·CLINICAL RESEARCH·

Expression and Clinical Significance of Vascular Endothelial Growth Factor C and D in Nasopharyngeal Carcinoma

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[ABSTRACT] **BACKGROUND & OBJECTIVE:** Recently, vascular endothelial growth factor-C (VEGF-C) and -D (VEGF-D) have been identified as specific lymphangiogenic factors, and their overexpression are related to lymphatic metastasis. This study was to investigate the expression and clinical significance of VEGF-C and VEGF-D in nasopharyngeal carcinoma (NPC). **METHODS:** The expression of VEGF-C, VEGF-D, vascular endothelial growth factor receptor-3 (VEGFR-3), and CD34 in 66 specimens of NPC and 19 specimens of nasopharyngitis tissue were detected by SP immunohistochemistry. Lymphatic microvessel density (LMVD) and microvessel density (MVD) were calculated. **RESULTS:** The high expression rate of VEGF-C was significantly higher in NPC than in nasopharyngitis tissues (54.5% vs. 26.3%, $P<0.05$). The high expression rate of VEGF-C was significantly higher in the NPC with regional lymph node metastasis than in those without, and higher in the NPC with high T stage than in those with low T stage ($P<0.05$). Both univariate and multivariate logistic regression analyses showed that high expression of VEGF-C was correlated to regional lymph node metastasis ($P<0.05$), but not to age, sex, 5-year survival, LMVD, and MVD ($P>0.05$). The positive rate of VEGF-D was significantly higher in NPC tissues than in adjacent non-cancerous tissues (69.7% vs. 42.1%, $P<0.05$). In NPC tissues, VEGF-D expression had no correlations to age, sex, T stage, regional lymph node metastasis, LMVD, and MVD ($P>0.05$), but was positively related to high expression of VEGF-C. The 5-year survival rate was significantly lower in VEGF-D-positive NPC than in VEGF-D-negative NPC (50.0% vs. 85.0%, $P<0.05$). **CONCLUSION:** In NPC, high expression of VEGF-C is closely correlated to regional lymph node metastasis; positive expression of VEGF-D shows no correlation to regional lymph node metastasis, but is positively related to high expression of VEGF-C and closely correlated to 5-year survival rate.

KEYWORDS: Vascular endothelial growth factor C (VEGF-C); VEGF-D; Nasopharyngeal neoplasm; Immunohistochemistry; Lymphatic microvessel density; Microvessel density; Survival rate

Introduction

Invasion and metastasis are important features of malignant tumors, both of which are the primary causes of treatment failure and clinical death of the patients. Lymphalic metastasis is an essential factor which affects the prognosis of many solid tumors. Above 50% tumour cells metastasize through lymphatic pathway. Therefore, whether lymph node is affected or not, is the assessment basis of staging, therapy, recurrence and prognosis of many tumors. Vascular endothelial growth factor-C (VEGF-C) and vascular endothelial growth factor-D (VEGF-D) have been recently identified as specific lymphangiogenic factors, and both of they can induce the generation and development of lymphatic vessels in solid tumors or pericancerous tissues. They closely correlate with lymphatic metastasis in malignant tumors. Nowadays, many studies on the expression and clinical significance of VEGF-C in head and neck tumors mainly focus on thyroid carcinoma, laryngeal squamous cell carcinoma, glossal squamous cell carcinoma, carcinoma of cheek, oral squamous cell carcinoma, etc., indicating that the expression of VEGF-C is closely correlated to lymphatic metastasis. The expression and clinical significance of VEGF-D in brain tumors, is seldomly reported. This study was to investigate the expression and clinical significance of VEGF-C and VEGF-D in nasopharyngeal carcinoma (NPC), and their correlation to clinical and pathologic factors such as lymphatic metastasis, etc., thus to provide new ideas for the diagnosis and study of NPC.

Materials and Methods

General data

A total of 66 paraffin embedded pathologically confirmed specimens of NPC from Cancer Centre, Sun Yat-sen University between Feb. 2000 and Mar 2000 were collected. According to the classification of WHO 1991 for NPC, 65 were classified as nonkeratinizing squamous cell carcinoma and 1 was undifferentiated carcinoma. Nineteen paraffin embedded non-NPC specimens taken in the same period were used as controls. These 19 specimens were pathologically confirmed as chronic inflammation. The clinical and pathological data of all cases were collected. The patients were followed up since they were discharged until their death, or until they were lost. The cut-off time was June, 2005.

Reagent

Rabbit anti-human VEGF-C, rabbit anti-human VEGFR-3 and anti-CD34 were purchased from Zhongshan goldenbidge biotechnology Co., Ltd.. Rabbit anti-human VEGF-D was purchased from Wuhan Boster Biological Technology Ltd.. SP immunohistochemical kit and DAB kit were both purchased from Zhongshan goldenbidge biotechnology Co., Ltd..

Test methods

SP immunohistochemistry method was used. All specimens were conventionally embedded in paraffin, sliced into continuous sections of 5μ m. After deparaffinization, endogenous peroxydase was inhibited by an incubation with 3% H_2O_2 for 10 min. Normal rabbit serum was applied to the slides and incubated for 10 min at room temperature, followed by the incubation with primary antibodies such as VEGF-C (1:50), VEGF-D (1:50), VEGFR-3 (1:50) and CD3 (1:100) in a moisturized box at 4overnight. After being sufficiently washed in PBS, the specimens were incubated with biotin labeled goat anti rabbit IgG or secondary antibody at 37℃ for 30min. Then the specimens were incubated with horseradish peroxidase labeled avidin at 37℃ for 30min, followed by DAB color development, counterstaining with haematoxylin, dehydration through graded alcohol, clearing with xylene and sealing with neutral gum. The stained slides were observed under a light microscope. According to reagent instruction, the specimen of colon carcinoma was taken as a positive control. The negative control was designed by using PBS instead of primary antibody.

Result assessment

VEGF-C and VEGF-D were expressed in cytoplasm. Positive expression was determined if there were obvious light-yellow to brownish yellow granules in cytoplasm. No staining or little nubilous light yellow granules were considered as negative. In a typical region, 5 fields were selected randomly. The positive rate was attained by counting 100 cells per field. Then the mean percentage of positive-stained cells was calculated. Based on the rate of positive-stained cells, the positive expression of VEGF-C and VEGF-D was indicated as follows: (-) denoted that there were no positive-stained cells or the positive rate was <5%; (+) denoted that the positive rate was 5%-25%; (++) denoted that the

positive rate was 26%-50%; (+++) denoted that the positive rate was > 50%.

Weidners method was applied [1] for the microvessel quantitation. Firstly, 5 most densely stained fields were determined under low power lens (× 40 and × 100), then the brown-stained single cell or cell cluster was observed under a × 200 light microscope. This kind of cell or cell cluster was considered as a vessel. All the vessels with muscular layer were excluded. VEGFR-3 positive lumina were considered as micro lymphatic vessels and CD-34 positive lumina were considered as microvessels. Lymphatic microvessels and capillary vessels were respectively counted. In each specimen, 5 fields were observed. The mean value was taken as lymphatic microvessel density (LMVD), and microvessel density (MVD).

Statistical analysis

Chi square test, Fishers exact test, T test, analysis of variance, univariate and multivariate logistic regression analyses, etc. were performed with SPSS 12.0. The *P* value of less than 0.05 was considered as statistically significant.

Results

Immunohistochemical results

The expression of VEGF-C, VEGF-D was mainly found in the cytoplasm of NPC cells, which were light-yellow to brownish yellow granules (Figure 1 and 2). Positive lymphatic microvessels and microvessels were observed at the verge of or around the tumor tissues, which were brown-stained clusters or vessel lumens (Figure 3 and Figure 4).

Expression of VEGF

Different VEGF-C expression in NPC and the control group

(-) was considered as negative of VEGF-C expression. (+), (++) or (+++) were considered as positive expression of VEGF-C. The positive rate of VEGF-C in NPC group

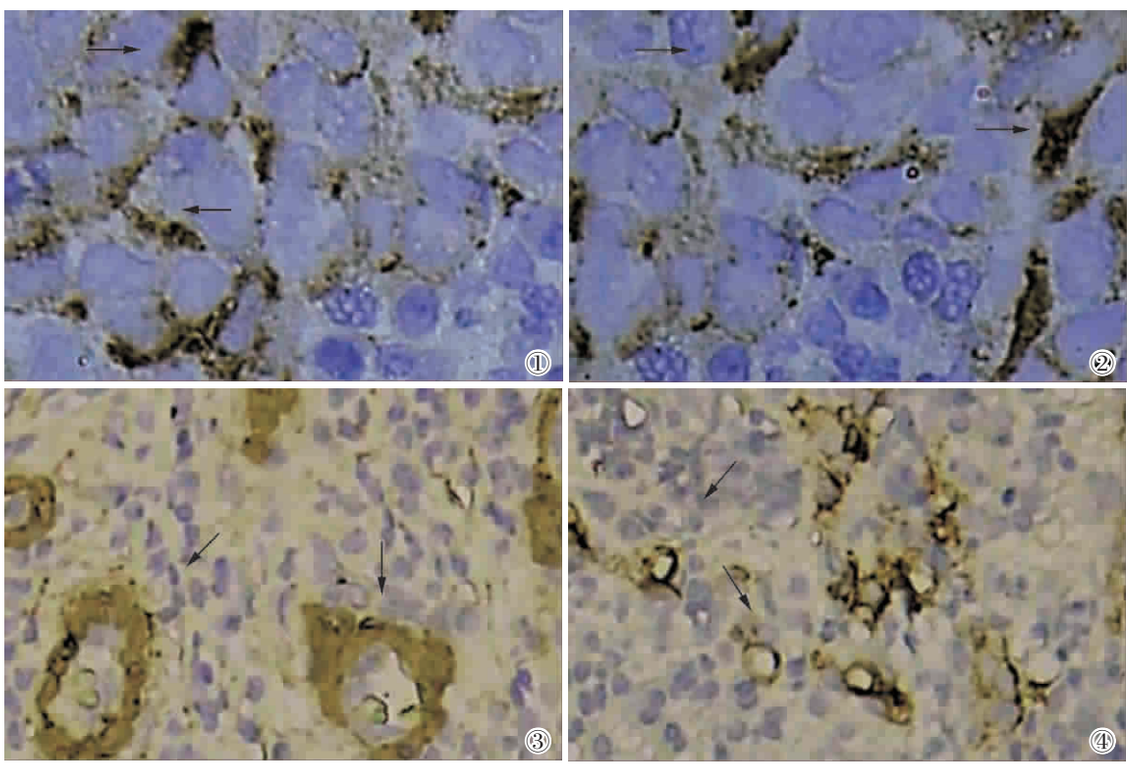

Figure 1 Positive expression of vascular endothelial growth factor-C (VEGF-C) in nasopharyngeal carcinoma (SP ×400)

Figure 2 Positive expression of VEGF-D in nasopharyngeal carcinoma (SP ×400)

Figure 3 Vessels with positive expression of vascular endothelial growth factor receptor-3 (VEGFR-3) in nasopharyngeal carcinoma (SP ×200)

Figure 4 Vessels with positive expression of CD34 in nasopharyngeal carcinoma (SP×200)

Table 1 Correlations of high expressions of VEGF-C to clinicopathologic features of nasopharyngeal carcinoma

Item		VEGF-C		*P*
		Low expression	High expression	
Sex	Male	24(46.2%)	28(53.8%)	0.826
	Female	6(42.9%)	8(57.1%)	
Age (years)	<60	26(47.3%)	29(52.7%)	0.507
	≥60	4(36.4%)	7(63.6%)	
T stage	T1	4(80.0%)	1(20.0%)	0.007
	T2	7(35.0%)	13(65.0%)	
	T3	6(26.1%)	17(73.9%)	
	T4	13(72.2%)	5(27.8%)	
Lymph node metastasis	Positive	19(38.0%)	31(62.0%)	0.032
	Negative	11(68.8%)	15(31.3%)	
Survival time (years)	<5	9(34.6%)	17(65.4%)	0.154
	≥5	21(52.5%)	19(47.5%)	
LMVD		22.83±12.77	22.54±12.83	0.926
MVD		34.59±17.91	30.34±14.20	0.287

VEGF-C, vascular endothelial growth factor-C; LMVD, lymphatic microvessel density; MVD, microvessel density.

Table 2 Correlations of high expressions of VEGF-D to clinicopathologic features of nasopharyngeal carcinoma

Item		VEGF-D		*P*
		Negative	Positive	
VEGF-C	Low expression	15(50.0%)	15(50.0%)	0.001
	High expression	5(13.9%)	31(86.1%)	
Sex	Male	16(30.8%)	36(69.2%)	1.000
	Female	4(28.6%)	10(71.4%)	
Age (years)	<60	19(34.5%)	36(65.5%)	0.152
	≥60	1(9.1%)	10(90.9%)	
T stage	T1	1(20.0%)	4(80.0%)	0.431
	T2	6(30.0%)	14(70.0%)	
	T3	5(21.7%)	18(78.3%)	
	T4	8(44.4%)	10(55.6%)	
Lymph node metastasis	Positive	15(30.0%)	35(70.0%)	1.000
	Negative	5(31.3%)	11(68.8%)	
Survival time (years)	<5	3(11.5%)	23(88.5%)	0.007
	≥5	17(42.5%)	23(57.5%)	
LMVD		22.77±13.98	22.63±12.28	0.968
MVD		32.96±17.68	31.97±15.43	0.819

was 92.4% (61/66), while in the control group was 84.2% (16/19) ($P>0.05$).

VEGF-C (-) or (+) was considered as low expression of VEGF-C, and (+), (++) or (+++) were considered as high expression of VEGF-C. The high expression rate of VEGF-C in NPC group and in the control group was 54.5% (36/16) and 26.3% (5/19), respectively ($P<0.05$).

Correlations of high expression of VEGF-C to clinicopathologic features

The correlation of high expression of VEGF-C to sex, age, T stage, regional lymph node metastasis, five-year survival time, LMVD and MVD, and other pathological factors are listed in Table 2.

There was a significant positive correlation between the expression of VEGF-C and VEGF-D in NPC tissues ($P<0.01$); the 5-year survival rate was significantly lower in VEGF-D-positive NPC (50%, 23/46) specimens than in VEGF-D-negative ones (85%, 17/20) ($P<0.05$). In NPC tissues, VEGF-D expression had no correlations to age, sex, T stage, regional lymph node metastasis, LMVD, and MVD ($P>0.05$).

Density of lymphatic microvessels

LMVD in NPC and non-cancerous tissues, and the correlation of LMVD to high expression of VEGF-C and VEGF-D, sex, age, T stage, regional lymph node metastasis, five-year survival time are listed in Table 3. There was no significant difference of LMVD between NPC group and the control group. Furthermore, LMVD had no correlations to the other clinicopathologic features.

Correlation of regional lymph node metastasis to other clinicopathologic features

Table 3 Correlations of LMVD to clinicopathologic features of nasopharyngeal carcinoma

Item		Cases	LMVD	*P*
Group	Nasopharyngitis	19	22.53± 9.52	0.964
	Carcinoma	66	22.67±12.71	
VEGF-C	High expression	36	22.54±12.83	0.926
	Low expression	30	22.83±12.77	
VEGF-D	Positive	46	22.63±12.28	0.968
	Negative	20	22.77±13.98	
Lymph node metastasis	Positive	50	23.01±13.45	0.706
	Negative	16	21.62±10.36	
T stage	T1	5	26.24±11.17	0.137
	T2	20	17.93± 9.90	
	T3	23	26.65±16.42	
	T4	18	21.88± 8.77	
Survival time	<5 years	26	23.63±12.86	0.627
	≥5 years	40	22.05±12.73	

Both univariate and multivariate logistic regression analyses showed that regional lymph node metastasis in NPC tissues was only correlated to high expression of VEGF-C (P=0.024), but not to the expression of VEGF-D, sex, age, T-stage, 5-year survival and LMVD.

Discussion

VEGF family consists of a series of secretory glycoproteins. The family members are specific mitogens for endothelial cells. These members can selectively promote the mitosis of blood-vessel endothelial cells and/or lymphatic endothelial cells; stimulate the proliferation of endothelial cells; trigger the growth of blood vessels; increase vessel permeability, especially microvessel permeability and chemotaxis; and thus form a fresh rete vasculosum [2]. VEGF-C is the first-discovered lymphatic growth factor of VEGF family, which has been detected in most human malignant tumors, such as prostate carcinoma, colorectal cancer, gastric cancer, breast carcinoma, lung cancer, leukoma, malignant melanoma, fibroma, *etc*.. Its expression is significantly higher in malignant tissues than in normal tissues. VEGF-C is closely correlated to the generation of lymphatic vessels, lymph node metastasis and prognosis of the disease. Several studies on squamous cell carcinoma of the head have indicated that, in comparison with normal epithelium, the expression of VEGF-C is significantly increased, and the increased VEGF-C has a prognostic value for cervical lymph node metastases[3,4]. In our study, it was indicated that the high expression rate of VEGF-C was significantly higher in NPC than in nasopharyngitis tissues; and those with high expression of VEGF-C usually had a high rate of lymph node metastasis. Both univariate and multivariate logistic regression analyses showed that regional lymph node metastasis was closely correlated to high expression of VEGF-C. Currently, most studies report that high expression of VEGF-C in malignant tumors triggers lymphatic metastasis mainly through VEGF-C, which is generated in malignant cells and plays a role in adjusting hyperplasia and permeability of lymphatic vessels, so that lymphatic metastasis can occur more easily through the following ways: (1) activating VEGFR-3 in lymphatic endothelial cells, thus triggering lymphatic endothelial cell proliferation. In this way, lymphatic vessel hyperplasia in pericancerous tissues is induced and cell-to-cell adhesion is decreased. Permeability of lymphatic vessels is therefore increased for more metastastic chances. (2) Up-regulated tumor-cell adhesion: there are 3 types of adhesive molecule families on endothelial cells, that is, immunoglobulin superfamily (including growth factor receptors), integrin family and selectin family. Through the receptors on the surface of lymphatic endothelial cells, the tumor cells which can secrete VECF-C adhere to lymphatic endothelial cells [6]; (3) as a chemotactic factor, driving the tumor cells to migrate to lymphatic vessels [7]; (4) adjusting lymphatic vessels, through activating the generation of nitrogen monoxidum, because lymph fluid flows into lymph node through intermittent contractions of lymphatic vessels [8]. Besides that, our study has indicated that in NPC group, those with high expression of VEGF-C are usually in a high T stage (no strict positive correlations except T4). The possible reason might be that VEGF-C has an effect on promoting the growth of tumor cells. It has been reported that the excessive expression of VEGF-C in human breast cancer MCF-7 cells promoted the growth of tumor cells, but had no effect on angiogenesis in tumor tissues; the binding of VEGF-C and VEGFR-3 can be inhibited by dissolvable VEGFR-3-Ig, thus to inactive VEGF-C.

VEGF-D is another identified lymphangiogenic-factor. Up to now, only few studies on VEGF-D have been carried out, and the conclusions vary. Some studies have found that VEGF-D has positive correlation to lymphatic metastasis: Stacker et al [10] found that VEGF-D could not only induce the formation of lymphatic vessels in tumor tissues, but also result in the metastasis of tumor cells to regional lymph nodes; in a study on oral squamous cell carcinoma, VEGF-D was higher in tumor-positive lymph nodes than in tumor-negative lymph nodes[11]; in a study of prostatic carcinoma, it was showed that in the primary tumors with sentinel lymph node metastasis, the expression of VEGF-D was significantly higher than in those without lymph node metastasis [12]. However, some studies have drawn a different conclusion: O-charoenrat et al found that the expression of VEGF-A and VEGF-C in tumor cells was higher than that in the normal ones, while the expression of VEGF-D was decreased. In comparison with the normal epithelium tissues,

the expression of VEGF-A mRNA and VEGF-C mRNA in tumors was increased, while the expression of VEGF-D mRNA was significantly decreased. Strauss et al [3] also found that in head and neck squamous cell carcinoma accompanied with cervical lymph node metastasis, the expression of VEGF-D was lower than in those without metastasis. In our study, it was showed that the expression of VEGF-D in NPC tissues was higher than that in non-NPC tissues; and the high expression rate of VEGF-C was significantly correlated to regional lymph node metastasis. Currently, because little about VEGF-D is known, the mechanism of how VEGF works remains unknown. According to some scholars, the possible reason is that: though VEGF-D can stimulate lymph cell generation in normal and tumor tissues, it does not play a role in the development of lymphatic system; or during the term, VEGF-C or other unknown factors take effect as a supplement to VEGF-D. Our study shows that in NPC patients with positive VEGF-D expression, the five-year survival rate was significantly lower than in those without VEGF-D expression, implying that VEGF-D may be a predictive factor of long-term survival of the patients.

In adults, VEGFR-3 is mainly localized in lymphatic endothelial cells and used as a mark for lymphatic vessels. It is reported that the expression of LMVD and VEGF-C in tumors is closely correlated to lymph node metastasis: for example, Sedivy et al [14] find that in oral squamous cell carcinoma tissue, LMVD is significantly increased in comparison with the control tissues, and it is positively related to the expression of VEGF-C, tumor grading, lymph node status and potential metastasis; in the studies of esophageal carcinoma, prostatic carcinoma, colon carcinoma, pulmonary carcinoma, etc., it is also indicated that the high expression of VEGF-C/VEGFR-3 is positively related to lymphatic vessel proliferation and lymph node metastasis in pericancerous tissues. However, other studies show that the expression of VEGFR-3 has no correlations to T stage, pathologic grading age, the expression of VEGF-C and lymph node metastasis [15,16]. In our study, it is showed that there is no difference between LMVD of NPC tissues and that of non-NPC tissues. Further more, LMVD of NPC tissues has no correlations to the expression of LMVD and VEGF-C, and to regional lymph node metastasis. The possible reasons are as follows: (1) Though the expression of VEGFR-3 is mainly localized in lymphatic endothelial cells of adults, it is showed that VEGFR-3 also can be expressed not only in a small number of blood vessel endotheliums in tumors [17], but also in some fenestrated capillary endotheliums and sinusoidal capillary endotheliums of normal adults[18]. As we know that the capillaries and venules of nasal mucosa have high permeability, and the endothelium is typically fenestrated. It has been found that VEGFR-3 can be expressed in the blood vessels (including artery, vein and capillary endotheliums) and lymphatic vessels of mucous membrane of nose [19]. Therefore, LMVD of NPC tissues has no positive correlation to the expression of VEGF-C and lymph node metastasis. (2) Studies show that the expression of VEGFR-3 can be increased in tumor, inflammation and other pathological status, thus endothelial cell proliferation is induced [20]. However, in our study, all the tissues in the control group are chronic inflammatory tissues, instead of normal mucous membrane of nose. There is no significant difference of LMVD in the control group and NPC group. (3) It may be affected by the factors such as: sampling or cutting site, the limitation of sensitivity of the study method, long-term (more than 5 years) fixation, etc.. (4) It may be associated with non-specific staining of polyclonal antibodies. This study may reflect the biological diversity of tumors. As for the exact mechanism of it, the observation of more samples and more in-depth studies are needed.

[REFERENCES]

[1] Weidner N. Current pathologic methods for measuring intratumoral microvessel density within breast carcinoma and other solid tumors [J]. Breast Cancer Res Treat, 1995,36(2):169-180.

[2] Veikkola T, Alitalo K. VEGFs, receptors and angiogenesis [J]. Semin Cancer Biol, 1999,9(3):211-220.

[3] Strauss L, Volland D, Kunkel M, et al. Dual role of VEGF family members in the pathogenesis of head and neck cancer (HNSCC): possible link between angiogenesis and immune tolerance [J]. Med Sci Monit, 2005,11(8):280-292.

[4] O-charoenrat P, Rhys-Evans P, Eccles S A. Expression of vascular endothelial growth factor family members in head and neck squamous cell carcinoma correlates with lymph node metastasis [J]. Cancer, 2001,92(3):556-568.

[5] Joukov V, Pajusola K, Kaipainen A, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases [J]. EMBO J, 1996,15(2):290-298.

[6] Bevacqua S J, Welch D R, Diez de Pinos S M, et al.

Quantitation of human melanoma, carcinoma and sarcoma tumor cell adhesion to lymphatic endothelium [J]. Lymphology, 1990,23(1):4-14.

[7] Oh S J, Jeltsch M M, Birkenhager R, et al. VEGF and VEGF-C: specific induction of angiogenesis and lymphangiogenesis in the differentiated avian chorioallantoic membrane [J]. Dev Boil, 1997,188(1):96-109.

[8] Crowe M J, Von Der Weid P Y, Brock J A, et al. Co-ordination of contractile activity in guinea-pig mesenteric lymphatics [J]. J Physiol, 1997,500(Pt 1):235-244.

[9] Karpanen T, Egeblad M, Karkkainen M J, et al. Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth [J]. Cancer Res, 2001,61(5):1786-1790.

[10] Stacker S A, Caesar C, Baldwin M E, et al. VEGF-D promotes the metastasis spread of tumor cells via the lymphatic [J]. Nat Med, 2001,7(2): 186-191.

[11] Shintani S, Li C, Ishikawa T, et al. Expression of vascular endothelial growth factor A, B, C, and D in oral squamous cell carcinoma [J]. Oral Oncol, 2004,40(1):13-20.

[12] Stearns M E, Wang M, Hu Y, et al. Expression of a flt-4 (VEGFR3) splicing variant in primary human prostate tumors. VEGF D and flt-4t (Delta773-1081) overexpression is diagnostic for sentinel lymph node metastasis [J]. Lab Invest, 2004,84(6):785-795.

[13] Baldwin M E, Halford M M, Roufail S, et al. Vascular endothelial growth factor D is dispensable for development of the lymphatic system [J]. Mol Cell Biol, 2005,25(6):2441-2449.

[14] Sedivy R, Beck-Mannagetta J, Haverkampf C, et al. Expression of vascular endothelial growth factor-C correlates with the lymphatic microvessel density and the nodal status in oral squamous cell cancer [J]. J Oral Pathol Med, 2003,32(8):455-460.

[15] Zhou H Z, Gu X M, Hu M, et al. Study on the formation of lymphaducts induced by check cancer [J]. Jun Yi Jin Xiu Xu Yuan Xue Bao, 2003, 24(20): 106-108.

[16] Yu D H, Wen Y M, Wang C M, et al. Correlation of VEGFR expression to lymphocyte metastasis in oral carcinoma [J]. Lin Chuang Kou Qiang Yi Xue Za Zhi, 2001, 17 (4): 243-245.

[17] Partanen T A, Alitalo K, Miettinen M. Lack of lymphatic vascular specificity of vascular endothelial growth factor receptor 3 in 185 vascular tumors [J]. Cancer, 1999,86(11):2406-2412.

[18] Partanen T A, Arola J, Saaristo A, et al. VEGF-C and VEGF-D expression in neuroendocrine cells and their receptor, VEGFR-3, in fenestrated blood vessels in human tissues [J]. FASEB J, 2000,14(13):2087-2096.

[19] Saaristo A, Partanen T A, Arola J, et al. Vascular endothelial growth factor-C and its receptor VEGFR-3 in the nasal mucosa and in nasopharyngeal tumors [J]. Am J Pathol, 2000,157(1):7-14.

[20] Skobe M, Brown L F, Tognazzi K, et al. Vascular endothelial growth factor-C (VEGF-C) and its receptors KDR and flt-4 are expressed in AIDS-associated Kaposi's sarcoma [J]. J Invest Dermatol, 1999,113(6):1047-1053.