Significance of Expression of VEGF In Patients With Diffused Large B Cell Lymphoma

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Abstract **Objective** To study whether expression of VEGF is associated with clinical characteristics or prognosis in patients with diffused large B cell lymphoma. **Methods** An immunohistochemical method of LSAB was used to do the VEGF staining in 67 biopsy samples. Chi-Square test, method of Kaplan-Meier and Log-Rank test were used to do the statistical analysis of the correlation between clinical characteristics, prognosis and VEGF expression. **Results** The expression rate of VEGF in patients accompanied with B-symptoms was 79.2%, and it was 51.2% in patients without B-symptoms (P=0.024). Among the 63 patients whose response to chemotherapy could be evaluated, the CR rate of VEGF in negative group was significantly higher than that in positive grou (P=0.001). Median progression free survival and median overall survival were 40 months and 52 months of VEGF negative group, significantly better than that in VEGF positive group (13 months and 39 months, P=0.004 and 0.029). the expression rate of VEGF was not related with gender, age, and stages of the patients (P>0.05). **Conclusion** Expression of VEGF is associated with B-symptoms, a poor response to chemotherapy and a worse prognosis in patients with diffused large B cell lymphoma, there was not any relationship between VEGF and gender, age or stage had been observed in this study.

Key words VEGF; Diffused large B cell lymphoma; Clinical characteristics; Prognosis

Diffused large B cell lymphoma (DLBCL), originated from the canceration of B lymphocyte, is the most common type of aggressive Non-Hodgkin´s lymphoma (NHL), the clinical manifestation, therapeutic efficacy and outcome of this disease differs at a big range. If we could find any indicatrix that related to the clinical characteristics or prognosis of DLBCL, we might be at the positive position in clinical works.

The relationship between angiogenesis and malignant neoplasma is more and more concerned. We have noticed angiogenesis in many physiological and pathological processes, such as female menstrual cycle and wound healing etc. During tumorigenesis, the vasculature can become activated to grow new capillaries in response to an appropriate stimulus[1]. New vessels may bring tumor cells oxygen and nutrition, and carry metabolic product at the same time. In addition, the dissemination of malignant tumors is dependent on angiogenesis[2], DLBCL, as well.

Angiogenesis is regulated by a balance of various enhancing and inhibiting angiogenic factors [3]. We have found decades of angiogenic factors, including fibroblast growth factor, vascular endothelial growth factor (VEGF), angiopoietins, epithelial growth factor, platelet-derived growth factor, transforming growth factor-β, etc. VEGF specific affects endothelial cell, plays the most important role in angiogenesis.

It was observed that sVEGF (serum VEGF) level is associated with a poor World Health Organization performance status, a high International Prognostic Index, a high serum lactate dehydrogenase (LDH) level, and a lower 5-year survival rate in patients with NHL [4]. In present study, we detected the expression of VEGF in situ and tried to find out the association between VEGF and clinical characteristics of patients with DLBCL.

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MATERIALS AND METHODS

Patients

Patients who had been diagnosed as DLBCL and treated in HuaXi hospital during January 2002 to March 2005 were eligible. 67 cases had the pathological samples that could be used to experience immunohistochemical stain once more. Of them, 39 (58.2%) were men and 28 (41.8%) were women, the median age was 59 years old. 24 (35.8%) patients accompanied with B symptoms (weight loss, unexplained fever, or night sweats). There were 10 (14.9%) patients of stage I, 32 (47.8%) patients were of stage II, 11 (16.4%) patients were of stage III, and 14 (20.9%) patients were of stage IV.

Immunohistochemical evaluation of VEGF expression

Expression of VEGF protein was determined immunohistochemically in 67 patients´paraffin blocks by biopsy. Histological slides (5μm) were deparaffined in xylol and passed through graded alcohols. Antigen retrieval was done by heated at 800 W in a microwave oven for 5 minutes. The sections were treated with 0.3% hydrogen peroxide (H_2O_2) in methanol for 10 minutes to quench the endogenous peroxidase activity within the tissue. Nonspecific binding sites were blocked with 0.1% BSA and 20% heat-inactivated goat serum in PBS for 10 minutes at 37℃. The sections were incubated at room temperature for 1 hour with a monoclonal VEGF antibody (DAKO Company, Denmark, dilution: 1:100). Then slides we incubated with secondary biotinylated antibody (DAKO Company, Denmark, dilution: 1:100) for 1 hour at room temperature before being washed for 15 min with three changes of PBS. Peroxidase activity was visualised using 3,3-diaminobenzidine (DAB, brown colour) with 0.01% H_2O_2 as activator. Rinsing the sections with distilled water terminated colour development. Sections were counterstained with haematoxylin. Negative control studies were performed in which normal goat serum was used instead of the primary antibody. No significant staining was observed in the negative control sections. As positive controls, formalin-fixed, paraffin-embedded sections from human lung cancer tissue were stained.

Buffy grana distributed steady intracytoplasm was defined to be the VEGF positive cell. We examed 10 high power fields randomly, the quantity of VEGF positive cells larger than 10% was judged as the positive expression of VEGF. (Figure 1, Figure 2)

Treatment and follow-up

Regimen of CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) was chosen to be the first line chemotherapy, all of the 67 patients administered at least 1 cycle of treatment. 2 patients accepted only 1 cycle of chemotherapy because of the rapid tumor progression result in death, the other 65 patients had com-

Table 1 Expression of VEGF and clinical characteristics

Clinical characteristics	Groups	Case number	Positive rate of VEGF	Chi-Square	*P*
Gender	Male	39	26(66.7%)	1.177	0.278
	Female	28	15(53.6%)		
Age	<60	42	27(64.3%)	0.453	0.501
	⩾60	25	14(56%)		
Stage	Ⅰ	10	5(50%)	2.665	0.446
	Ⅱ	32	18(56.3%)		
	Ⅲ	11	7(63.6%)		
	Ⅳ	14	11(78.6%)		
Accompanied with B symptoms	Yes	24	19(79.2%)	5.087	0.024
	No	43	22(51.2%)		

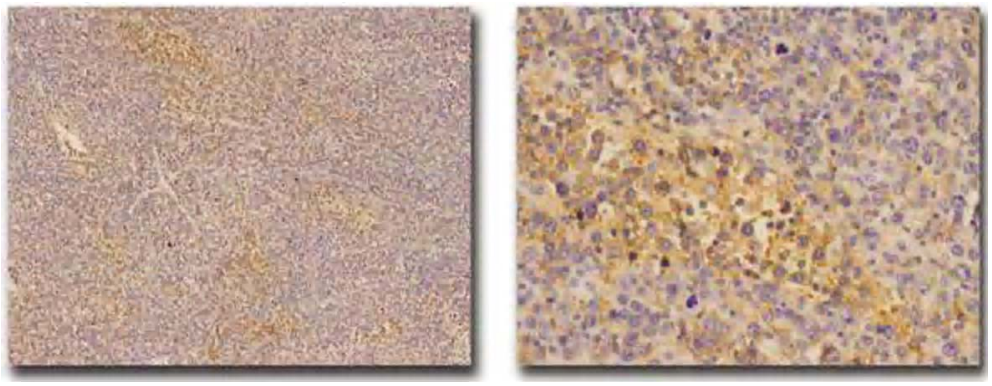

Fig. 1 These two images show DLBCL tissue expresses VEGF by Immunohistochemical staining (×100, ×420)

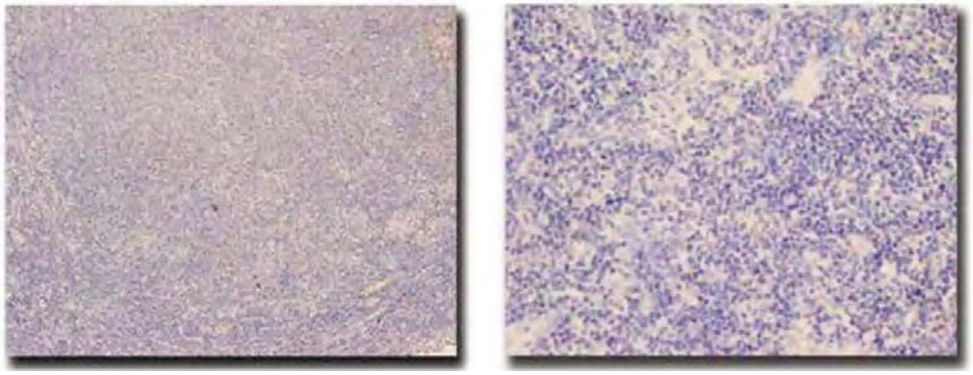

Fig. 2 These two images show VEGF negative DLBCL tissue (×100, ×400)

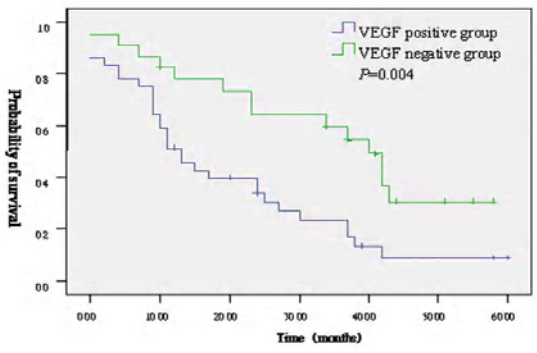

Fig. 3 Expression of VEGF and progression free

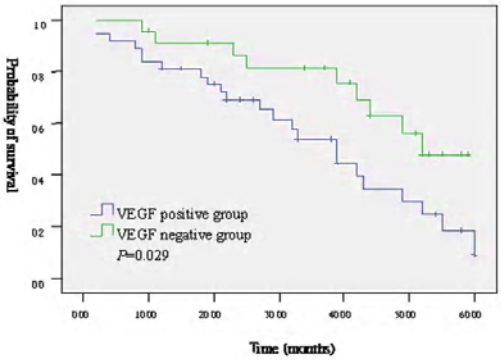

Fig. 4 Expression of VEGF and overall survival

pleted 4 cycles of chemotherapy in the first course. Response to treatment was estimated after 4 cycles of chemotherapy, the 2 patients accepted only 1 cycle were evaluated as progression disease (PD), there were another 4 patients refused the evaluation for their personal reasons.

Follow−up of present study ceased in March 2007, median time of follow−up was 41 months (range 24−62 months). 7 patients (10.4%) had dropped out, who were excluded of the survival analysis.

Statistical analysis

Patients were divided into several groups according to their gender, age, stage and B symptoms. Chi − Square test was used to analyze VEGF positive rate in different groups. The complete remission (CR) rates of

2 groups (VEGF positive group and VEGF negative group) were also analyzed by Chi−Square test. Kaplan−Meier process and Log−Rank test was used to do the survival analysis. All of the statistical work referred in this study was done by SPSS13.0 software. (SPSS Inc. Chicago)

RESULTS

Expression of VEGF and clinical characteristics

Among 67 patients, 26(66.7%) samples of male were VEGF positive; and 15 (53.6%) samples of female were positive, after Chi−Square test, Chi−Square=1.177, *P*=0.278. According to the definition of old people by WHO, we divided the 67 patients into 2 groups (younger than 60 or not). 27 (64.3%) samples of young patients were of VEGF positive, while old patients had 14(56%) positive samples. Chi−Square=0.453, *P*=0.501. positive rate of VEGF in different stage was displayed as following: stage I: 50%, stage II: 56.3%, stage III: 63.6%, stage IV: 78.6%, Chi−Square=2.665, *P*=0.446.

We found out the precise information about B symptoms (weight loss, unexplained fever, or night sweats) in all patients´ medical records. In the 24 patients accompanied with B symptoms, 19 (79.2%) were VEGF positive, while there were only 22 (51.2%) positive among the 43 B symptoms free patients. Chi−Square=5.087, *P*=0.024(Table 1).

Expression of VEGF and response to chemotherapy

Whether obtaining CR or not during the first course of treatment is strongly associated with the prognosis of NHL. In this study, obtaining CR within the first 4 cycles of chemotherapy was chosen as a symbol of good therapeutic efficacy.

There were 4 (6.0%) patients´ tumor response could not be evaluated for their personal reasons. Among the other 63 patients, 2 groups (VEGF positive and VEGF negative) were divided. 13(32.5%) patients achieved CR in VEGF positive group, while the CR rate was 82.6% of VEGF negative group. Chi−Square=14.670, *P*=0.000.

Expression of VEGF and survivalof the petients

A total of 60 patients were brought into survival analysis. Median progression free survival (PFS) of 37 patients with VEGF positive expression was 13 months (95%CI: 8.16~17.84 months), 27 months shorter than that of the 23 patients with VEGF negative expression, Chi−Square=8.244, *P*=0.004 (Figure 3) . Median overall survival of VEGF positive group was 39 months (95%CI: 28.81~49.20 months), 13 months shorter than that of VEGF negative group, Chi−Square=4.772, *P*=0.029 (Figure 4).

DISCUSSION

VEGF, a member of platelet derived growth factor family, its gene located in the short arm of 6th chromosome, has 8 exons and 7 introns [5, 6]. Ferrarra depurated VEGF from cattle appendix cerebri follicle cell culture fluid in 1989. In recent years, some polypeptide factors which are similar in function and autoploidy in constitution with VEGF have been found, they all together construct the VEGF family, including placental growth factor, VEGF−B (VEGF related factor), VEGF−C (VEGF related protein), VEGF−D and VEGF−E.

When endothelial cell affected by VEGF, multiple function has been generated. (1) promote cell mitosis intensively; (2) increase vasopermeability, saturate plasma protein to district extra vessel. The protein might become sustentation for neogenetic vessel and tumor matrix, this action would enhance malignant cells entering circulation, advantage tumor metastasis [7, 8]; (3) regulate vascular tone; (4) induce endothelial cell producing vessel bioactive molecule [9] to resist thrombogenesis [10]; (5) inhibit tumor cell apoptosis [11]. We can observe VEGF overexpression in almost all the neoplasma, stimulating tumor angiogenesis in a paracrine action.

Some studies aimed directly at solid tumors showed the association between VEGF expression and clinical characteristics. The level of VEGF Expression in adenocarcinoma tissue is significantly higher than that in squamous cell lung cancer tissue.Among lung cancers of the same cell type, poorly differentiated tumors have the higher percentage of VEGF positive, at the same time, overexpression of VEGF is found to be associated with lymphonodes and distant metastasis in lung cancer[12]. It´s

reported overexpression of VEGF and mutant P53 protein is associated with distant metastasis in gastric cancer [13]. High serum VEGF concentration of patients with colorectal cancer is associated with the advanced staging, higher serum CEA concentration, and lymphonodes or liver metastasis [14]. Anthony et al. detected expression of VEGF in 46 biopsy samples of breast cancer by in situ hybridization and found that expression of VEGF is associated with characteristics such as lymphonodes metastasis, distant metastasis and short survival period[15].

Recent data suggested that similar event occurs in lympho −hematopoietic system malignancies. Serum VEGF concentration in patients with Hodgkin´s lymphoma was found significantly higher than that in normal individuals, the higher sVEGF concentration often predicts the poor survival. The prognosis of the patients whose sVEGF concentration decrease after treated was better than the patients with the stable sVEGF concentration [16]. 82 patients with defferent subtype non − Hodgkin´s lymphoma were detected serum VEGF concentration, and those had high sVEGF concentration experienced the poor outcome [17]. It´s also found that simultaneous high sVEGF and bFGF concentration is associated with advanced staging and LDH level in patients with non−Hodgkin´s lymphoma[16]. A recent study enrolling 110 patients with non−Hodgkin´s lymphoma showed sVEGF concentration of patients with diffuse large B cell lymphoma was about 2 times higher than mantle cell lymphoma or other subtype of low aggressive grade[18].

In present study, we had detected in situ expression of VEGF in pretreatment biopsy samples gained from patients with DLBCL. The VEGF positive rate of 86.1% was observed in patients accompanied with B symptoms, which was significantly higher than that in those without B symptoms. VEGF negative patients had more chance to obtain CR after the first course of chemotherapy, meanwhile, expression of VEGF in situ predicts poor response to chemotherapy. Hazar found there was significantly higher response rate (CR+PR) after treatment among patients of in situ VEGF negative versus VEGF positive [19]. Results of these 2 studies are comparable. There was another impressive result we found in current study: patients whose samples expressed VEGF had a shorter PFS and OS.

In conclusion, results of present study indicated expression of VEGF in situ is associated with B symptoms and a poor outcome in patients with DLBCL. We strongly suggest doing immunochemical stain of VEGF in biopsy samples that were diagnosed as DLBCL, this may help you to handle the disease more accurately, choose the proper regimen, and prepare for the potential anti−angiogenesis therapy.

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