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The Relationship between the Expression of Vascular Endothelial Growth Factor, Microvessel Density and the Progress, Metastasis of Tumor in Head and Neck*

血管内皮生长因子表达及微血管密度与头颈肿瘤发展和转移的关系

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Abstract To investigate whether the expression of vascular endothelial growth factor (VEGF) and microvessel density (MVD) is related to the progression and metastasis of head and neck tumor, the VEGF expression and MVD were assessed by immunohistochemistry using rabbit anti-human VEGF antibody and rabbit anti-human factor Ⅷ-related antigen antibody. The VEGF expression and MVD in malignant tumors of head and neck were higher than those in benign tumors and non-tumor tissues in head and neck tissue ($P < 0.05$). The VEGF expression and MVD in malignant tumor with metastasis was significantly increased as compared with those without metastasis ($P < 0.05$). There was a close positive correlation between VEGF expression and MVD ($r = 0.398$, $P < 0.05$). Thus, the VEGF expression is closely correlated with angiogenesis. It is revealed that the increases in VEGF expression and MVD may promote the progression and metastasis of head and neck tumor. Thus, VEGF and MVD may have prognostic value in head and neck tumor.

Key words vascular endothelial growth factor, angiogenesis, head and neck tumor, tumor metastasis

摘要: 研究血管内皮细胞生长因子(VBGV)的表达及微血管密度(MVD)与头颈肿瘤发展及转移的关系。应用免疫组织化学 S-P法,检测 32例头颈恶性肿瘤、20例头颈良性肿瘤、16例头颈部无瘤组织石蜡标本组织中的血管内皮细胞生长因子(VEGF)的表达、微血管密度(MVD)。头颈恶性肿瘤组织 VEGF的表达及 MVD明显高于头颈良性肿瘤及头颈无瘤组织($P < 0.05$),转移组比较非转移组高($P < 0.05$)。此外,在头颈肿瘤的发展及转移中 VEGF的表达及 MVD具有显著的正相关关系($r = 0.398$, $P < 0.05$)。VEGF与头颈肿瘤血管生成有密切关系; VEGF的表达和 MVD的增高对头颈肿瘤发展及转移有促进作用,其检测有可能作为头颈肿瘤预后的指标。

关键词: 血管内皮生长因子 血管生成 头颈肿瘤 肿瘤转移
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Angiogenesis is defined as the growth of new blood vessels from the existing vascular bed. Angiogenesis is normally under tight regulatory control and is transiently observed only under particular circumstances such as reproduction, development, and wound healing. Excessive angiogenesis, however, occurs in several pathological

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conditions such as cancer, diabetic blindness, rheumatoid arthritis, and psoarisis. Several angioenic growth factors, such as vascular endothelial growth factor (VEGF), transforming growth factor-α (TGF-α) participate in the growth, invasion and metastasis of tumor[1-4].

VEGF is a growth factor for endothelial cells as well as a vascular permeability factor that increases the permeability of capillary vessels to different macromolecules. VEGF is a homodimeric glycoprotein with relative molecular weight of 45,000. The expression of VEGF has been shown to be upregulated by hypoxia. VEGF could (1) increase cytoplasmic Ca^{2+} level up to four-fold, (2) increase the release of von Willebrand factor[5], and (3) maintain the survival of endothelial cells. The receptors for VEGF (FLK-1, KDR, and FLT-4) are exclusively expressed in endothelial cells. Since tumor growth and metastasis largely depend on angiogenesis, VEGF has played a more important role in tumor angiogenesis[6]. Recent studies demonstrated that there was a close relationship between the expression of VEGF and the growth, invasion, and metastasis of tumor[7]. Thus, the inhibition of VEGF might prevent the growth, invasion and metastasis of tumor[8]. In the present study, we aim to investigate whether VEGF is expressed in head and neck tumor. The correlation between the expression of VEGF and angiogenesis in head and neck tumor is studied by immunohistochemistry.

1 Materials and Methods

1.1 Tissue Samples

From 1998 through 2000, biopsy tissue specimens were obtained from 63 patients with malignant tumors, benign tumors and non-tumors at the Department of Otorhinolaryngology of The First Affiliated Hospital of Guangxi Medical University in China. All subjects gave their informed consent to participate in the study and the biopsy tissue samples employed for this study were in accordance with Helsinki Declaration. All the specimens were fixed by formalin with neutral pH and embedded in paraffin. The patients studied in this study were summarized in Table 1. The clinical classification of 32 patients with malignant tumors was determined following the Union International Control Cancer (UICC) classification, with the resultant numbers: 9 patients in stageⅠ, 6 in stageⅡ, 9 in stageⅢ, and 8 in stageⅣ. The patients with head and neck malignant tumors were further divided into two groups: metastasis (20 patients, 15 men, 5 women) and non-metastasis group (12 patients, 10 men, 2 women).

Table 1 Summary of profile of patients studied

Group of tissue specimens	No. of cases*	Age**
Malignant tumor	32(24 and 8)	52(18~74)
squamous cell carcinoma	21	
adenocarcinoma	5	
non-Hodgkin's lymphoma	2	
sarcoma	1	
Malignant hemangioma	1	
Malignant fibroma	1	
Malignant melanoma	1	
Benign tumor (Papilloma, mixed tumor of salivary gland, chordoma, meningioma, hemangioma, angioleiomyoma, neurofibroma, osteoma)	15(8 and 7)	38(2~67)
Non-tumor	16(9 and 7)	53(18~67)

* The numbers in the parentheses represent male and female, respectively. ** The numbers represent the average age (range of age).

1.2 Materials

Rabbit anti-human factorⅧ-related (F8-RA) antibody (ZA-0111), rabbit anti-human VEGF antibody (SC-507), and an S-P immunohistochemistry reagent kit were purchased from Beijing Tyuzan Konsu (Beijng, China).

1.3 Immunohistochemistry

Immunohistochemistry was preformed as previously described[9]. The formalin-fixed, paraffin-embedded specimens were cut into 4μm thick sections and were laid on poly-L-Lysine-coated slides. Deparaffinized sections were treated with 3% hydrogen peroxide for 10min inactivate endogenous peroxidase activity. For antigen unmasking, the sections were placed in a container and covered with 10 Mm sodium citrate (pH 6.0), and then were heated by microwave at 95℃ for 5min. For staining with F8-RA antibody, the sections were further related by digestion with pancreatin. The sections were incubated with normal goat serum for 20min and incubated at 4℃ overnight with rabbit anti-human F8-RA antibody (ZA-0111), with rabbit antihuman VEGF antibody(SC-507), or with normal rabbit serum serving as a negative control. The sections were then washed with phosphate-buffered

saline (PBS, pH 7.2) and incubated for 20min with biotinylated goat anti-rabbit immunoglobulins at 37℃. After being washed three times with PBS, the sections were incubated for 20min with avidin-biotin peroxidase complex reagent at room temperature. Immunocomplex was visualized by addition of synthesized substrate, 3′3-diaminobenzidine tetrachloride dissolved in 0.03% hydrogen peroxide. The sections were counterstained with hematoxylin and mounted.

1.4 Evaluation of Immunohistochemistry

The cells positively expressing VEGF were judged by their cytoplasm stained brown by SC-507 antibody (Fig. 1A~D). The VEGF-positive tissues were classified into three groups based on the percentage of positive cells out of 100 tumor cells, which were serially counted in a field. The cell counting was repeated in four different microscopic × 400 fields. Three groups consisted of level Ⅰ, 1% to 60% (of the tumor cells); level Ⅱ, 61% to 80%; and level Ⅲ, greater than 80%.

1.5 Calculation of the Microvessel Density (MVD)

The microvessels were defined as small vessels, which (1) had endothelial cells stained brown by F8-RA antibodies, (2) were adjacent to tumor cells, (3) were bounded clearly by surrounding connective tissues (Fig. 1, E and F). The microvessels that had a luminal diameter greater than the total diameter of eight erythrocytes and that were located in relatively thick muscle tissue or in sclerotic lesion were all excluded. To calculate the MVD, four different microscopic × 100 fields, in which a large number of microvessels were well stained, were chosen and the MVDs were counted under microscopic × 400 fields. A maximal number of MVDs were chosen for further statistical analysis.

1.6 Statistical Analysis

The values of data were statistically analyzed by Student τ test, one-way analysis of variance, q test, and exact probabilities in 2× 2 table using PEMS, statistic software developed by the Department of Statistics, Huaxi College Medicine in China.

2 Results

As shown in table 2, the percentage of positive VEGF expression in malignant tumors of head and neck tissue tested was 78.1% (25/32), whereas in the benign tumors and tissues without tumor of head and neck were 46.7% (7/15) and 12.5% (2/16), respectively. The percentage of positive expression of VEGF in malignant tumor was significantly higher than those in benign tumors of head and neck tissue

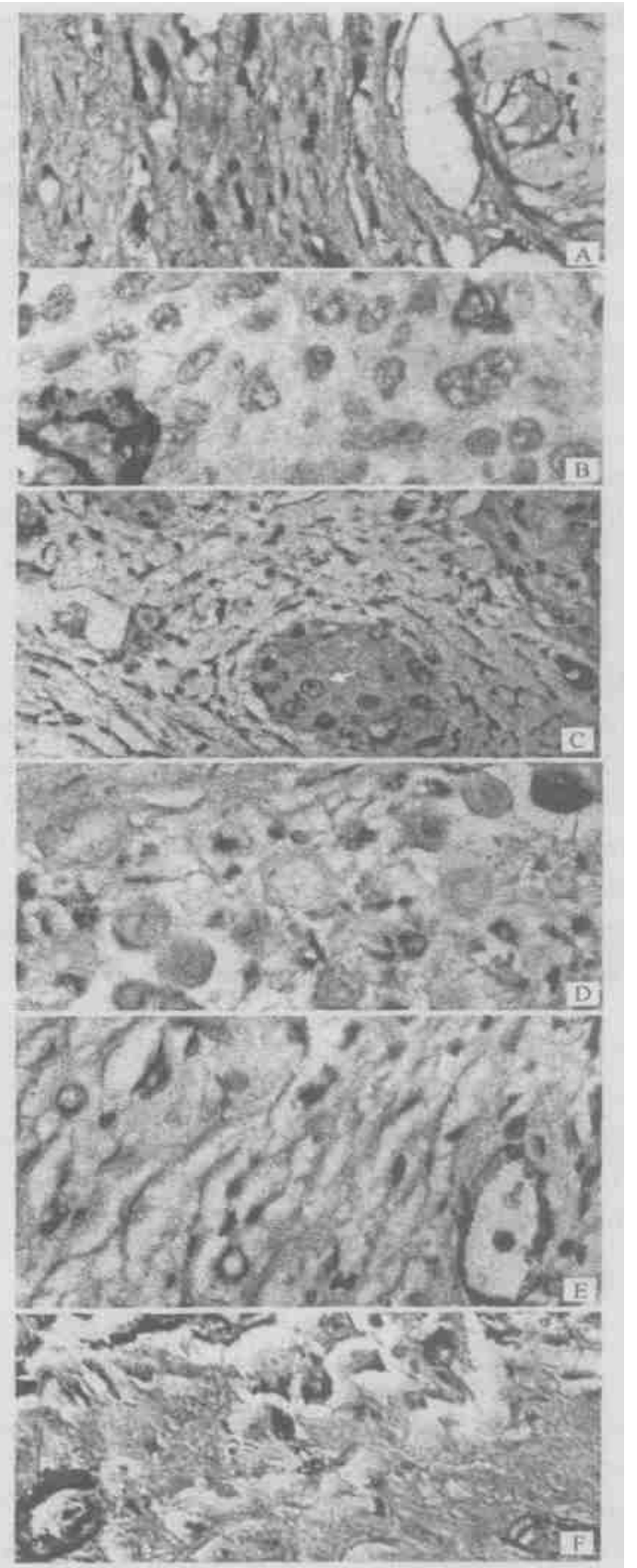

Fig. 1 Immunohistochemistry of specimens derived from tumor of head and neck

A. VEGF-positive tumor cells in malignant tumor of head and neck (× 400); B. VEGF-positive tumor cells in head and neck benign tumor (× 400); C. VEGF-positive tumor cells in head and neck malignant tumor with metastasis (× 400); D. VEGF-positive tumor cells in head and neck malignant tumor without metastasis (× 400); E. Anti-factor Ⅷ-related antigen antibody in head and neck malignant tumor with metastasis (× 400); F. Anti-factor-Ⅷ-related antigen antibody in head and neck malignant tumor without metastasis (× 400).

with a statistical significance ($P < 0.05$). The MVD in malignant tumors of bead and neck tissue was increased with a statistical significance as compared with those in benign tumors and non-tumor tissue in head neck ($P < 0.05$) (Table 2). Furthermore, the percentage of positive VEGF expression in the metastasis group (84.6%, 11/13) was higher than that in non-metastasis group (58.3%, 7/12) ($P < 0.05$) (Table 3). In addition, the MVD of metastasis group of malignant tumors was higher than that in non-metastasis group ($P < 0.05$) (Table 3). There was a close positive correlation between VEGF and MVD ($r = 0.398$, $P < 0.05$).

Table 2 The expression of vascular endothelial growth factor (VEGF) and microvessel density (MVD) in three groups of head and neck tissue

Tissue type of head and neck	No. of cases	Positive expression of VEGF(%)*	MVD**
Without tumors	16	2(12.5)	2.38±1.16
Benign tumor	15	7(46.7)	11.90±1.05
Malignant tumor	32	25(78.1)	20.31±1.37

* Statistical significance was tested by x^2 test, $P = 0.031$ (3 vs. 2); $P = 0.001$ (3 vs. 1); $P = 0.036$ (2 vs. 1)

** Values represent the means± standard deviation.

Statistical significance was tested by q test, $P < 0.05$ (3 vs. 2); $P < 0.01$ (3 and 1); $P < 0.05$ (2 and 1).

3 Discussion

In the present study, we demonstrated that there

Table 3 Comparisons of metastasis group with non-metastasis group on the VEGF expression the MVD in head and neck malignant tumors

Group	No. of cases	Positive expression of VEGF(%)*	MVD**
With metastasis	13	11(84.6)	25.15±1.43
Without metastasis	12	7(58.3)	12.00±0.57

* Statistical significance was tested by x^2 test, two groups at $P = 0.036$. ** Statisitical significance was tested by t test, two groups at $P < 0.05$.

was a close positive correlation between VEGF expression and MVD count in malignant tumors of head and neck (Table 2), suggesting that VEGF play a more important role in tumor angiogenesis of head and neck. We also found that both the VEGF expression and the MVD in malignant tumors of head and neck tissues were higher than those in benign tumors and non-tumors of head and neck tissues. This implies that the VEGF expression and the MVD in head and neck tumors increase as the stage of tumor progresses. In addition, the VEGF expression and MVD in malignant tumors with metastasis were higher than those in malignant tumor without metastasis (Table 3). Thus, metastasis of malignant tumor in head and neck is possibly related to angiogenesis as expected. In another words, VEGF seems to play a more important role in the progression and metastasis of head neck tumors.

Growth and metastasis of solid tumors depend on angiogenesis[10]. Tumor angiogenesis may be regulated by angiogenic growth factors that are secreted by tumor cells under specific conditions such as hypoxia. Recently, several angiogenic factors have been identified, and VRGF is an important one of such factors[11,12]. VEGF acts on endothelial cells to increase microvascular permeability. In addition, VRGF is a selective mitogen for endothelial cells and may directly stimulate the growth of new blood vessels. It was demonstrated that expression of VEGF was closely associated with the promotion of angiogenesis in malignant tumors[13]. Moreover, anti-VEGF monoclonal antibody administration led to a dose-and time-dependent inhibition of growth of subcutaneous xenografts and to a marked reduction in number and size of metastasized tumor in experimental liver metastases[8]. Taken together, VEGF may be a potentially important target for therapy of tumor.

4 Conclusion

The expression of VEGF is closely correlated with angiogenesis of head and neck tumor. VEGF and MVD may have prognostic value in head and neck tumor. The suppression of VEGF may inhibit angiogenesis; thereby inhibit growth and metastasis of head and neck tumor.

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本项目成果应用和发布后对养禽业影响最大的就是改变或者完善企业原来的 MD防制措施,即由原来的不免疫或免疫 HVT疫苗,只注重免疫、不注重生物安全等等,逐渐完善到现在的既免疫 CVI988/Respens疫苗、又注重生物安全的正确防制措施。养禽业特别是大型龙头企业中 MD的危害已得到有效的控制,肿瘤病的危害已从上世纪 90年代中后期的平均 7%~10%的临床发病率降低至现在的平均 2%左右。这是一个具有重大意义的进展,不管是在养禽业的经济效益的层面上,还是在家禽的健康以及其它疾病的有效控制上。

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