

# Vascular endothelial growth factor and microvascular density in esophageal and gastric carcinomas

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## Abstract

**AIM:** To observe the relationship between the expression of vascular endothelial growth factor (VEGF), microvascular density (MVD) and the pathological characteristics of esophageal and gastric carcinomas.

**METHODS:** S-P immunohistochemical staining was used to investigate the expression of VEGF in all the specimens. The antibody against factor VIII-related antigen was used to display vascular endothelial cells, and MVD was examined by counting the factor VIII-positive vascular endothelial cells.

**RESULTS:** The positive rates of VEGF expression in esophageal carcinoma and gastric carcinoma were 81.36 % and 67.5 % respectively, and the MVD averaged  $41.81 \pm 8.44$  and  $34.36 \pm 9.67$  respectively, which were higher than those in benign diseases. The expression of VEGF and MVD were closely correlated with the degree of differentiation, lymphatic metastasis, but not related to depth of cancer invasion. In early stage gastric carcinoma, the rate of expression of VEGF and MVD was lower than that in progressive gastric carcinomas.

**CONCLUSION:** The expression of VEGF is correlated with tumor angiogenesis, and VEGF plays an important role in new blood vessels formation, the expression of VEGF and MVD play an important role in tumor growth and metastasis. MVD and the expression of VEGF may be two important indexes for patients' prognosis.

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## INTRODUCTION

The growth and metastasis of solid tumor, a complex biological event, are affected by many factors. Recent research found that the growth and metastasis of tumors needed constant angiogenesis, which could provide a way for tumor metastasis through vessels, and could affect the prognosis of patients<sup>[1]</sup>. Angiogenesis is not an active process by itself, and it is controlled by some angiogenic factors and some angiogenic inhibitors<sup>[2]</sup>. Of all the angiogenic factors, vascular endothelial growth factor (VEGF) is a potent, multifunctional cytokine that exerts several important and possibly independent actions on vascular

endothelium. That is its property and capacity to induce angiogenesis, which has excited the greatest interest in VEGF<sup>[3]</sup>.

In this study, we used immunohistochemical method to detect VEGF expression and MVD in 59 cases of esophageal carcinoma and 80 cases of gastric carcinoma. We studied the relationship between VEGF expression and MVD and pathological features, which will help to understand the role of VEGF and angiogenesis in the growth of esophageal and gastric cancers.

## MATERIALS AND METHODS

### Materials

The resected specimens from 59 cases of esophageal cancer and 80 cases of gastric cancer were obtained from our hospital from January 2000 to June 2002. Of 59 cases of esophageal carcinoma, 57 were male and 2 were female, with a mean age of 57 (38 to 79). Of 80 cases of gastric carcinoma, 55 were male, 25 were female, with a mean age of 59 (35 to 69). All these specimens were clearly classified by experienced pathologists based on the depth of invasion, metastasis of lymph nodes and degree of differentiation. We collected specimens of 20 normal esophageal tissues and 20 gastric tissues as control. All of them had not received any radiotherapy or chemotherapy.

### Reagents and methods

Rabbit anti-human VEGF polyclonal antibodies (RAB-0243), rabbit anti-human VIII polyclonal antibodies (RAB-0070) and ready-to-use SP immunohistochemical reagent box were purchased from Fujian Maxin Co. Ltd. Formalin-fixed, paraffin-embedded specimens were available and sectioned sequentially with a thickness of 4  $\mu$ m. The sections carrying the detected antigen were stained with SP immunohistochemical method.

In this study we used lung cancer specimen that was known as positive of VEGF expression to be positive control, and with the first antibody substituted by PBS as negative control.

### Results

**Criteria of positive staining VEGF** According to the criteria proposed by Volms *et al*<sup>[4]</sup>, brown granules in the cytoplasm of tumor cells or vascular endothelial cells were identified to be positive VEGF. The sections were graded respectively according to the density (1) and the percentage (2) of positively stained tumor cells into score 0, 1, 2 and 3. If the sum of two scores (1) and (2) were 0-2, the section was considered as negative, whereas 3-6 was considered as positive VEGF.

**MVD** According to the criteria proposed by Weidners *et al*<sup>[5]</sup>, when the cytoplasm of vascular endothelial cells was stained brown or brownish yellow, it was positive. The microvessels were counted according to the number of single endothelial cell or endothelial cell cluster showing brownish yellow granules in the cytoplasm. The sections were observed first under the low power ( $\times 40$ ), then the most dense area of microvessel sections was selected under the high power ( $\times 200$ , the surface area of every vision field being 0.785 mm<sup>2</sup>). The number of microvessel in three vision fields were counted and averaged as MVD of this specimen.

**Statistical methods**

Statistic analysis was performed by using the  $\chi^2$  test to dispose the expression of VEGF and the pathological features. *t* test was used to detect the relationships between the expression of VEGF and MVD, and between MVD and pathological characteristics.

**RESULTS**

**The relationship between the expression of VEGF and pathological features of esophageal carcinoma**

Of 59 cases of esophageal carcinoma, 11 cases were negative and 48 cases were positive, and the positive rate was 81.36 % (48/59). Of 20 normal esophageal tissues, 4 cases were positive, and the positive rate was 20 %. The rate of expression of VEGF in esophageal carcinoma was higher than that in normal esophageal tissue ( $\chi^2=24.99, P<0.001$ ). The expression of VEGF was closely related to pathological grade, that is, the poorer differentiation of the tumor, the higher expression of VEGF ( $\chi^2=7.08, P<0.05$ ). The cases having lymph node metastasis had significantly higher VEGF expression than those having no lymph node metastasis ( $\chi^2=5.59, P<0.05$ ). The VEGF expression was not related to invasion depth of tumor (Table 1).

**Table 1** Relationship between expression of VEGF and MVD and pathological features of esophageal carcinoma

Pathological characteristics	n	VEGF				MVD	
		-	+++	$\chi^2$	P	$\bar{x}\pm s$	P(t)
<b>Degree of differentiation</b>							
Well differentiated	27	9	18	7.08	<0.05	38.52±9.22	<0.05
Poorly differentiated	32	2	30			43.43±7.61	(t=2.07)
<b>Depth of invasion</b>							
Invading muscularis	37	7	30	0.01	>0.05	40.38±8.31	>0.05
Invading serosa	22	4	18			42.53±9.24	(t=0.88)
<b>LN metastasis</b>							
-	35	10	25	5.59	<0.05	38.22±8.54	<0.05
+	24	1	24			45.5±7.04	(t=3.23)

**The relationship between the expression of VEGF and pathological features of gastric carcinoma**

Of 80 cases of gastric carcinomas, 26 cases were negative and 54 cases were positive, and the positive rate was 67.5 % (54/80). There was no positive stain in 20 cases of gastric tissues. The expression of VEGF was closely related to degree of differentiation ( $\chi^2=11.31, P<0.01$ ) and lymph node metastasis ( $\chi^2=9.32, P<0.01$ ). As in esophageal carcinoma, the expression of VEGF had no significant difference between the different depths of invasion ( $\chi^2=0.40, P<0.05$ ). The expression of VEGF in early stage carcinoma was significantly lower than that in progressive stage cancer ( $\chi^2=19.67, P<0.001$ ) (Table 2).

**The relationship between MVD and pathological features in esophageal carcinoma and gastric carcinoma**

In the two carcinomas, MVD was higher than that in normal tissue, and MVD was closely related to differentiation degree of tumor and metastasis of lymph nodes, but not related to depth of invasion. In gastric carcinoma, MVD was significantly different between the different stages of carcinoma. (Table 1 and Table 2).

**The relationship between VEGF expression and MVD in the two cancers**

In this study, MVD was closely related to the expression of

VEGF in gastric carcinoma and esophageal carcinoma, that is, the stronger the expression of VEGF, the higher the MVD. This suggested that VEGF be related to MVD and angiogenesis. VEGF and MVD were closely related to tumor growth (Table 3).

**Table 2** Relationship between expression of VEGF and MVD and pathological features of gastric carcinoma

Pathological characteristics	n	VEGF				MVD	
		-	+++	$\chi^2$	P	$\bar{x}\pm s$	P(t)
<b>Degree of differentiation</b>							
Well differentiated	43	21	22	11.31	<0.001	32.85±6.14	<0.01
Poorly differentiated	37	5	32			38.63±5.10	(t=4.53)
<b>Depth of invasion</b>							
Not invading serosa	39	14	25	0.40	>0.05	34.94±9.26	>0.05
Invading serosa	41	12	29			33.68±9.12	(t=0.30)
<b>Tumor stage</b>							
Early stage	21	15	6	19.67	<0.001	32.14±5.89	<0.05
Progressive stage	59	11	48			35.46±6.58	(t=2.04)
<b>LN metastasis</b>							
-	52	23	29	9.32	<0.01	33.06±5.33	<0.05
+	28	3	25			35.74±7.58	(t=2.07)

**Table 3** Relationship between VEGF expression and MVD in the two cancers

VEGF expression in esophageal carcinoma	n	MVD	
		$\bar{x}\pm s$	P(t)
-	11	43.45±6.98	<0.05
+++	48	31.30±8.74	(t=4.33)
<b>VEGF expression in esophageal carcinoma</b>			
-	26	31.08±9.54	<0.05
+++	54	36.83±8.87	(t=2.65)

**DISCUSSION**

The importance of tumor angiogenesis in the growth and infiltration of tumor has been well known since J Folkman first proposed the hypothesis “Growth of solid tumor and the formation of metastasis are dependent on the formation of new blood vessels” in 1971. Growth of solid tumors is dependent on the induction of new blood vessels<sup>[6]</sup>. In order to maintain the unlimited growth of tumor, tumor tissue must depend on the constant and wide formation of new blood vessels, which is essential for tumors to grow beyond minimal size, providing oxygenation and nutrient perfusion as well as removal of waste products<sup>[7]</sup>. In normal organism, angiogenesis is strictly controlled, but in tumors, angiogenesis is uncontrolled and immature<sup>[8]</sup>. Controlled by angiogenic factors and angiogenic inhibitors, tumor cells, endothelial cells and other cells can produce and release VEGF protein if the local microenvironment is changed by hypoxia, *etc*<sup>[9]</sup>. Some researches proved that in tumors with foci of relative hypoxia, VEGF mRNA may be expressed not only by malignant cells but also by stromal cells<sup>[10,11]</sup>. Different tumor needs different angiogenesis factors, such as bFGF, which is a very important angiogenesis factor in fibrosarcoma. In gastroenteric tumors VEGF proved to play a key role.

VEGF, also known as VPF (vascular permeability factor), is secreted by some tumor cells. It combines with its receptors on endothelial cells. It can render venules and small veins hyperpermeable to circulatory macromolecules, and induce angiogenesis, which can induce tumor growth<sup>[12]</sup>.

The human VEGF gene has been assigned to chromosome 6p21.3. Its coding region spans approximately 14kb. Native VEGF is a basic, heparin-binding homodimeric glycoprotein<sup>[13,14]</sup>. VEGF target cell is endothelial cell. On the one hand it renders microvascular hyperpermeable, so that plasma proteins and fibrinogen leak, can stimulate angiogenesis and new stroma formation. On the other hand, VEGF stimulates the endothelial cell of microvessels to proliferate, migrate and alters their pattern of gene expression<sup>[15]</sup>.

More and more researches proved the important role of VEGF in tumor growth. Recently, Meada *et al*<sup>[16]</sup> found that VEGF expression was consistent with MVD, that is, MVD of gastric carcinoma with positive expression of VEGF was higher than that with negative expression of VEGF, and MVD was higher in the area where VEGF expression was positive. Toi *et al*<sup>[17]</sup> found that the positive expression of VEGF and factor VIII were detected in the samples of breast carcinoma which were poorly differentiated, with invasive growth and lymph node metastasis. So strong expression of VEGF and factor VIII may indicate a poor prognosis.

In our study, a strong correlation was found between VEGF expression and increased tumor microvasculature, malignancy and metastasis in esophageal carcinoma and gastric carcinoma. These results indicate that VEGF and angiogenesis promoted by VEGF play important roles in cancer growth, infiltration and metastasis in esophageal and gastric carcinoma. It also implied that VEGF expression and MVD have prognostic significance. We also found that there was an obvious heterogeneity in VEGF expression and new vessel formation in cancer tissue. New tumor vessels were deficient in constant basement membrane. This proved that the new vessels were hyperpermeable. This may facilitate the tumor cells to penetrate through the blood vessels and metastasis. VEGF expression manifested that positive cells located at the center of tumor or at the edge of the necrosis area, this may be explained by the hypoxia, which can stimulate VEGF expression and its biological activity.

In the study of the correlation of VEGF expression and MVD, we proved that VEGF was closely related with MVD in the cancer tissues of both esophageal and gastric carcinoma, this result proved VEGF could induce formation of new blood vessels. Thus VEGF expression and MVD may play important roles in tumor biological behaviors, progression and prognosis.

In conclusion, VEGF overexpression and active angiogenesis exist in esophageal carcinoma and gastric carcinoma. VEGF and MVD are closely relevant to lymph node metastasis, tumor differentiation and clinical stage. VEGF and MVD may act as two valuable indexes of tumor prognosis. These conclusions may provide an important theoretical evidence for cancer therapy through antiangiogenesis.

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