

RESEARCH ARTICLE

Relationships of uPA and VEGF Expression in Esophageal Cancer and Microvascular Density with Tumorous Invasion and Metastasis

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Abstract

Objective: To investigate uPA and VEGF expression in esophageal cancer and relations with tumorous invasion and metastasis. **Methods:** Immunohistochemistry was used to detect uPA and VEGF expression in the normal epithelial tissue of esophageal mucosa and cancer tissue and detect CD34 labeled micrangium and analyze the relationships with clinical pathological features and tumor angiogenesis. **Results:** Positive rates for uPA and VEGF protein expression were significantly greater in esophageal cancer than normal epithelial tissue ($P < 0.05$), the two being linked ($P < 0.05$). In addition, uPA and VEGF protein expression of the high microvessel density (MVD) group was significantly lower than in the low MVD group ($P < 0.05$), with relation to clinical pathological staging, differentiation and lymph node metastasis ($P < 0.05$). **Conclusion:** In esophageal cancer tissue, uPA and VEGF proteins are overexpressed and promote tumor angiogenesis, indicative of a poor prognosis.

Keywords: uPA - VEGF - esophageal cancer - angiogenesis - immunohistochemistry

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Introduction

The invasion and metastasis characteristic of esophageal cancer not only is the postoperative recurrent nature, but also causes many patients to lose an operation opportunity. Therefore, the invasion and metastasis is still the refractory key. The invasion and metastasis of therioma is one of main causes resulting in treatment failure and death of tumor patients. Degradation of extracellular matrix and basement membrane caused by fibrin degradation and vascular formation effect are the key steps of tumorous invasion and metastasis. uPA (Urokinase-type Plasminogen Activator) can activate a variety of fibrinolytic enzymes, degrade extracellular matrix and basement membrane and promote tumorous infiltration and metastasis (Schmitt et al., 1997, 2011; Yoshizawa et al., 2011). The growth, infiltration and metastasis process of tumor depends on tumorous angiogenesis. In addition, angiogenesis itself had a certain tissue invasion, and tumor cells can invade the surrounding tissues along the open tissue fissure of new micrangium.

VEGF (vascular endothelial growth factor) can specifically affect vascular endothelial cell and induce division and proliferation of endothelial cells. Also, it induces angiogenesis in vivo. At present, it is the known

strongest pro-angiogenesis factor (Katoh and Katoh, 2006; Pengchong and Tao, 2011; Liu et al., 2012). There are a fewer researches on the significance of uPA and VEGF expressions in esophageal cancer and their influences on tumor angiogenesis. This study used immunohistochemistry SP method to detect uPA and VEGF protein expressions in esophageal cancer, analyzed their significance by combining clinical pathological features of esophageal cancer and investigated the influences of uPA and VEGF on tumor angiogenesis and the relations of them with tumorous invasion and metastasis.

Materials and Methods

Clinical data

Normal epithelial tissues of esophageal mucosa (18 cases) and esophageal cancer tissues (68 cases) were collected from the patients receiving exairesis in the Second Affiliated Hospital of Medical College of Xi'an Jiaotong University from October, 2008 to October, 2009. Tissue typing and clinical staging of each case of esophageal cancer complied with the diagnosis and treatment specification of esophageal cancer prepared by Ministry of Health. This study was conducted in

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Table 1. Expression of uPA and VEGF in Normal Esophageal Tissue and Esophageal Carcinoma

Group	Number	uPA					VEGF						
		-	+	++	Positive rate (%)	χ^2	P	-	+	++	Positive rate (%)	χ^2	P
Esophageal cancer tissue	68	20	17	31	70.6			25	16	27	63.2		
Normal mucosa epithelial tissue	18	13	3	2	27.8	11.63	<0.05	14	2	2	22.2	9.78	<0.05

accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of XI'an Jiaotong University. Written informed consent was obtained from all participants.

Immunohistochemistry

The specimen was fixed with 10% formaldehyde, embedded with paraffin wax and continuously cut into sections of 4um. One section was let alone to carry out haematoxylin-eosin (KL) staining for return visit. In addition, immunohistochemistry SP method was used for staining, and CD34 was taken as a marker of micrangium. Main reagents were rabbit anti-human uPA, VEGF and CD34 monoclonal antibodies, purchased from Boster Biological Engineering Company Limited. Working concentrations: uPA (1: 100), VEGF (1: 100), CD34 (1: 50).

The first antibody was replaced with PBS as the blank control, and known uPA, VEGF and CD34 positive staining sections were taken as the positive control.

Result assessment criteria

All the pathological sections were independently read by two experienced doctors in department of pathology by means of the blind method. In case of inconsistent result, the principle of consultation unity was used. Tissue sections showed that granules of blown yellow to blow color present in cytoplasm were positive markers. In terms of Iseki K criteria (Iseki et al., 1999), an comprehensive assessment was conducted according to the staining intensity and the number of positive cells and converted into the positive index: a staining intensity (0=none, 1=weak, 2= moderate, 3= strong); b number of positive cells (0=0 to 5% positive staining cells, 1=5% to 50% positive staining cells, 2=50% to 100% positive staining cells). If the total score of a and b was 0 to 1, the positive index was 0; If the total score of a and b was 2, the positive index was 1; If the total score of a and b was 3, the positive index was 2; If the total score of a and b was 4 or 5, the positive index was 3. Moreover, if the positive index was less than 2, it was negative (-); If the positive index was no less than 2, it was positive (+); if the positive index was no less than 3, it was strongly positive (++). Both (+) and (++) were regarded as positive.

CD34 protein positive staining was located in vascular endothelial cell membrane, presenting blown-yellow granules. Positive stained individual endothelial cell or endothelial cell cluster could act as a separate and countable micrangium. Assessment criteria of MVD (microvascular density) referred to the method proposed by Bosari et al. (1992): firstly scan the whole section with 40 times of optical microscope to seek vascular high density area and then select 5 visual fields in this area by

use of 200 times of optical microscope to count the number of blown stained micrangiums. The result was expressed as the mean.

Statistical analysis

SPSS17.0 statistical analysis software package was used for statistical analysis. χ^2 test and Fisher exact probability test were used for analysis of count data. If $P < 0.05$, a significant difference could be observed.

Results

Positive staining of uPA and VEGF

uP A and VEGF positive staining were located in cytoplasm and were blown-yellow to blown, presenting diffuse and granular staining (Figure 1). Staining intensities were different. In addition, there were a small amount of fibroblasts and vascular endothelial cells in tumors, and weaker positive expressions of uPA were visible. Also, some endothelial cells presented weaker VEGF expression.

uPA and VEGF expressions in different esophageal tissues

Positive rates of uPA protein expression in the normal

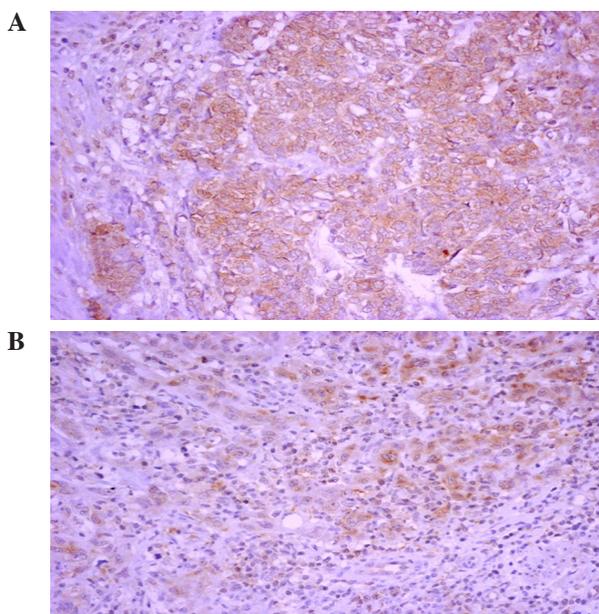


Figure 1. Expression of uPA and VEGF in Esophageal Carcinoma (x200) A) uPA; B) VEGF

Table 2. Correlations of uPA Expression to VEGF Expression in Esophageal Carcinoma

Group	Case	VEGF		χ^2	P
		Negative	Positive		
uPA negative	20	13	7		
uPA positive	48	12	36	9.72	<0.05

Table 3. Correlations of uPA and VEGF Expression to MVD in Esophageal Carcinoma

Group	Number	uPA					VEGF						
		-	+	++	Positive rate (%)	χ^2	P	-	+	++	Positive rate (%)	χ^2	P
High MVD	36	6	10	20	83.3			7	10	19	80.6		
Low MVD	32	14	7	11	56.3	6.13	<0.05	18	6	8	43.8	10.12	<0.05

Table 4. Association Between uPA, VEGF Expression and the Pathologically Biological Behavior of Esophageal Carcinoma

Group	Number	uPA					VEGF						
		-	+	++	Positive rate (%)	χ^2	P	-	+	++	Positive rate (%)	χ^2	P
Age													
< 50 year old	7	2	2	3	71.4			3	2	2	57.1		
≥ 50 year old	61	18	15	28	70.5	0.05	>0.05	22	14	25	63.9	0.41	>0.05
Gender													
Male	45	13	11	21	71.1			17	10	18	62.2		
Female	23	7	6	10	69.6	0.06	>0.05	8	6	9	65.2	0.14	>0.05
Pathological type													
Squamous carcinoma	54	16	13	25	70.4			20	13	21	63		
Others	14	4	4	6	71.4	0.12	>0.05	5	3	6	64.3	0.08	>0.05
Clinicopathological stages													
I-II	39	15	12	12	61.5			17	12	10	56.4		
III-IV	29	5	5	19	82.8	8.17	<0.05	8	4	17	72.4	7.75	<0.05
Differentiation degree													
High	18	11	4	3	38.9			11	4	3	38.9		
Middle	34	7	10	17	50	14.5	<0.05	10	9	15	67.6	11.66	<0.05
Low	16	2	3	11	87.5			4	3	9	81.3		
Lymph node metastasis													
Yes	31	4	9	18	87.1			5	10	16	83.9		
No	37	16	8	13	56.8	7.6	<0.05	20	6	11	45.9	10.48	<0.05

epithelial tissue of esophageal mucosa and the esophageal cancer tissue were respectively 27.8% and 70.6%, and uPA expression in the esophageal cancer tissue was significantly higher than that in the normal epithelial tissue of esophageal mucosa ($P < 0.05$). Positive rates of VEGF protein in the normal epithelial tissues of esophageal mucosa and the esophageal cancer tissue were respectively 22.2% and 63.2%, VEGF expression in the esophageal cancer tissue was significantly higher than that in the normal epithelial tissue of esophageal mucosa ($P < 0.05$) (Table 1).

Relation of uPA protein expression with VEGF protein expression in esophageal cancer

In the group containing 20 cases with uPA negative expression, there were 13 cases with VEGF negative expression, accounting for 65.0%. In the group containing 48 cases with uPA positive expression, there were 36 cases with VEGF positive expression, accounting for 75.0%. Therefore, uPA and VEGF expressions had a consistency ($P < 0.05$) (Table 2).

Relationships of uPA and VEGF protein expressions with MVD

Mean MVD of 68 cases of patients with esophageal cancer was 42.4 ± 11.6 . With 42.4 as the boundary, 68 cases were divided into the high MVD group (> 42.38) and the low MVD group (≤ 42.38). In the high MVD group, there were 30 cases with uPA positive and the positive rate was 83.3%. In the low MVD group, there were 18 cases with

uPA positive and the positive rate was 56.3%. Between the two groups, there was a significant difference ($P < 0.05$). In addition, there were 29 cases with VEGF positive in the high MVD group and the positive rate was 80.6%. In the low MVD group, there were 14 cases with VEGF positive and the positive rate was 43.8%. Between the two groups, there was a significant difference ($P < 0.05$, Table 3).

Relationships of uPA and VEGF protein expressions in esophageal cancer tissue with the clinical pathological features

uPA and VEGF expressions were unrelated to age, gender and pathological type ($P > 0.05$). However, they were related to the clinical pathological staging, and those of the group of in III-IV stage were significantly higher than those of the group in I-II stage ($P < 0.05$); Also, they were related to the differentiation extent of tissue. Lower the differentiation extent was, expression positive rate was higher ($P < 0.05$); In addition, they were related to lymphatic metastasis, and uPA and VEGF expressions of the group with lymphatic metastasis were significantly higher than those of the group without lymphatic metastasis ($P < 0.05$, Table 4).

Discussion

The invasion and metastasis process of thieroma is one of main causes resulting in treatment failure and death of tumor patients. Degradation of extracellular matrix and

basement membrane caused by fibrin degradation and vascular formation effect are the key factors tumorous invasion and metastasis process.

In the gradation process of extracellular matrix and basement membrane, PAs (Plasminogen Activators) plays a key role. Especially uPA can activate plasminogen into plasmin, degrade the components of extracellular matrix and basement membrane (such as LN, FN) and activate matrix metalloproteinase 1, 3, 9 and 12 and thus further damages extracellular matrix and basement membrane and promotes tumorous invasion and metastasis (Mazar, 2008; Huang et al., 2010). uPA - mediated plasmin degradation system plays an important role in the infiltration and metastasis process of tumor.

Tumorous growth, infiltration and metastasis depend on tumorous angiogenesis, and angiogenesis itself also has a certain tissue invasion. Tumor cells can invade the surrounding tissues along the open tissue fissure of new micrangium (Kang et al., 2003). Vascular proliferation process is accompanied with tumor cells or host cell secreting vascular growth factor. VEGF is the central regulatory factor of tumorous angiogenesis, and other vascular growth factors mostly directly or indirectly participate in or coordinate with angiogenesis through VEGF (Zhang et al., 1997; Zhang et al., 2011).

Many researches find that uPA and VEGF can express in a variety of tumor tissues, such as mastocarcinoma (Xiao et al., 2007), prostate cancer (Conn et al., 2009), gastric cancer (Qin et al., 2005) etc., and it is related to tumorous infiltration and metastasis. Also, it is a bad prognosis factor of therioma. Results of this study showed that uPA and VEGF protein expressions in the esophageal cancer tissue were significantly higher than those in the normal epithelial tissue of esophageal mucosa ($P < 0.05$). Also, uPA and VEGF proteins presented over expressions in the esophageal cancer tissue, indicating that uPA and VEGF are related to the occurrence and development of esophageal cancer.

In this study, it was showed that in the esophageal cancer tissue, uPA expression and VEGF expression had a consistence ($P < 0.05$), and positive rates of uPA and VEGF protein expressions in the high MVD group were significantly higher than those of the low MVD group ($P < 0.05$). MVD is a quantitative indicator of angiogenesis. Also, it is regarded as the mark of angiogenesis activity effect. It not only is associated with the feeding and nutrition supply of proliferated tumor cells, but also can represent their invasion and metastasis activity. According to this analysis, VEGF and uPA can achieve a synergistic effect in esophageal cancer angiogenesis, and VEGF and uPA interact. High-level uPA further induces the vascular invasion of tumor angiogenesis possibly by up-regulating VEGF or directly activating VEGF (Cavallaro et al., 2001; Isogai et al., 2001; Reuning et al., 2003; Viacava et al., 2003), and high-level VEGF further induces degradation of extracellular matrix possibly by up-regulating uPA level (Dvorak et al., 1995). Therefore, they form a positive feedback loop to greatly increase uPA product, degrade extracellular matrix and basement membrane of micrangium and provide an advantageous microenvironment for the migration and proliferation

of endothelial cell and tumor angiogenesis. At the same time, it is also useful for cancer cells detach shedding into vessels or spreading towards adjacent tissues, which creates a good condition for tumorous infiltration and metastasis.

This study showed that uPA and VEGF expressions were unrelated to age, gender and pathological type ($P > 0.05$), and uPA and VEGF expressions were related to the differentiation extent of tissue. Lower the differentiation extent was, expression positive rate was higher ($P < 0.05$), suggesting that with increase of cellular malignancy grade, uPA and VEGF protein expressions were in a rising trend. In addition, uPA and VEGF expressions were related with lymphatic metastasis, and uPA and VEGF expressions of the group with lymphatic metastasis were significantly higher than those of the group without lymphatic metastasis ($P < 0.05$). Also, uPA and VEGF expressions were related to the clinical pathological staging, and those of the group in III-IV stage were significantly higher than those of the group in I-II stage ($P < 0.05$). It is further proved that uPA and VEGF participate in the invasion and metastasis process of esophageal cancer. It is possible that cancer cells with high uPA and VEGF protein expressions increase the ability of degrading the components of extracellular matrix and basement membrane and the angiogenesis ability and have higher migration and invasion ability, which causes this part of cancer cells to more easily infiltrate deep tissues, vessels and lymphatic vessels and causes distant metastasis.

In a word, expression up-regulation of uPA and VEGF promotes ECM degradation and angiogenesis possibly through their respective actions and the interaction to influence the invasion ability of tumor cells and promote tumorous invasion and metastasis, and their expression extent reflects tumorous invasion and metastasis ability. Therefore, uPA and VEGF can be taken as the important indicators of predicting the biological behavior and prognosis of esophageal cancer.

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