Expression of Vascular Endothelial Growth Factor-D in Hypopharyngeal Carcinoma and Its Relationship with Lymphatic Metastasis

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Abstract **objective** To study the relationship between the expression of vascular endothelial growth factor-D (VEGF-D)and lymphatic metastasis in hypopharyngeal carcinoma. **Methods** Immunohistochemistry was used to examine the expression of VEGF-D in 64 cases of hypopharyngeal carcinoma. **Result** Expression rate of VEGF-D was 57.8% (37/64) in 64 cases of hypopharyngeal carcinoma. According to expression intensity of VEGF-D all cases were divided into three groups: negative group(27 cases), focal positive group (23 cases), and diffuse positive group (14 cases), whose metastatic rate to lymph nodes were 25.9%, 78.3% and 92.9% respectively. The difference was statistically significant ($p<0.01$), and there was a significant correlation between the expression of VEGF–D and lymphatic metastasis. But the expression of VEGF-d was not related with venous invasion. **Conclusion** the presence of VEGF-D in hypopharyngeal cancer may predict lymph node metastasis. In addition, restraining the expression of VEGF-D may be a new therapeutic strategy for hypopharyngeal cancer.

Key words VEGF-D; hypopharyngeal neoplasms; lymph node metastasis; immunohistochemistry

Lymphatic metastasis, the common biological behavior of malignant tumor, is the important reason causing the tumor patients die. At present, the molecular mechanism of lymphangiogenesis in malignant tumor and the tumor cells metastasis through lymph vessel are not clear. Some researches indicate that VGEF-D and its distinctive acceptor

VEGFR-3 play an important role in lymphangiogenesis and lymph metastasis. At the moment, no reports refers to the expression of VEGF–D in hypopharyngeal tumor. In this research, immunohistochemistry was used to examine the expression of VEGF-D in 64 cases of hypopharyngeal carcinoma, to study the relation between expression of VEGF-D and lymphatic metastasis in hypopharyngeal carcinoma.

MATERIAL AND METHODS

Cases and specimen Sixty-four specimens of hypopharyngeal carcinoma were collected from the Department of pathology, Qilu hospital handong University. All the patients with hypopharyngeal carcinoma didn′t receive any radiotherapy and chemotherapy before operation. Of 64 examples, male 56 cases and female 8 cases, their ages were from 42 to 78 years old with averaging 60. All specimens were diagnosed as squamous cell cacinoma by the pathologic histology inspection. Among them, well differentiation were 15 cases, meddle differentiation 40 cases and poorly differentiation 9 cases. According to UICC1997TNM standard, 18 cases were in I-Ⅱ stage, 46 cases in Ⅳ-Ⅵ stage. 38 cases exist with lymphatic metastasis and 26 cases without. All specimens were conventionally embedded in paraffin wax, and then slices with thick 5-um were made continuously.

Reagent Goat anti-human VEGF-D primary antibody, rabbit anti-goat VEGF-D secondary antibody, HIGH-SABC immunohistochemistry reagent box and DAB dye were purchased from the Beijing Zhongshan biological technology limited company.

Immunohistochemial staining Paraffin section were deparaffinized and immunohistochemical staining was performed with the method of

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HIGH-SABC, i.e. endogenous peroxidase was blocked by 0.3% H_2O_2, the section were incubated with 10% normal rabbit serum to achieve blockijng, then antibodies were added in proper order, color was developed by diaminobenzidine(DAB), counter-staining was done with haematoxylin and dehydrated gradually, then transparently seals the piece, at last examined it with microscope.

Judgment of the staining of VEGF-D When obvious yellowish brown particles appeared in the tumor cell, which were taken as positive staining. At first, the grades of the staining were made according to intensity of the staining. The colorless is marked as 0, light yellow marked 1, high yellow marked 2, and brown color is marked as 3. Then grades were made according to the percent of positive staining in carcinoma cells. When the tumor cells don´t have positive staining cell, which is qualified as 0, positive cells≤10% as 1, 11~50% as 2, 51~75% as 3, >75% as 4. Both product ≥3 are qualified as positive immunohistochemical expression. Particular classified as follows: grades 0, 1, 2 is Classified as negative group; 3, 4, 6 as focal positive group; 8, 9, 12 as diffuse positive group;

Statistics analysis SAS software and X^2 test was applied to analyze the all data.

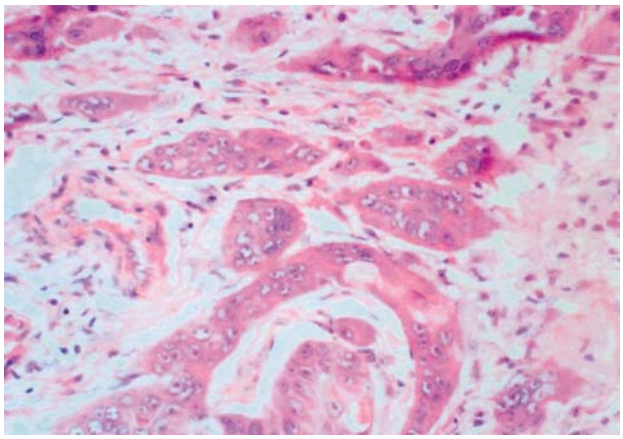
RESULTS

In 64 specimens, 37 examples obviously yellow or the yellowish brown particles, VEGF-D positive expression rate was 57.8% (37/64). Among them, focal positive 23 cases, diffuse positive 14 cases. The relations between expression of VEGF-D and the clinical materials was showed in table 1. The immunohistochemical staining of three groups were shown in Fig.1.

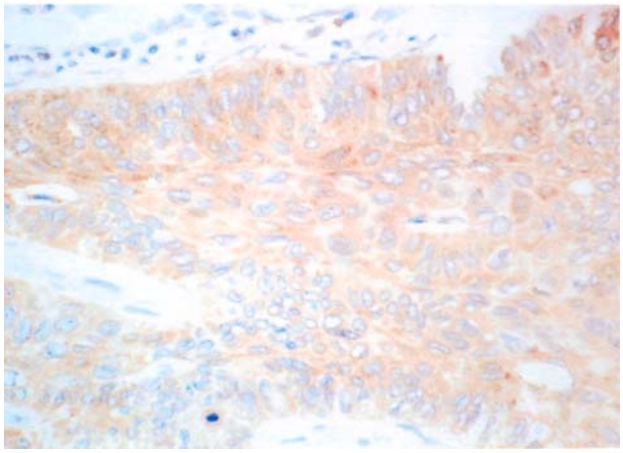
The results of this experiment demonstrated that the metastasis rate is 25.9% (7/27) in VEGF-D negative group, 78.3% (18/23) in local positive group, and 92.9 (13/14) in diffuse-positive group, which suggested that lymphatic rate increase gradually going with the increasing of positive expression of VEGF-D. The statistic analysis indicates that there are close relationship between the positive expressions of VEGFD and lymphatic metastasis of the hypopharyngeal carcinoma (P<0.01). At the same time, the positive expressions of VEGFD is related with stages of hypopharyngeal carcinoma (P<0.05), but it is not related to the age, the sex, the distant metastasis, the position of primary tumor and the tumor pathological grades.

Table 1. The relations between expression of VEGF and the clinical material

Sort	n	negative group (27)	focal group (23)	diffuse group (14)	*P*
Sex					
male	56	25	18	13	>0.05
female	8	2	5	1	
location of tumor					
Piriform region	50	24	16	10	>0.05
Post-cricoid region	10	2	5	3	
Post-wall region	4	1	2	1	
Pathologic differentiation					
well	15	5	4	6	>0.05
moderate	40	22	14	22	
poorly	9	0	5	4	
TNM by stages					
Ⅰ-Ⅱ	18	13	4	1	<0.05
Ⅲ-Ⅳ	46	14	19	13	
Lymphatic metastasis					
yes	26	20	5	1	<0.01
no	38	7	18	13	
Distant metastasis					
yes	62	27	23	12	>0.05
no	2	0	0	2	



A. VEGF-D negative immunohgistochemical staining;



B. VEGF-D focal positive immunohgistochemical staining;

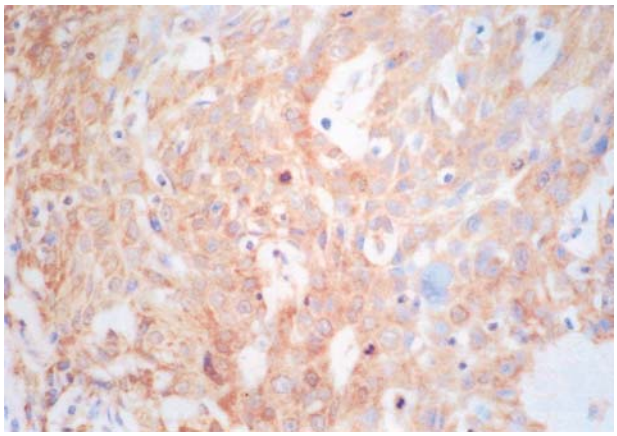


Fig.1 VEGF-D immunohistochemical staining in hypopharyngeal carcinoma

C. VEGF-D diffuse positive immunohgistochemical staining;

DISCUSSION

VEGF-D is a new member of VEGF family, it´s gene locate in chromosome XP22.31, span 50 ckb, the size of transcription product is 2.3kb. It exists mainly in heart, lung, skeletal muscles, colon, small intestine and so on. The literature reports that VEGF-D mainly bined with VEGFR-3, which is a tyrosine kinase receptor on endothelial cell surface, and may induce the endothelial cell proliferation, expansion, and migration, so it may regulates regeneration of endothelial cell of the blood and lymph vessels. It Play the important control role in the growth of embryo, tumor cell growth and metastasis. Nathanson et al. reported(6) that VGEF–3 positive lymph vessel´s density increased in the patients with breast cancer having axillary lymph node metastasis, which is four times of density in the patients without lymph node metastases. But the type IV collagen-immunostained microvessels density in node-positive patients is about 1/2 of that in node-negative patients, which indicates tumor lymphatic metastasis is formed through VEGF-3 positive lymph vessels. Because the tumor lymph vessels induced by VEGF-3 has special structure, the permeability of lymph vessel epithelial cells increased, which can causes the tumor cell to be easily to invade lymph vessel and form the distant lymphatic metastasis.

This experiment studies the expression of VEGF-D in hypopharyngreal carcinoma tissue with the immunohistochemistry technology. The results demonstrates that lymph node metastases rate is 25.9%, 78.3%, and 92.9% respectively in VEGF-D negative group, the local positive group and the diffuse positive group, the difference has the statistics significance (p<0.01). But it´s irrelevant with tissue differentiation type, distant metastasis and the position of primary tumor. This results consistently with the domestic and abroad scholars. Hirashi et al. (7) studied 83 examples of large intestine cancer specimen with lymphatic metastasis, discovered positive expression of VGEF-D in 26 examples, positive expression of VGEF-D has correlation with lymphatic metastases and has no relation with tissue´s differentiation or the liver metastasis. Kokoy et al.(8) also discovered the expression of VEGF-C, VEGF-D and VEGFR-3 in the ovary epithelial carcinoma was remarkably higher than that in the benign ovary tumor, the expression rate has the close correlation with lymph node metastasis but has nothing to do with tumor tissue differentiation types, tumor sizes and infiltration depth by the tumor.

The results of this experiment showed VGEF-D positive expression rate is higher than the other reports, which is possibly related with the tumor o-

riginal spot, malignant degree as well as the primary antibody used. This research also demonstrated that the later the tumor´s TNM stages is, the higher the positive expression of VGEF-D is. Therefore, VEGF-d may be used as indicator which forecasts whether the lower pharygeal cancer have lymphatic metastasis, and blocking expression of VEGF-D has the possibility to be one kind of new effective treatment.

REFERENCES

1. O-charoenrat P, Rhys-Evans P, Eccles SA, Expression of vascular endothelial family member in head and neck squamous cell carcinoma correlates with lymph node metasis. Cancer, 2001, 92:556–568.
2. Kenji S, Hajime K, Koji Y, et al. Suppression of VEGFR-3 signaling inhibits lymph node metastasis in gastric cancer. Cancer Sci, 2004, 95(4):328–333.
3. Kurebayashi J, Otsuki T, Kunisue Het a. Expression of vascular endothelial growth factor (VEGF) family members in breast cancer. Jph J Cancer Res, 1999, 90:977–981.
4. Makoto I, Joji K, Shinsuke K, et al. Expression of vascular endothelial growth factor for lymphatic metastasis in undifferentiated early gastric carcinoma. Jpn J Clin Oncol, 2003, 33(1):21–27.
5. Kaipainen A, korhonen J, Mustonen T, et al. Expression of the fms-like tyrosine kinase 4 gene becomes restricted to lymphatic endothelium during development. Proc Natl Acad Sci USA, 1995, 92: 3566–3570.
6. Nathanson SD, Zarbo RJ, Wachna DL, et al. Microvessels that predict axillary lymph nde metastases in patients with breast cancer. Arch Surg, 2000, 135(5): 586–593.
7. Hiroshi F, Genichi H, Shin-ichi H, et al. Expression of Vascular endothelial growth factor D is associated with lymph node metastasis in human colorectal carcinoma. Oncology, 2003, 64(4): 416–422.
8. Yokoyama Y, Charnock-jones DS, Licence D, et al. Vascular endothelial growth factor–D is an independent prognostic factor in epithelial ovarian carcinoma. British Journal of Cancer, 2003, 88(2): 237–224.
9. Makinen T, Jussila L, Veikkola T, et al. Inhibition of lymph angiogenesis with resulting lymphhedema in transtgenicmice expressing soluble VEGF receptor-3. Nat Med, 2001, 7920: 199–205.