Evaluation of tumor angiogenesis and thymidine phosphorylase tissue expression in patients with endometrial cancer

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The formation of new blood vessels in endometrial cancer tissue is a main process, which leads to tumor progression, and is connected with tumor expansion and invasiveness.

The aim of the study was evaluation of thymidine phosphorylase protein (TP) expression in human endometrial cancer cells by immunohistochemistry and comparison obtained data with intensity of angiogenesis process and clinicopathological factors as FIGO stage of disease and histopathologic grade. Endometrial cancer specimens were obtained from 55 postmenopausal patients (aged 52 to 74 years) underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. None of patients received preoperative pelvic irradiation. Histopathological typing and grading of the endometrial tumors (G-1, G-2, G-3) as well as myometrial invasion (<1/2, >1/2) were assessed using standard criteria, on hematoxylin-eosin sections. At the surgery, FIGO clinical stage of disease was determined.

Thymidine phosphorylase overexpression was observed in 23 of 55 (41.8%) cases of endometrial cancer. Although we found no statistically significant differences in TP expression between histopathologic grades, particular FIGO stages showed a significant trend of increase TP tumor overexpression. Thymidine phosphorylase overexpression cases demonstrate higher intensity of angiogenesis in comparison to negative samples and results are statistically significant for t-test ($p<0.0001$). The most intensive new blood vessel formation was observed in G-2 of tumor differentiation grade ($p=0.013$ for ANOVA test) Mean angiogenic points density (APD) values in cases of G-1 histopathologic grade reached 135.7; values of G-2 and G-3 grades reached 213.8 and 162.8, respectively. Mean intensity of angiogenesis in the first FIGO stage of disease reached 160.0 APD, in stage II 205.6 APD, and in the third 286.9, respectively. Angiogenesis was more intensive in cases of advanced tumors – analysis of variance (ANOVA) confirmed statistically significant differences in APD values between FIGO stage groups ($p=0.0007$).

In conclusion, thymidine phosphorylase expression correlates with increased microvessel density in endometrial cancer. The intensity of angiogenesis process increases according to FIGO stage of disease, which is connected with progressing of cancer disease. Thymidine phosphorylase can play an important role in endometrial cancer progression and could offer additional information about advance of disease.

Key words: endometrial cancer, angiogenesis, thymidine phosphorylase

The formation of new blood vessels in endometrial cancer tissue is a main process, which leads to tumor progression, and is connected with tumor expansion and invasiveness. Particular steps of angiogenesis process are induced by various angiogenic factors, produced and secreted by the tumor and non-malignant surrounding tissues, lymphocytes, macrophages, mast cell or endothelial cell [1–3]. Recent studies have shown many different angiogenic factors, but the main factor and its mechanism remain still unknown [4–6]. The most intensively studied angiogenic factors in endometrial cancer seem to be vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and angiopoetins (Ang-1 and Ang-2) as well as transforming growth factor beta (TGFβ).

Thymidine phosphorylase (TP) originally entitled as platelet derived endothelial cell growth factor (PD-ECGF) is consecutive angiogenic factor, being an object of research study of endometrial carcinoma [4, 7–9]. TP is 45 kDa

polypeptide is mainly produced and released by platelets. It is expressed in nonpathological tissues like lymph nodes, spleen, lung, liver, placenta and endometrium. TP tissue activity was also found in malignant gliomas, thyroid tumors, cancers of colon, breast, pancreas, gall bladder, kidney, lung and gynaecological tumors [8–12]. Thymidine phosphorylase does not stimulate the growth of endothelial cells, but only their chemotaxis. Additionally one of its metabolic product 2-deoxy-D-ribose, has also angiogenic activity. Immunohistochemical TP expression has been found to correlate with various clinicopathologic factors and prognosis in several types of cancer, like non-small cell lung cancer [13, 14], ovarian cancer [15–18], prostate cancer [19, 20] and endometrial cancer [8, 9]. However, TP expression in endometrial cancer has not been clarified.

The aim of the study was evaluation of thymidine phosphorylase expression in human endometrial cancer cells and correlation of obtained data with intensity of angiogenesis, FIGO stage of disease and histopathologic grade.

Patients and methods

Endometrial cancer specimens were obtained from 55 postmenopausal patients (aged 52 to 74 years) underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy at the Department of Gynecology in Medical University of Bialystok, in Poland. None of patients received preoperative pelvic irradiation. Histopathological typing and grading of the endometrial tumors (G-1, G-2, G-3) as well as myometrial invasion (<1/2, >1/2) were assessed using standard criteria, on hematoxylin-eosin sections. At the surgery, FIGO clinical stage of disease was determined. A group of 33 women (60%) were of I FIGO stage, 16 (29%) and 6 (11%) patients were of II and III stage, respectively. Tumor differentiation grade G-1 (well differentiated tumors) was found in 11 cases (20%), grades G-2 and G-3 were found in 32 (58%) and 12 (22%) cases.

Tissue samples were collected immediately after the surgery and fixed in buffered formalin solution and paraffin embedded on the same day. The local ethical committee accepted the study protocol and each patient provided a written consent for participation.

For evaluation of angiogenesis, immunohistochemical and morphometric methods were applied as reported in previous publications [21, 22]. Prepared slices were incubated with DAKO EPOS Anti-Human Von Willebrand Factor/HRP antibodies, AEC (3-amino-9-ethyl-carbozole) was used as chromogen. Negative controls were performed with DAKO EPOS Immunoglobulin/HRP. Both vessels and endothelial cells were counted on a light microscope under 100x magnification. The areas of the highest vessel and endothelial cell density (so called "hot spots") were chosen for counting. Counting was performed automatically using computerised morphometric appliance equipped with LUCIA-NIKON specialist software. Angiogenic points (AP) were defined as microvessels and single endothelial cells including closed capillaries. Angiogenic points density (APD) were defined as the density of AP per square mm.

Immunohistochemical staining for evaluation thymidine phosphorylase expression in tumor tissue was performed using NeoMarkers' Thymidine Phosphorylase/PD-ECGF Ab-1 MS-499 antibodies. Formalin-fixed, paraffin-embedded human endometrial carcinoma samples were stained with monoclonal antibodies using peroxidase-conjugate and AEC chromogen. Nuclear and cytoplasmic staining of tumor cells was noted. Specimens with positive reaction occurring in more than 5% of cells were treated as TP protein overexpression.

Statistical analysis. Comparisons based on contingency tables were performed using chi-square test, Fisher's exact test and Cochran-Armittage test for trend. Comparisons of intensity of angiogenesis values among groups defined by different levels of FIGO stages of disease, histological grade and TP were performed using ANOVA test, Bonferroni's multiple comparison test and t-test. The assumptions were checked using Shapiro-Wilk's and Bartlett's test. Since the variances were significantly different the logarithmic transformation was applied.

All the tests performed in the analyses were two-sided. The 0.05 significance level was adopted. The calculations were performed using SAS STAT v.8.2 package and PRISM.

Results

Thymidine phosphorylase overexpression was observed in 23 of 55 (41.8%) cases of endometrial cancer. It tended to be more frequent in cases of advanced cancer disease. Although we found no statistically significant differences in TP expression between histopathologic grade, particular FIGO stages showed a significant trend of increasing TP tumor overexpression (Cochran-Armitage test for trend, p values 0.05 and 0.035 for tumor differentiation grading and FIGO staging, respectively). According to increasing stage of cancer disease the number of thymidine phosphorylase overexpression cases for Fisher's exact test tended also to be risen (p=0.081) (Tab. 1).

Table 1. Evaluation of thymidine phosphorylase overexpression (TP=1) according to FIGO stage and histological grade (G)

	TP=1 n (%)	P value
G-1	2/11 (18.2)	0.102[a]
G-2	17/32 (53.1)	0.5[b]
G-3	4/12 (33.3)	
FIGO		0.081[c]
Ia+Ib+Ic	11/33 (33.3)	0.035[b]
IIa+IIb	7/16 (43.7)	
IIIc	5/6 (83.3)	

[a]p value for chi-square test, [b]p value for Cochran-Armittage test for trend, [c]p value for Fisher's exact test

Table 2 demonstrates the relationships between intensity of angiogenesis process (angiogenic points density per square mm), thymidine phosphorylase expression, histological grading and FIGO staging of disease. Thymidine phosphorylase overexpression cases demonstrate higher intensity of angiogenesis (mean APD value 239.4 points) in comparison to negative samples (mean APD 149.4). These results were statistically significant for t-test ($p<0.0001$).

The most intensive new blood vessel formation was observed in G-2 of tumor differentiation grade ($p=0.013$ for ANOVA test). Mean APD values in cases of G-1 histopathologic grade reached 135.7 points, G-2 and G-3 grades reached 213.8 and 162.8, respectively.

Mean Angiogenic Points' Density (APD) reached 160.0 points in I FIGO stage cases and 205.6 and 286.9 in stages II and III, respectively. As was already found in our previous study [21, 22], we observed that angiogenesis was more intensive in cases of advanced tumors – analysis of variance (ANOVA) confirmed statistically significant differences in APD values between FIGO stage groups ($p=0.0007$).

Bonferroni's multiple comparison tests of intensity of angiogenesis for histological grading and TP expression demonstrate Figures 1 and 2, respectively. The mean values on figures are signed by horizontal lines.

Multiple analysis of FIGO groups shows statistically dependent trends in comparisons I vs. II and I vs. III ($p<0.05$ and $p<0.01$ respectively). There is no statistical important correlation between FIGO II and III stage.

Discussion

The biology of tumor angiogenesis and its clinical significance has been studied in a variety of gynaecologic malignancies. Several studies have been reported in ovarian carcinoma [1, 3] and in cervical carcinoma [23, 24]. GUIDI et al [25] demonstrated that in early cervical lesions the microvessel density was significantly increased both in invasive carcinoma and in high grade intraepithelial lesions as compared with low grade intraepithelial lesions and benign squamous epithelium.

In our study, the intensity of angiogenesis was greater in advanced tumors. We confirmed statistically significant differences of APD values between particular groups of FIGO stage. The most intensive angiogenesis process was found in the most of clinically advanced cancer cases. No such dependence was found for tumor histologic grade.

ABULAFIA et al [26] reported that the angiogenesis was increased in complex endometrial hyperplasia as compared to controls of simple hyperplasia. The angiogenic capability of complex hyperplasia was comparable to the FIGO stage Ia endometrial cancer. An increased angiogenesis was found in cases of invasive (stages Ib and Ic) endometrial cancer as compared to complex hyperplasia or stage Ia endometrial cancer.

Thymidine phosphorylase activity in endometrial malignant tissue can suggest its feasibly important role in cancer progressing process. Because of huge number of angiogenic and antiangiogenic factors and their co-operation in formation of new blood vessels in tumor tissue the role TP expres-

Table 2. The relationship between intensity of angiogenesis process (APD), thymidine phosphorylase overexpression (TP=1), histological grade (G) and FIGO stage of disease

	n	mean APD ± SD	p
TP			$<0.0001^{a\#}$
0	32	149.4 ± 58.66	
1	23	239.4 ± 87.82	
G-1	11	135.7 ± 49.64	$0.013^{b\#}$
G-2	32	213.8 ± 92.05	
G-3	12	162.8 ± 59.09	
FIGO			$0.0007^{b\#}$
Ia+Ib+Ic	33	160.0 ± 79.22	
IIa+IIb	16	205.6 ± 57.39	
IIIc	6	286.9 ± 94.81	

[a]p value for t-test, [b]p value for ANOVA test, [#]the $p<0.05$ significance level

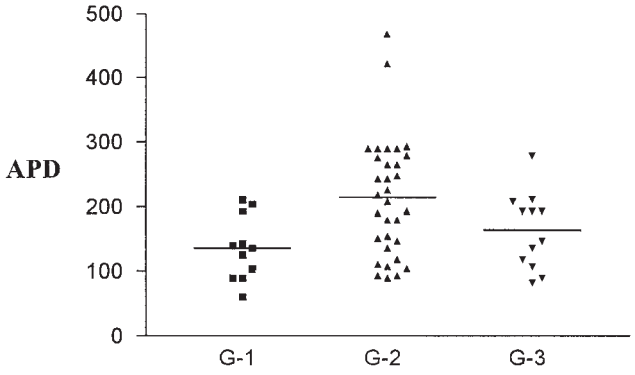

Figure 1. Angiogenic points density (APD) in particular histologic grades (G). The horizontal lines show mean values.

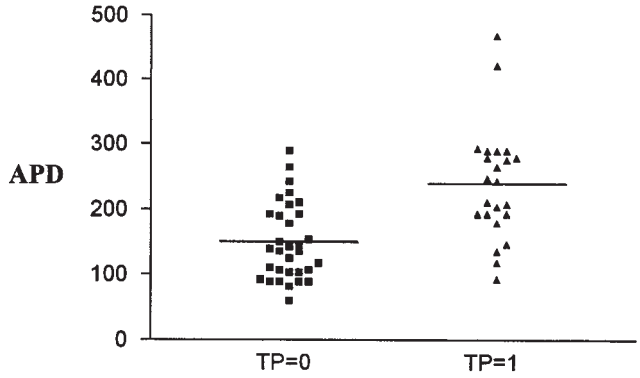

Figure 2. Intensity of angiogenesis (APD) in thymidine phosphorylase overexpression cases (TP=1). The horizontal lines show mean values.

sion seems to be limited, but can deliver additional information about cancer progression. TP has exclusive, synergistic or additive effect on angiogenesis in endometrial cancer, which has been already demonstrated [4, 8]. Thymidine phosphorylase tumor tissue expression in our study correlates with the intensity of angiogenesis. The number of positive TP cases increase according to advancement of the FIGO stage of disease, which is connected with cancer progression. The correlation between TP expression and prognosis remains controversial, because of a few research studies as exemplified by patient with gastric, colorectal and lung cancer, where the inversely dependence was shown [10–12].

TANAKA et al [27] noted a positive expression of thymidine phosphorylase in 41% of endometrial cancer cases. Most of tumor stromal cells expressing TP were shown also to co-express CD68 molecule. The study showed a significant correlation with a high intensity of angiogenesis and TP overexpression in either cancer cells or tumor stromal cells. They observed that a stromal macrophages/fibroblasts exhibited high TP expression and independently of whether cancer cells showed the positive TP expression. The results of the study suggest that high intensity of angiogenesis correlated with TP overexpression and production of thymidine phosphorylase by neighbouring tumor-infiltrating macrophages may play a role in the regulation of local invasion and distant metastatic behaviour.

TSUKAGOSHI et al [4] observed the TP angiogenic capacity in ovarian cancer cells and suggest that thymidine phosphorylase mediate tumor angiogenesis and could be the main factor responsible for blood vessel formation in cancer tissues and it's progression and metastasis.

The association between thymidine phosphorylase expression and positive prognosis was reported in patients with node positive breast cancer. FOX et al [28] observed upregulated the TP expression in breast epithelium and endothelium and suggested that spread of the breast cancer cells via lymphatic pathway as it happens in endometrial cancer progression, is dependent on thymidine phosphorylase expression. No such correlation was found in FUJIWAKI et al study [9].

In conclusion, thymidine phosphorylase expression correlates with increased microvessel density in endometrial cancer. The intensity of angiogenesis process increases according to FIGO stage of disease, which is connected with progression of cancer disease. Regardless of the increasing evidence for tumor growth dependence on angiogenesis, thymidine phosphorylase can play an important role in endometrial cancer progression and deliver additional information about progression of disease.

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