses several growth factors involved in angiogenesis, including epidermal growth factor (EGF), transforming growth factor (TGF-β) and vascular endothelial growth factor (VEGF). VEGF is one of the most common promoters of angiogenesis, being expressed even by normal endometrium. As an angiogenetic factor, VEGF stimulates the proliferation of endothelial cells and also increases vascular permeability and protein extravasations.

Material and Methods

In this study, we observed the relation between MVD, the expression of VEGF and clinical as well as morphological factors related to endometrial carcinoma. Patients' charts were selected from the Hospital of Obstetrics and Gynecology "Salvator Vuia" Arad; we included 54 cases of endometrial carcinoma that were treated by total hysterectomy with bilateral annexectomy. Lymphadenectomy was performed only in cases that had positive lymph nodules, diagnosed before surgery through abdominal computer tomography and lymphangiography. The mean age of patients was 57 years with a minimum of 38 and maximum of 81 years.

For the immunohistochemical evaluation of tumor angiogenesis we used monoclonal antibodies anti-CD34, clone QBEnd10 (DAKO, Glostrup, Denmark), through LSAB technique. Sections of 4 μm were boiled for 20 minutes in retrieval solution at 95–99^{0}C and then treated with primary antibody, secondary antibody and with Streptavidin for 30 minutes. The visualization system used DAB and counterstaining was performed

with Hematoxylin. As positive control for CD34, we used a pyogenic granuloma, while for negative control we replaced primary antibody with buffer solution.

We measured MVD by microscopically examination of sections from representative zones that had the biggest number of capillaries and venules according to the method described for the first time by Weidner N *et al.* After initial examination with a small objective (×100) and selection of zones with increased MVD, we counted the microvessels with an objective ×200. Immunoreactive endothelial cells and nests of endothelial cells clearly separated by nearby microvessels were also counted, while the identification of vessel lumen was not necessary for the identification of a microvessel. Micro-vascular density represents the medium number of vessels counted on five microscopic fields with an objective of ×200.

In order to evaluate the expression of VEGF we used monoclonal antibody antihuman VEGF, clone VG1 using LSAB+ technique. Sections were pre-treated beforehand by boiling with Dako–Cytomation Target Retrieval solution pH 9 (DAKO) for 15 minutes. Primary antibody was applied in dilution 1:25 with an incubation period of one hour. The visualization system included DAB solution, counterstained with Hematoxylin.

In order to describe intensity and immunostaining grade for VEGF we used a semi-quantitative score based on two parameters: percentage of positive cells and intensity of coloration. Those parameters were quantified by numbers from 1 to 3 as follows:

▪ Intensity of staining:

– 0=negative response;

– 1=weak intensity;

– 2=moderate intensity;

– 3=strong intensity;

▪ Percentage of positive cells:

– 0=negative (0% immunopositive cells);

– 1=positive immunoreaction in <25% of tumor cells cytoplasm;

– 2= positive immunoreaction in 26–50% of tumor cells cytoplasm;

– 3= positive immunoreaction in >50% of tumor cells cytoplasm.

Final score was obtained by summing the two parameters, with the following interpretation for the immunohistochemical reaction:

▪ a negative immunoreaction for a score between 0 and 2;

▪ a slightly positive immunoreaction for a score between 3 and 4;

▪ a strongly positive immunoreaction for a score between 5 and 6.

Statistical analysis was performed using the Epi 3.2.2, OpenEpi 2.3 and Epi Info 6.04 and consisted in computing the frequency count and percentages for qualitative variables, the mean and standard deviation for quantitative variables. The comparison of the percentages and the means were performed using the chi-square test and the unpaired Student *t*-test *p*-value <0.05 was considered significant.

Results

Quantification of intratumor micro-vascular density by immunoreaction with CD34

Normal endometrium is histologically characterized by permanent vascular remodeling, main involving steroid-sensitive spiral arteries and subepithelial capillary plexus during the entire menstrual cycle. Angiogenesis is reduced during the menstrual phase followed by a rapid grow in the proliferative phase and then by a graduate decreasing until the end of the cycle.

There was observed an intense tumor angiogenesis in the studied endometrial carcinoma with highest values for the invasion front. Inside the tumor stroma, among pseudo-glands or solid beaches there was a positive reaction for anti-CD34 of numerous microvessels together with groups of endothelial cells ("hot spot"). MVD had variable values between 16 and 138 with an average of 61.95, greater than compared to MVD of endometrial mucosa from proliferative stage (49.0). Vascular architecture appeared modified compared to normal endometrial mucosa, with variable vessels regarding size, with irregular lumen, partially thronged and chaotically distributed (Figure 1). There were also present isolated endothelial cells CD34 positive or arranged in small groups without evident vascular lumen and "hot spots" without red cells (Figure 2).

Our results showed that there is a significant statistical difference between MVD according to patients' age (p=0.0409 S, Table 1).

Table 1 – ***Relation between MVD and clinico-morphological prognostic factors of endometrial carcinoma***

	No. of cases	Mean MVD	Standard deviation
Normal histologic proliferative endometrium	10	49.00	10.20
Endometrial carcinoma			
G_1	24	59.63	6.79
G_2	21	64.36	14.87
G_3	9	62.95	8.87
Stage I FIGO	26	59.90	7.11
Stage II FIGO	17	65.93	15.28
Stage III FIGO	11	61.95	8.71
Negative lymph nodules	49	62.36	11.35
Positive lymph nodules	5	57.88	4.76
Age <60 years	35	63.00	4.99
Age >61 years	19	59.90	5.55

There is a strong correlation between the histological grade and MVD, the later one increasing as tumor differentiation grade decreases (Figures 3 and 4). The mean MVD counts of patients with well-differentiated G_1 endometrial carcinoma (59.63) was significantly lower than patients with G_2 (64.36) or G_3 (62.95) (p=0.00159).

Mean values of MVD quantified for patients in stage I FIGO (59.9) are smaller than those of patients in stage II FIGO (65.93) as well as stage III FIGO (61.95), with insignificant statistical difference (p=0.20039).

There were differences between mean values for

MVD calculated on patients without lymph nodules metastases (62.36) as compared to cases with positive lymph nodules (57.88), but not statistically significant (p=0.3890).

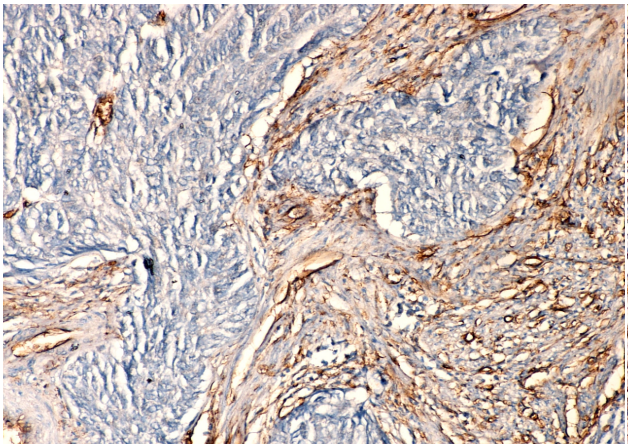

Figure 1 – ***Deeply modified vascular architecture in endometrioid carcinoma (CD34, DAB immunoreaction, ob. ×20).***

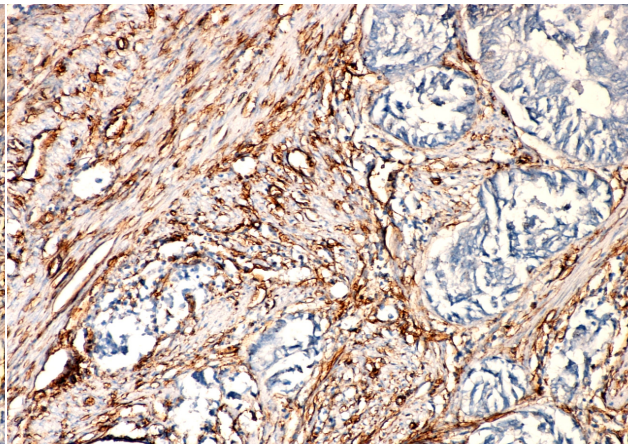

Figure 2 – ***Many microvessels and "hot spot" in the tumor invasion front (CD34, DAB immunoreaction, ob. ×20).***

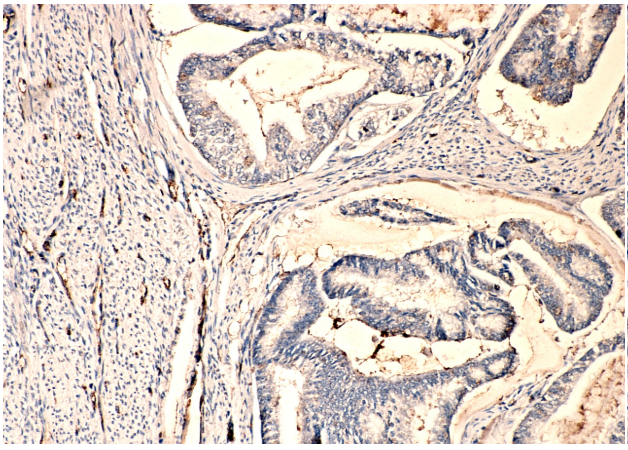

Figure 3 – ***Endometrioid carcinoma, low grade (CD34, DAB immunoreaction, ob. ×10).***

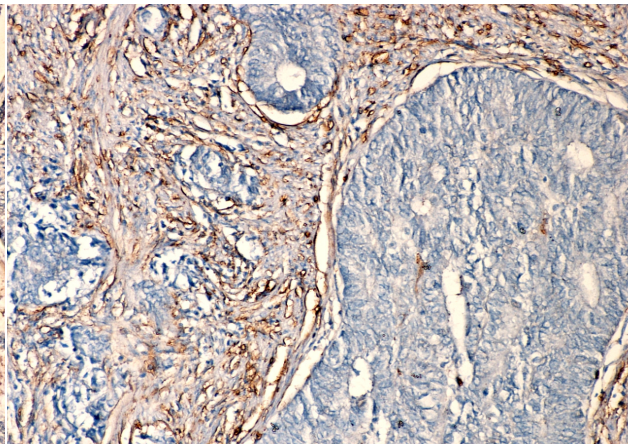

Figure 4 – ***Endometrioid carcinoma, high grade (CD34, DAB immunoreaction, ob. ×20).***

Immunohistochemical expression of VEGF in endometrial carcinoma

VEGF is expressed in the cytoplasm of normal endometrial cells, neoplasic ones, endothelial cells and stroma.

Positive immunoreactions for VEGF showed a coloration of variable intensity inside epithelial tumor cells' cytoplasm as well as focal for stromal cells (Figure 5). Inside the normal proliferate endometrium, there were negative VEGF immunoreactions (30% of cases), weekly positive (40% of cases) and intense positive (30%) (Table 2). In the endometrial carcinoma compared to normal endometrium, there were discovered a higher percentage of positive immunoreactions for VEGF (88.88% of cases, p=0.278).

Among clinical prognostic factors, we discovered that age does not influence the expression of VEGF (p=0.579).

It exists a correlation between the tumor histological grade and the obtained VEGF score, but not statistically significant (p=0.467). The expression rate for VEGF was 83.34% in the well-differentiated endometrial carcinoma (Figure 6) and 93.33% in the moderately and poorly differentiated carcinoma (Figures 7 and 8).

Table 2 – ***Correlation of VEGF with clinico-morphological prognostic factors***

	No. of cases	Expression	VEGF	
		Negative n (%)	*Weak + n (%)*	*Intense + n (%)*
Age <60 years	35	5	4	26
Age >61 years	19	1	4	14
Normal proliferative endometrium	10	3 (30)	4 (40)	3 (30)
Endometrial carcinoma				
Total	54	6 (11.11)	8 (14.81)	40 (74.07)
G_1	24	4 (16.67)	4 (16.67)	16 (66.67)
$G_2 + G_3$	30	2 (6.67)	4 (13.33)	24 (80.00)
Stage I	25	4 (16.00)	3 (12.00)	18 (72.00)
Stage II + III	29	2 (6.89)	5 (17.24)	22 (75.86)
Negative LN	49	6 (12.24)	6 (12.24)	37 (75.51)
Positive LN	5	0 (0.00)	2 (40.00)	3 (60.00)

The quantification of VEGF expression according to the stage of the disease shows slightly different values for stage I FIGO (84.0%) as compared to stage II and III FIGO (93.1%), not statistically significant (p=0.530).

By comparing VEGF expression to the status of lymph nodules, we obtained positive VEGF reactions in 87.75% of cases without metastasis together with all

five cases with positive lymph nodules (small number of cases that could not be statistically interpreted).

The analysis of correlation between MVD and the VEGF expression reveals that tumors with positive VEGF had a mean MVD value of 65.74, bigger than that of negative VEGF endometrial carcinoma (55.60), with a significant statistical difference (p=0.0352) (Table 3).

Table 3 – ***Correlation between VEGF expression and mean MVD in endometrial carcinoma***

No. of cases	VEGF expression	Mean MVD	Standard deviation
48	positive	65.74	11.23
6	negative	55.60	5.86

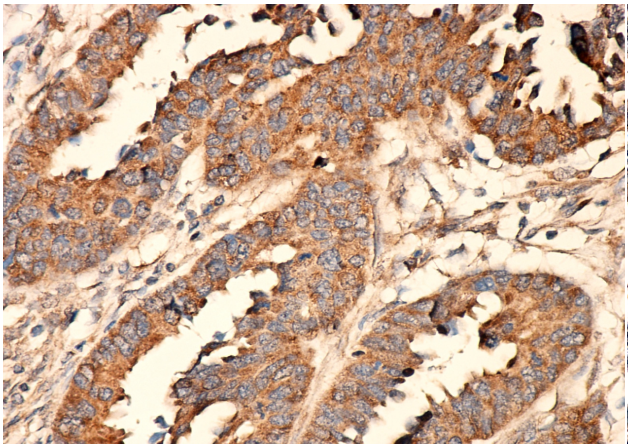

Figure 5 – ***Positive VEGF immunoreaction in the tumor cells' cytoplasm and focal in stromal cells (DAB, ob. ×40).***

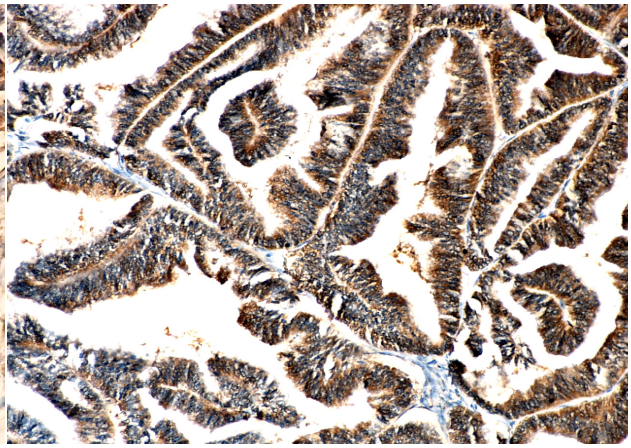

Figure 6 – ***Positive VEGF immunoreaction in villo-glandular carcinoma, FIGO grade 1 (DAB, ob. ×10).***

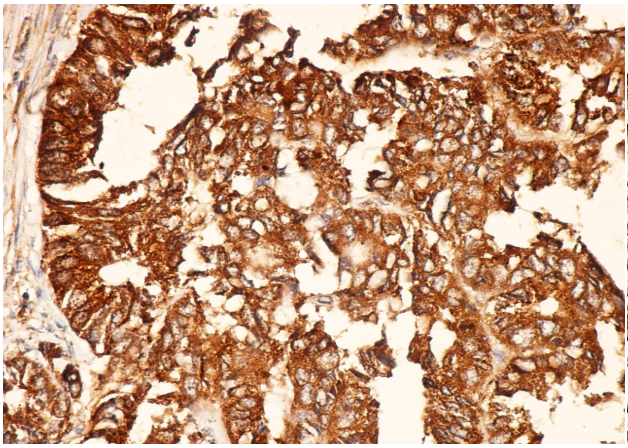

Figure 7 – ***Intensely positive VEGF immunoreaction in the cytoplasm of glandular cells of G_3 endometrial adenocarcinoma (DAB, ob. ×40).***

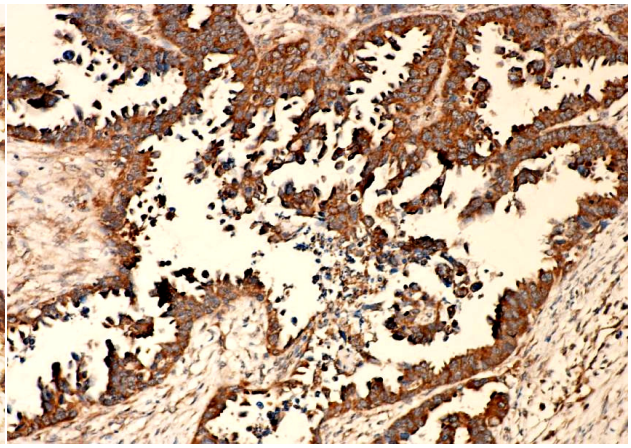

Figure 8 – ***Immunohistochemical staining of VEGF in serous carcinoma, high grade (DAB, ob. ×10).***

Discussion

Angiogenesis is an essential phase in the uncontrolled cell proliferation, invasion and metastasis described initially by Judah Folkman in 1969 consisting in the forming and remodelation of new vessels.

Folkman J *et al.* (1971) quantified the number of tumoral vessels, endothelial cell hyperplasia and specific malign aspects of tumors on usual colored samples obtaining MAGS score which allows the classification of tumors in week or rich vascularized [1, 2].

After 1980, the most applied method meant to describe angiogenesis, was introduced by Weidner N ("hot spot" method). This method is based on immunohistochemical identification of tumor vessels as well as their quantification by choosing samples with highest vascular density. The method is applied even nowadays and confirms reach vascularization of tumor tissues compared to normal tissues; the vascular micro-density obtained through this method represents an important prognostic factor in cancers [3].

Endometrium is a dynamic organ in which angiogenesis develops naturally with the lowest expression during menstruation phase, followed by rapid growth at the beginning of the proliferative phase and then followed by gradual decrease to the end of the cycle [4]. Excessive angiogenesis is noticed in a variety of pathological conditions such as endometriosis, hyperplasia of the endometrium and solid tumors [5, 6].

Even though studies demonstrate that MVD, as part of angiogenesis, represents an important prognostic factor in various malign tumors, including genital ones [7–9], in literature there are also contradictory data regarding the association between aggressiveness of endometrial carcinoma and MVD measured with the pan-endothelial markers [10–12].

Angiogenesis was quantified in our study by

antibody CD34 and expressed through MVD. It was correlated to the tumor differentiate grade (as the histological grade decreases, the number of new vessels increases), respectively the stage of the disease (mean values for MVD are smaller in patients with stage I FIGO compared to stage II and III FIGO) as well as lymph nodules status. The differences between mean values of MVD were statistically significant only for the cases of appreciating the tumor histological grade (p=0.0159).

The study performed by Erdem O *et al.* showed that [13] the mean value of MVD, quantified with anti-CD34 were higher in the cases with carcinoma as compared to hyperplasia or normal proliferative endometrium, though not statistically significant. MVD in endometrial carcinomas G_2 and G_3 was higher compared to G_1, though comparing G_2 and G_3 with proliferative endometrium, hyperplasia and G_1 carcinoma differences were not statistically significant. Referring to stadialization of endometrial carcinoma, MVD measured in stage II and III raised above values from stage I, but with no significant differences between tumors with high grade respectively low grade. There were no significant differences between MVD and lymph nodules status.

Giatromanolaki A *et al.* performed a study on 121 endometrial carcinomas and had proven that MVD and the expression of VEGF represent independent prognostic factors on patients with endometrial cancer [14].

In the majority of studies [15] regarding endometrial carcinoma, increased angiogenesis correlates with a modest prognosis.

VEGF represents a mitogen for endothelial cells that induces the formation of lumen's vessels and also increases vascular permeability and protein extravasation [16]. The expression of VEGF on normal endometrium, detected in both glandular epithelium and stroma, is low during proliferative phase but increases during secretory phase reaching its maximum in the menstrual phase [4, 17]. Zhang L *et al.* proved an intense VEGF expression in the basal layer of the endometrium rather than in the functional one [18]. In the stromal cells, the expression of VEGF is reduced compared to glandular epithelium cells and does not modify during menstrual cycle [19]. Some studies demonstrated the growth of VEGF under the influence of estradiol and progesterone [20–22] or even an increased expression as a result to their combination compared to individual administration [23].

Many studies sustain that there is an increased production of VEGF in endometrial carcinoma and hyperplasia of the endometrium compared to normal endometrium; the VEGF expression contributes to the role of angiogenesis in the transition towards carcinoma. Still, there are contradictory results regarding prognostic relevance of VEGF and its receptors in the evolution of endometrial carcinoma [24–26].

Our results show a correlation between the VEGF expression and the histological grade, the FIGO stage of the disease and the lymph nodules status, but with no statistical significance. The correlation between VEGF expression and MVD demonstrate statistical significant differences: MVD=65.74 in positive VEGF tumors, MVD=55.6 in negative VEGF tumors, p=0.0352S.

Saito M *et al.* performed a study on 85 endometrial adenocarcinoma and obtained a significant VEGF expression higher for well-differentiated tumors as well as moderately ones compared to those that were poorly differentiated [27]. It was reported that estrogen increases VEGF concentration [28], so this should imply that the expression of VEGF should be higher in well-differentiated endometrial adenocarcinomas estrogen-dependent. The results of this study suggest that the expression of angiogenic factors is different for endometrial adenocarcinoma compared to normal endometrium, but those factors do not participate directly to the disease progression.

There are several other studies in which expression of VEGF in endometrial tumors did not correlate to none of the previously studied histopatological variables: histological type, histological grade, the depth of the invasion and vascular invasion. VEGF expression correlated to increased MVD in the invading tumor front [14]. Seki N *et al.* observed an increased level of VEGF that correlated significantly to rich vascularization of endometrial carcinomas [29]. In contradiction, according to Fujisawa T *et al.*, VEGF does not correlate with the number of microvessels from the endometrial carcinoma [30].

Conclusions

Immunoreactions for CD34 antigen represent a good method to quantify angiogenesis in endometrial carcinoma.

The MVD value from the front of tumor invasion presented a significant increase compared to normal proliferative endometrium (p<0.01).

There was a significant correlation between MVD and histological grade, as intratumor vascularization increases ones the tumor is less differentiated. Increased MVD could reflect an advanced stage of endometrial carcinoma and that is why it can be considered an important prognostic marker for patients diagnosed with endometrial tumors.

VEGF expression correlated with MVD, as positive VEGF tumors presented significantly high levels of MVD compared to negative VEGF cases.

References

[1] FOLKMAN J, *Tumor angiogenesis: therapeutic implications*, N Engl J Med, 1971, 285(21):1182–1186.

[2] RASILA KK, BURGER RA, SMITH H, LEE FC, VERSCHRAEGEN C, *Angiogenesis in gynecological oncology – mechanism of tumor progression and therapeutic targets*, Int J Gynecol Cancer, 2005, 15(5):710–726.

[3] WEIDNER N, *Tumoural vascularity as a prognostic factor in cancer patients: the evidence continues to grow*, J Pathol, 1998, 184(2):119–122.

[4] TORRY DS, HOLT VJ, KEENAN JA, HARRIS G, CAUDLE MR, TORRY RJ, *Vascular endothelial growth factor in cycling human endometrium*, Fertil Steril, 1996, 66(1):72–80.

[5] MCLAREN J, *Vascular endothelial growth factor and endometriotic angiogenesis*, Hum Reprod Update, 2000, 6(1):45–55.

[6] HEALY DL, ROGERS PAW, HII L, WINGFIELD M, *Angiogenesis: a new theory for endometriosis*, Hum Reprod Update, 1998, 4(5):736–740.

[7] WEIDNER N, *Intratumor microvessel density as a prognostic factor in cancer*, Am J Pathol, 1995, 147(1):9–19.

[8] WEIDNER N, SEMPLE JP, WELCH WR, FOLKMAN J, *Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma*, N Engl J Med, 1991, 324(1):1–8.
[9] KAKU T, KAMURA T, KINUKAWA N, KOBAYASHI H, SAKAI K, TSURUCHI N, SAITO T, KAWAUCHI S, TSUNEYOSHI M, NAKANO H, *Angiogenesis in endometrial carcinoma*, Cancer, 1997, 80(4):741–747.
[10] KIRSCHNER CV, ALANIS-AMEZCUA JM, MARTIN VG, LUNA N, MORGAN E, YANG JJ, YORDAN EL, *Angiogenesis factor in endometrial carcinoma: a new prognostic indicator?*, Am J Obstet Gynecol, 1996, 174(6):1879–1882; discussion 1882–1884.
[11] SALVESEN HB, IVERSEN OE, AKSLEN LA, *Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study*, J Clin Oncol, 1999, 17(5):1382–1390.
[12] ERDEM O, ERDEM M, DURSUN A, AKYOL G, ERDEM A, *Angiogenesis, p53, and bcl-2 expression as prognostic indicators in endometrial cancer: comparison with traditional clinicopathologic variables*, Int J Gynecol Pathol, 2003, 22(3):254–260.
[13] ERDEM O, ERDEM M, ERDEM A, MEMIS L, AKYOL G, *Expression of vascular endothelial growth factor and assessment of microvascular density with CD 34 and endoglin in proliferative endometrium, endometrial hyperplasia, and endometrial carcinoma*, Int J Gynecol Cancer, 2007, 17(6):1327–1332.
[14] GIATROMANOLAKI A, SIVRIDIS E, BREKKEN R, THORPE PE, ANASTASIADIS P, GATTER KC, HARRIS AL, KOUKOURAKIS MI; TUMOUR AND ANGIOGENESIS RESEARCH GROUP, *The angiogenic "vascular endothelial growth factor/flk-1(KDR) receptor" pathway in patients with endometrial carcinoma: prognostic and therapeutic implications*, Cancer, 2001, 92(10):2569–2577.
[15] SIVRIDIS E, *Angiogenesis and endometrial cancer*, Anticancer Res, 2001, 21(6B):4383–4388.
[16] DVORAK HF, BROWN LF, DETMAR M, DVORAK AM, *Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis*, Am J Pathol, 1995, 146(5):1029–1039.
[17] TORRY DS, TORRY RJ, *Angiogenesis and the expression of vascular endothelial growth factor in endometrium and placenta*, Am J Reprod Immunol, 1997, 37(1):21–29.
[18] ZHANG L, SCOTT PA, TURLEY H, LEEK R, LEWIS CE, GATTER KC, HARRIS AL, MACKENZIE IZ, REES MC, BICKNELL R, *Validation of anti-vascular endothelial growth factor (anti-VEGF) antibodies for immunohistochemical localization of VEGF in tissue sections: expression of VEGF in the human endometrium*, J Pathol, 1998, 185(4):402–408.
[19] GARGETT CE, LEDERMAN FL, LAU TM, TAYLOR NH, ROGERS PA, *Lack of correlation between vascular endothelial growth factor production and endothelial cell proliferation in the human endometrium*, Hum Reprod, 1999, 14(8):2080–2088.
[20] ARCHER DF, NAVARRO FJ, LESLIE S, MIRKIN S, *Effects of levonorgestrel, medroxyprogesterone acetate, norethindrone, and 17beta-estradiol on vascular endothelial growth factor isomers 121 and 165 in Ishikawa cells*, Fertil Steril, 2004, 81(1):165–170.
[21] MUELLER MD, VIGNE JL, PRITTS EA, CHAO V, DREHER E, TAYLOR RN, *Progestins activate vascular endothelial growth factor gene transcription in endometrial adenocarcinoma cells*, Fertil Steril, 2003, 79(2):386–392.
[22] HUANG JC, LIU DY, DAWOOD MY, *The expression of vascular endothelial growth factor isoforms in cultured human endometrial stromal cells and its regulation by 17beta-oestradiol*, Mol Hum Reprod, 1998, 4(6):603–607.
[23] SHIFREN JL, TSENG JF, ZALOUDEK CJ, RYAN IP, MENG YG, FERRARA N, JAFFE RB, TAYLOR RN, *Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis*, J Clin Endocrinol Metab, 1996, 81(8):3112–3118.
[24] FINE BA, VALENTE PT, FEINSTEIN GI, DEY T, *VEGF, flt-1, and KDR/flk-1 as prognostic indicators in endometrial carcinoma*, Gynecol Oncol, 2000, 76(1):33–39.
[25] YOKOYAMA Y, SATO S, FUTAGAMI M, FUKUSHI Y, SAKAMOTO T, UMEMOTO M, SAITO Y, *Prognostic significance of vascular endothelial growth factor and its receptors in endometrial carcinoma*, Gynecol Oncol, 2000, 77(3):413–418.
[26] HOLLAND CM, DAY K, EVANS A, SMITH SK, *Expression of the VEGF and angiopoietin genes in endometrial atypical hyperplasia and endometrial cancer*, Br J Cancer, 2003, 89(5):891–898.
[27] SAITO M, SATO Y, WATANABE J, KURAMOTO H, KABA S, FUKUDA T, *Angiogenic factors in normal endometrium and endometrial adenocarcinoma*, Pathol Int, 2007, 57(3):140–147.
[28] CHARNOCK-JONES DS, SHARKEY AM, RAJPUT-WILLIAMS J, BURCH D, SCHOFIELD JP, FOUNTAIN SA, BOOCOCK CA, SMITH SK, *Identification and localization of alternately spliced mRNAs for vascular endothelial growth factor in human uterus and estrogen regulation in endometrial carcinoma cell lines*, Biol Reprod, 1993, 48(5):1120–1128.
[29] SEKI N, KODAMA J, HONGO A, MIYAGI Y, YOSHINOUCHI M, KUDO T, *Vascular endothelial growth factor and platelet-derived endothelial cell growth factor expression are implicated in the angiogenesis of endometrial cancer*, Eur J Cancer, 2000, 36(1):68–73.
[30] FUJISAWA T, WATANABE J, AKABOSHI M, OHNO E, KURAMOTO H, *Immunohistochemical study on VEGF expression in endometrial carcinoma – comparison with p53 expression, angiogenesis, and tumor histologic grade*, J Cancer Res Clin Oncol, 2001, 127(11):668–674.

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