STUDY ON THE CLINICAL-PATHOLOGICAL SIGNIFICANCE OF MICROVESSEL DENSITY AND VASCULAR ENDOTHILIAL GROWTH FACTOR EXPRESSION IN PRIMARY LIVER CANCER

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ABSTRACT

Objective: To evaluate the clinical-pathological significance of intratumoral microvessel density (MVD) and Vascular Endothelial Growth Factor (VEGF) expression in primary liver cancer (PLC). Methods: A retrospective study from 63 postoperative patients all with small PLC (diameter ≤ 5 cm) was done. One group of 29 patients developed recurrence or metastasis within 2 years. The other group of 34 patients had no evidence of recurrence or metastasis within 2 years. Three sections were taken from each patient. One for H.E. staining, the other two for VEGF and Bio-UEA-I immunohistochemical staining respectively. MVD was counted by endothelial cells, which were highlighted by Bio-UEA-I. Results: The MVD of the recurrence (or metastasis) group (49.6±29.7) were significantly greater than the other group (22.7±28.2) (P<0.01); The VEGF positive rate of the recurrence group was 86.2% (25/29), the rate of the other group was 47.1% (16/34). The difference between the 2 groups was stafistically significant (P<0.01). The stage of the tumor, the positive rate of satellite nodules and the positive rate of the portal vein embolus were all significantly different between the 2 groups. Conclusion: Besides tumor stage, satellite nodule and portal vein embolus, the MVD and VEGF are also of prognostic significance.

Key words: Primary liver cancer (PLC), Vascular endothelial growth factor (VEGF), Prognosis, Microvessel density (MVD).

Recent studies revealed that tumor recurrence and metastasis is associated with the intratumoral microvessel density (MVD). The MVD of many tumors (such as breast, stomach, renal and colon cancer) is correlated with postoperative recurrence and metastasis, and it is an independent prognostic indicator.[1–4]

Several angiogenic factors, such as basic fibroblast growth factor (BFGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF) etc are secreted by tumor cells. VEGF is a potent angiogenic factor. The expression of VEGF in several tumors (such as breast, stomach, renal, and colon cancer) is closely related to MVD, and has prognostic significance.[4,5–7]

The removed tissues of primary liver cancer (PLC) were used for the immunohistochemical staining of MVD and VEGF. Their relationship as well as their prognostic value and clinicopathological significance were studied retrospectively.

MATERIALS AND METHODS

Patients and Methods

The 63 samples of small PLC, which were surgically removed and pathologically proved, were selected from our institute from 1992 to 1995. The selected cases were all small PLC (single nodule, diameter ≤ 5 cm) and had the following characters: no residual tumor on the resection margin, no tumor embolus in the portal vein, no metastatic lympho-node at hepatic portal. Among the 63 patients, 29 developed recurrence or metastasis within 2 years and 34 had no evidence of recurrence and metastasis. There was no significant difference between the 2 groups regarding age, sex, HBV positive rate, AFP concentration, tumor diameter, tumor differentiation, tumor capsule, and degree of cirrhosis. The recurrence and metastasis were defined as: (1) the occult metastasis was not found before operation, but were proved several months after the operation; (2) the usually called recurrence and metastasis, means the recurrence and metastasis were really occurred after the operation. The follow-up means included measurement of AFP, B-ultrosonography, CT scanning, hepatic arterial angiography and lipiodol-CT.

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The Immunohistochemical Staining of Tumor Vessels and VEGF

Paraffin-embedded tumor samples containing peri-tumor liver tissues were used. The sections were 5 μm thick. The biotinylated ulex europaeus agglutinin-I (Bio-UEA-I, Vector Company, diluted as 1:500) was used for staining of endothelial cells. For VEGF staining, anti-VEGF antibody (Oncogene Company, diluted as 1:20) was used according to the ABC assay. The negative controls were done when the TBS buffer was used to replace the UEA-I and anti-VEGF. The blood vessels of the portal area of the peri-tumor tissue served as the positive controls of the UEA-I. The peri-tumor hepatic cells were seen as the positive controls of the VEGF.

Assessment of the Results

MVD was assessed by the way Weidner reported.[3] The stained sections were scanned by light microscope at low (40 ×) magnification, and areas of highest neovascularization (so-called "hot spots") were found. The "hot spots" could occur anywhere within the tumors, but most frequently appeared at the capsules of the tumors. It was reported that the recurrence and metastasis of tumors were not correlated to the average MVD, but were correlated to the MVD of the "hot spots".[3] After the "hot spots" were identified, the numbers of the brown-staining endothelial cells were counted on a 200 × field. Two pathologists who did not review to the clinical data did the evaluations in the meantime under a multiple-ocular-microscope. Any brown-staining endothelial cell or endothelial-cell cluster, clearly separate from adjacent microvessels, tumor cells and other connective-tissue elements, were considered a single, countable microvessel. Distinct clusters of brown-staining endothelial cells, which might be from the same microvessel "snaking" its way in and out of the section, were considered as 2 microvessels. Vessel lumens, although usually present, were not necessary for a structure to be defined as a microvessel; and red cells were not used to define a vessel lumen too. Results were expressed as the highest number of microvessels in any single 200 × field. An average of multiple fields was not performed.

VEGF expression was characterized as negative and positive according to its staining intensity. Another pathologist who was blinded to clinical data did these evaluations.

Other Pathological Indicators

The differentiation of PLC was judged by the Edmondson-Steiner system. The invasion of portal vein was defined as: under the microscope, the tumor tissue involved the larger vessels accompanied with the discontinuous stained of the endothelial cells; or the tumor embolus could be found in the portal vein. Satellite nodules were those scattered along the main tumor and were pathologically proved as PLC. No capsule was found by naked eye, or incomplete capsules seen under the microscope were regarded as no capsules. The HBV positive was dependent on any one of the 5 HBV-markers. The degree of cirrhosis was divided into large (≥ 3 mm) or small (< 3 mm) nodular cirrhosis by the size of the cirrhotic nodule.

Statistics

The *t*-test was used for the measurement data of Gaussian distribution and homoscedasticity. The K-W nonparametric statistics was used for the other measurement data. The chi square test was used for the enumeration data.

RESULTS

The MVD of the Two Groups

The MVD of the recurrence (or metastasis) group (49.6±29.7) was significantly greater than that of the other group (22.7±28.2), (P<0.01). MVD was graded into 4 groups: grade I as <20; grade II as 20–39; grade III as 40–59; grade IV as ≥60. In the recurrence group, the number of the grade I–IV cases were 4, 10, 7, 8 respectively, and these numbers were 25, 3, 2, 4 respectively in the other groups. The difference between the 2 groups was significant statistically (P<0.01).

The Expression of VEGF

VEGF was stained mainly in the cytoplasm of tumor cells and weakly expressed in tumor endothelial cells and hepatic sinusoid endothelial cells. The intensity of VEGF expression in the peri-tumor areas was higher than that of the tumor areas. The VEGF positive rate was 100% (63/63) in the peri-tumor areas, but only 65.1% (41/63) in the tumor areas. The VEGF positive rate of the recurrence group was 86.2% (25/29), and the other group was only 47.1% (16/34). The difference between the 2 groups was significant (P<0.01).

Other Clinico-pathological Indicators Had Prognostic Value

As shown in the Table 1, the stage of tumor, the positive rate of satellite nodules, the positive rate of portal vein embolus were significantly different between the 2 groups (P<0.05, P<0.05, P<0.01). But the presence of a tumor capsule, the degree of cirrhosis, the tumor differentiation and other indicators had no significant differences (P>0.05).

The Relationship between MVD and VEGF

The VEGF positive rate of the MVD grade I was 53.6% (15/28). It was evidently lower than that of the MVD grade II–IV (76.5%, 26/35). This result suggested that MVD might correlate to VEGF. But statistical analysis showed no significant correlation between them.

DISCUSSION

Much data confirmed that tumor angiogenesis is a crucial event in the growth of solids tumors. The activation of tumor angiogenesis factors may occur at the very early stage of tumor growth. Weidner et al. first reported that MVD is an independent prognostic indicator in primary breast cancer.[8] In addition, the prognostic value of MVD was also confirmed in other type of tumors including stomach, renal, colon, non-small cell lung, bladder, soft-tissue and germ cell tumors.[2,4,6,7]

Several bioassay tests have been developed for the quantitative analysis of tumor angiogenesis, and most of them do not give reliable results. In contrast, the immunohistochemical assay for endothelial antigens has allowed a reliable quantitative assessment of angiogenesis. Distribution of MVD was similar among different reports.[2,8]

Table 1. The clinico-pathological data of the two groups ($\bar{x}\pm s$)

Clinical data	Non-recurrence group (n=34)	Recurrence group (n=29)	*P*
Diameter (cm)	3.5±0.9	3.7±1.0	>0.05
Capsule			
No	15	12	>0.05
Have	19	17	
Cirrhosis			
No/small	21	13	>0.05
Large	13	16	
Differentiation			
II	16	14	>0.05
III–IV	18	15	
Tumor stage			
II	26	14	<0.05
III	8	15	
Satellite nodules			
No	30	19	<0.05
Have	4	10	
Portal embolus			
No	29	15	<0.01
Have	5	14	
MVD	22.7±28.2	49.6±29.7	<0.01
MVD grade			
I	25	4	
II	3	10	
III	2	7	<0.01
IV	4	8	
VEGF			
Negative	18	4	<0.01
Positive	16	25	

MVD is thought to be a marker representing the effects of angiogenesis activities. MVD not only is relevant to the oxygen and nutrition supply for proliferating tumor cells, but also represents the activity of invasion and metastasis. Newly generated microvessels are the first target of invasiveness and metastasis of tumor cells. The new microvessels are leaky and easy to be involved with tumor cells.[9] Therefor, it has been suggested that MVD might predict tumor recurrence and metastasis and act as a prognostic indicator.

In our study, the MVD of the recurrence group was significantly greater than that of the control group. The VEGF positive rate of the recurrence group was 86.2%, significantly higher than that of the non-recurrence group (47.1%, $P<0.01$). The total VEGF expression rate was 65.1%, which was similar to that of the breast cancer (50%). The stage of tumor, the positive rate of satellite nodules, the positive rate of portal vein embolus were significantly different between the 2 groups ($P<0.05$,

$P<0.05$, $P<0.01$). But the tumor capsule formation, the degree of cirrhosis, the tumor differentiation and other indicators had no significant differences ($P>0.05$). These results were similar to other reports. The results suggested that besides tumor stage, satellite nodules and portal vein embolus, the MVD and VEGF expression be also of prognostic significance.

The results showed that the lower MVD grade (grade I) had a lower VEGF positive rate (53.6%, 15/28), the higher MVD grade (grade II–IV) had a higher VEGF positive rate (76.5%, 26/35). This suggested that MVD might correlate to VEGF, but statistic analysis showed no significant correlation between them ($P>0.05$). This might be caused by two reasons: the first was that the angiogenesis of the PLC might depend on the other factors besides VEGF, the second might be it is related to the small amount of cases in this study. It still needs further study.

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