Original article

Clinical Study on the Expression of Thymidine Phosphorylase and Vascular Endothelial Growth Factor in Endometrial Carcinoma

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ABSTRACT **Objective:** To study the expression of thymidine phosphorylase (TP) and vascular endothelial growth factor (VEGF) in endometrial carcinoma and its correlation with clinicopathologic features and angiogenesis. **Methods:** The specimens obtained from 37 patients with endometrial carcinoma, 10 cases with atypical hyperplasia were studied against 18 cases with normal endometrium as control group. The cellular expression of TP and VEGF in cancer tissues were assessed by immunohistochemical staining, intratumoral microvessel counts (MVC) was determined by immunohistochemistry using monoclonal antibodies to factor VIII related antigen. **Results:** The TP expression of atypical hyperplasia and endometrial carcimon were mainly observed in stromal cells or stroma and adenocyte simultaneously (80% and 94.6%, respectively). The expression of VEGF was observed in the endothelial cell of cancer with a positive staining of 86.5%. MVC in atypical hyperplasia was significantly higher than that in normal endometrium, but lower than that in endometrial carcinoma. TP and VEGF expression was significantly correlated with MVC and some clinicopathologic features. **Conclusion:** TP and VEGF are overexpressed in endometrial carcinoma, with their expression correlated with clinicopathologic features including myometrial invasion and histological grade. MVC may be involved in the tumourigenesis and development of endometrial cancer.

KeyWords: Endometrial carcinoma; Thymidine phosphorylase; Vascular endothelial growth factor; Microvessel counts; Angiogenesis

Thymidine phosphorylase (TP), an enzyme occurring in the remedy pathway of nucleotide synthesis, promotes angiogenesis. Vascular endothelial growth factor (VEGF), which can increase the permeability of capillary vessel and induce the migration of endothelial cell and angiogenesis, is associated with angiogenesis closely. The aim of the present work was to study the correlation of expression of TP and VEGF with clinicopathologic features in endometrial carcinoma.

Materials and methods

Patients

Endometrial cancer specimens were obtained from 37 patients aged from 33 to 76 years (mean age: 57.3 years) who were surgically treated at Jinan Central Hospital between January 1999 and January 2003. Patients who had undergone preoperative chemotherapy, hormonal therapy, or irradiation were excluded. All of 37 patients underwent total abdominal extended hysterectomy or radical hysterectomy and bilateral salpingo-oophorectomy or pelvic lymph node lymphadenectomy. Tumors were classified according to the International Federation of Gynecology and Obstetrics (FIGO) surgical staging system as follows: stage I, n=24; stage II, n=5, and stage III, n=8. Tumors were classified histologically according to the World Heath Organization (WHO) criteria. Atypical hyperplasic specimens were taken of 10 cases. And normal endometrial specimens of 18 cases that have not been subjected to hormonal therapy were obtained from diagnostic curettage. All specimens were examined by pathologist as inerrable.

Immunohistochemistry of TP and VEGF Expression and Microvessel Count

Immunohistochemical analysis was performed by the streptavidin-biotin-peroxidase method. Anti-TP mouse monoclonal antibody (Maixin, Fuzhou), which was purified from mouse cancer, was used to identify TP; anti-human VEGF multiclonal antibody (Maixin, Fuzhou), which was purified from rabbit and streptavidin-biotin-peroxidase cassette. And anti-mouse factor VIII-related antigen multiclonal antibody (Zhongshan, Beijing) was used to stain the endothelium to determine the MVC.

Each formalin-fixed paraffin-embedded tissue sections (4μ m) was mounted and subjected to staining for H&E, TP, VEGF and factor VIII-related antigen according to the instructions of the reagents as appropriate. The first antibody was substituted by PBS and positive stained endometrial cancer tissues respectively in

This work is supported by Fund of Nanjing Medical University (No.09NJMUM109).

ISSN: 1538-5124/$ - see front matter

negative and positive controls.

Assessment of TP and VEGF expression and evaluation of MVC

TP positivity was defined as the definitude staining of cytoplasm and/or the nuclear compartment in more than 5% of 200 tumor cells. The proportion of cells staining was categorized as 0, no staining; 1, <25% positive staining; 2, 26% to 75% positive staining; and 3, >75% positive. The intratumoral MVC (factor VIII-positive cells) was determined by light microscopy in the most vascular areas, according to the criteria of Weidner, et al (1).

VEGF positivity was indicated as a brown cytoplasmic staining of tumor cells and vascular endothelial cells. The result was judged according to the following two aspects simultaneously: (1) the proportion of cells staining in hot spots, 0, no staining; 1, <25% positive staining; 2, 26% to 50% positive staining; and 3, >50% positive; and (2) intensity of the stained cells: 0, negative; 1, weak, straw yellow; 2, medium, brown; 3, strong, dark brown. The score obtained by adding up (1) and (2) was judged as follows: 0-2, negative(-); 3-4, slightly positive(+); and 5-6, positive(++).

Statistical Analysis

The x^2 test analysis of variance were used to determine the association between TP, VEGF expression, MVC and the menopausal status, histologic type, surgical stage and myometrial invasion. P (x^2) was used to determine the association between TP expression and VEGF expression. A level of $P<0.05$ was accepted as statistically significant.

Results

Relationship between TP Expression and Clinicopathologic Factors

Endometrial cancer cells were positive for TP in 35 (94.6%) of 37 patients, with 28 patients showing overexpression. The usual pattern of TP expression was cytoplasmic and occasionally nuclearic, which is often stronger in the edge of invasive cancer. The cells staining in stroma may have been fibroblasts or macrophages. 10 of 19 G_2 patients and 7 of 7 G_3 patients exhibited high TP expression in the specimens. The pattern of TP expression in endometrial carcinoma and atypical hyperplasia was stromal cells-positive and endometrial glandular epithelial cells-positive; whereas the normal glandular epithelium was nuclear-positive, proliferative endometrial glandular epithelial cells (n=7) were either weakly stained or non-immunoreactive; the immunostaining was more intense in the glandular epithelium of the luteal phase.

Endometrial cancer cells were positive for VEGF in 32 (86.5%) of 37 patients, which is higher than the controls (27.8%). The VEGF expression, which was found to belong to the cytoplas-

Table 1
Relationship between the Expression of TP, VEGF, MVC and Clinicopathologic Factors

Clinicopathologic Factors		No. of patients	TP expression				P value	VEGF expression			P value	MVC
			−	+	++	+++		−	+	++		
Menopausal status	Premenopausal	15	2	3	5	5	>0.05	3	5	7	>0.05	60.1± 13.2
	Postmenopausal	22	0	4	3	15		2	5	15		68.4± 14.3
Histological grade	Grade1	11	1	5	2	3	<0.05	2	4	5*	<0.05	55.± 16.2*
	Grade2	19	1	2	6	10		2	5	12		59.3± 14.8
	Grade3	7	0	0	0	7		1	1	5		79.4± 16.3
Surgical stage	I	24	2	2	6	14	>0.05	3	7	14	>0.05	61.± 13.6*
	Ⅱ	5	0	2	1	2		1	2	2		66.5± 11.3
	Ⅲ	8	0	3	1	4		1	1	6		70.7± 14.5
Myometrial invasion	None	4	2	2	0	0	<0.05	0	1	3*	<0.05	53.± 14.2*
	<1/2	11	0	0	5	6		3	3	5		60.4± 14.8
	>1/2	22	0	5	3	14		2	6	14		72.3± 13.7

Intergroup comparison, * $P<0.05$

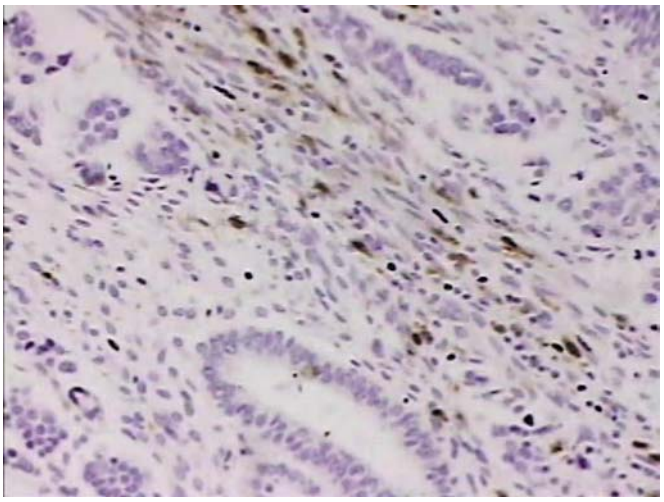

Fig.1 Expression of TP in endometrial carcinoma (10×10)

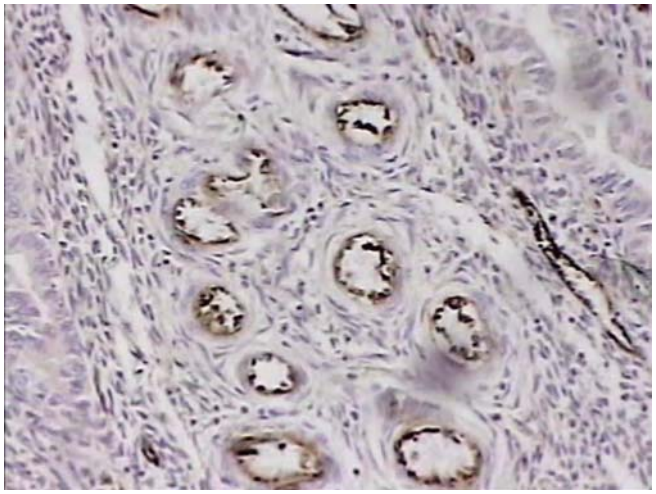

Fig.2 The microvessels of endometrial carcinoma(10×10)

mic pattern, was positively correlated with histological grade and myometrial invasion, but not associated with menopausal status and surgical stages.

MVC in endothelial cells increased gradually from normal glandular epithelium (40.56) to atypical hyperplasia (59.2) and endometrial cancer (67.46). The difference between them was statistically significant.

Relationship between TP Expression and VEGF Expression

TP expression is positively correlated with VEGF expression in endometrial carcinoma, their role in angiogenesis is harmonious. In addition, both TP expression and VEGF expression are positively correlated with histological grade and myometrial invasion of endometrial carcinoma.

Discussion

The process of tumor growth and metastasis is vessel dependent. TP itself is chemotactic to endothelial cells and is angiogenic in vivo; it can stimulate endothelial cell proliferation and chemotation and is related to metabolism of nucleic acid, with one of its degradation products, 2-deoxy-D-ribose, to be also angiogenic. Subsequent study indicated that platelet-derived endothelial-cell growth factor (PD-ECGF) can be identified by TP antibody (2), confirming that TP is identical to PD-ECGF as an endothelial-specific mitogen suggesting that TP plays an important role in maintaining the stabilization of blood vessels and promoting the repair of endothelial cells. Increasing of blood vessels when TP is overexpressed may be caused by stabilizing and maintaining the current vessel network. In fact, recent studies have indicated that TP is overexpressed in many tumors.

VEGF, also known as vascular permeability factor (VPF), is a highly specific mitogen on endothelial cells, which could induce the growth of capillary networks surrounding the tumor (3); it also is a potent inducer of vascular permeability and chemotactically directs the recruitment of monocytes/macrophages. Its expression was found to be constitutively expressed in normal and neoplastic

Table 2
Relationship between the expression of TP and VEGF

		TP expression				
		--	+	++	+++	Total
VEGF expression	--	1	2	1	1	5
	+	1	4	3	2	10
	++	0	1	4	17	22
	Total	2	7	8	20	37

human ovarian cells and tissues (4).VEGF expression has been reported to be correlated with MVC and survival in invasive breast carcinoma (5).

Relationship between TP Expression, VEGF Expression and Clinicopathologic Factors

The TP staining cells in stromal macrophages/fibroblasts of endometrial carcinoma are significantly correlated with histological grade, myometrial invasion and MVC in the present study, but not related to surgical stages. This is consistent with the results of previous studies (6). The positive rate of TP staining cells in poorly-differentiated grades is significantly higher than that in well-differentiated grades, suggesting that TP may accelerate the angiogenesis of poorly-differentiated endometrial cancer: the more TP staining cells, the more neovascularization. TP expression in stroma may be one of the prognostic factors. TP expression is also positively correlated with myometrial invasion. There was no correlation between TP expression and menopausal status or surgical stage.

VEGF immunoreactivity was detected in about 86.5% of endometrial cancer in the present study, which is close to the results (92%) of Qiao H (7).VEGF expression in endometrial cancer of well-differentiated grade (G_1) was significantly higher than that in poorly-differentiated grade (G_2 and G_3 respectively); this indicates that VEGF is a stimulus for endothelial cell proliferation by autocrine action in endometrial carcinoma, and is, therefore, associated with high malignancy. VEGF expression is evident at the invading tumor front, with myometrial invasion of >1/2 being significantly higher than that of <1/2. In a work analyzing the clinic and pathologic features of 53 endometrial carcinoma patients (8), it was found that G_3 and VEGF overexpression in cytokinesis are independent risk factors for recurrent endometrial carcinoma; G_1, G_2 and normal VEGF expression have more opportunity of disease-free survival; VEGF expression in endometrial cancer has good correlation with progression and metastasis and may be a prognostic factor of disease-free survival. In another study regarding the surgical stage, prognosis and VEGF expression in endometrial carcinoma (9), it was found that VEGF expression is positively correlated with progression and metastasis, and VEGF expression may be an independent prognostic factor of endometrial cancer. VEGF and MVC have been found to be among the prognostic factors of endometrial cancer (10).

Relationship between TP Expression, VEGF Expression and MVC

MVC increased gradually from normal endometrium to atypical hyperplasia and ($p<0.01$) endometrial carcinoma; in atypical hyperplasia and it increased along with the staging: mile<moderate<severe, and in endometrial carcinoma MVC of myometrial invasion >1/2 was significantly higher than that of myometrial invasion <1/2. The results indicate that atypical hyperplasia and endometrial carcinoma are all angiogenic; MVC of endometrial carcinoma increased significantly with advanced surgical stage, higher histological grade and deeper myometrial invasion. Positive staining of TP was exhibited in the macrophages and fibroblasts at the invading tumour front, showing the angiogenesis of tumor vessel in this region is the most activated and the vessel growth factor which it releases can participate in immune reactivity. Tumor-associated macrophages contain a much higher amount of TP than macrophages adjacent to the normal stroma. TP expression is related to macrophages. MVC in tumours with overexpression of TP in cancer cells or stromal macrophages was significantly higher than that with negative TP expression. This indicates that TP has an important role in angiogenesis of endometrial carcinoma. Later study showed that thymidine phosphorylase expression correlated with increased microvessel density in endometrial cancer. The intensity of angiogenesis process increased stage of disease, Thymidine phosphorylase could offer additional information about advance of disease (11). MVC was correlated significantly with VEGF expression in this work, which is consistent with previous results (12).

Li et al suggest that VEGF is the central tache of angiogenesis, while other vessel growth factors influence neovasculization by promoting VEGF (13). In the present study, it is found that TP and VEGF expression are compatible. Expression of TP and VEGF both are correlated with histological grade and myometrial invasion of endometrial carcinoma. The VEGF expressional pattern was cytoplasmic, whereas TP expressed in stromal cells of endometrial carcinoma, most of which were fibroblasts or macrophages and were associated with tumor invasion. A high TP activity in the stromal cells was associated with a high density of activated macrophages which further promotes endometrial tumour angiogenesis (14). Previous studies have indicated that when TP and VEGF co-expressed, they play a cooperative role in the induction of angiogenesis (15). Further study is necessary to clarify the mechanism of aniogenesis promotion by TP and VEGF in endometrial carcinoma. Even so, it can be speculated that tumour-infiltrating macrophages may be a new potential target of anti-angiogenesis therapy.

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