• LIVER CANCER •

Prognostic significance of preoperative circulating vascular endothelial growth factor messenger RNA expression in resectable hepatocellular carcinoma: A prospective study

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Received: 2003-10-15   **Accepted:** 2003-12-15

Abstract

AIM: To investigate the prognostic value of vascular endothelial growth factor messenger RNA (VEGF mRNA) in the peripheral blood (PB) of patients with hepatocellular carcinoma (HCC) undergoing curative resection.

METHODS: Using a reverse-transcription polymerase chain reaction (RT-PCR)-based assay, VEGF mRNA in the PB was determined prospectively in 50 controls and in 50 consecutive patients undergoing curative resection for HCC.

RESULTS: Among the isoforms of VEGF mRNA, $VEGF_{165}$ and $VEGF_{121}$ were expressed. By multivariate analysis, a higher level of $VEGF_{165}$ in preoperative PB correlated with a risk of HCC recurrence with borderline significance (P=0.050) and significantly with recurrence-related mortality (P=0.048); while $VEGF_{121}$ did not. Other significant predictors of HCC recurrence included cellular dedifferentiation (P=0.033), an absent or incomplete capsule (P=0.020), vascular permeation (P=0.018), and daughter nodules (P=0.006). The other significant parameter of recurrence related mortality was cellular dedifferentiation (P=0.053). The level of circulating VEGF mRNA, however, did not significantly correlate with tumor size, cellular differentiation, capsule, daughter nodules, vascular permeation, necrosis and hemorrhage of tumors.

CONCLUSION: The preoperative level of circulating VEGF mRNA, especially isoform $VEGF_{165}$, plays a significant role in the prediction of postoperative recurrence of HCC.

INTRODUCTION

Angiogenesis, known to be essential for the survival, growth, invasion, and metastasis of tumor cells, is a complex multistep process. There are extracellular matrix remodeling and binding of angiogenic factors to specific endothelial cell (EC) receptors, leading to EC proliferation, invasion of the basement membrane, migration, differentiation, and formation of new capillary tubes. Anastomosis of these new vessels develops into a vascular network[1,2].

Several factors with angiogenic activity have been identified, but one of the most potent, direct acting and specific is vascular endothelial growth factor (VEGF), also known as vascular permeability factor and vasculotropin[3].

Hepatocellular carcinoma (HCC), a leading cause of death in Taiwan and many Asian countries, is a highly vascular tumor dependent on neovascularization. Some authors have reported markedly elevated VEGF protein levels in HCC patients with remote metastases compared with those without metastasis, suggesting that VEGF may be a marker for metastasis in HCC[4-6]. Most such studies, however, depended on enzyme immunoassay to determine VEGF protein concentrations. To our knowledge, little is known about the prognostic significance of VEGF mRNA expression in the prediction of postresection recurrence of HCC. We conducted this prospective study to investigate the correlation between preoperative VEGF mRNA expression in peripheral blood (PB) and postoperative recurrence of HCC.

MATERIALS AND METHODS

Study population

From July 2001 to April 2003, 50 patients (31 men and 19 women, with a mean age of 56.2±13.3 yr) of 58 consecutive patients with HCC undergoing curative hepatectomy were enrolled in this prospective study. Patients who had previously had a hepatectomy or preoperative neoadjuvant ethanol injection or hepatic arterial chemoembolization (TACE) were excluded. The surgical procedures performed included 38 major resections (8 extended right lobectomies, 10 right lobectomies, 8 left lobectomies and 12 two-segmentectomies) and 12 minor resections (10 segmentectomies, 1 subsegmentectomy, and 1 wedge resection). A control group including 30 healthy volunteers without liver disease (15 men, 15 women, mean age 40 yr) and 20 patients with chronic liver disease but without evidence of HCC also gave PB samples.

PB samples for the detection of VEGF mRNA were obtained by forearm venipuncture one day prior to surgery from all 50 patients. After discharge, the patients were assessed regularly to detect tumor recurrence with abdominal ultrasonography (every 2-3 mo during the first 5 yr, then every 4-6 mo thereafter), serum alpha fetoprotein (AFP) and liver biochemistry (every 2 mo during the first 2 yr, then every 4 mo during the following 3 yr, and every 6 mo thereafter), abdominal computed tomography (CT) (every 6 mo during the first 5 yr, then annually), and chest X-ray and bone scans

(every 6 mo). Hepatic arteriography was obtained if the other studies suggested possible cancer recurrence. Detection of tumor on any imaging study was defined as recurrence.

Clinicopathological parameters analyzed included sex (male vs female), age, the presence of liver cirrhosis, hepatitis B virus (HBV) infection (hepatitis B surface antigen), hepatitis C virus (HCV) infection (anti-hepatitis C virus antibody), serum AFP level (<20 ng/mL vs 20 to 1 000 ng/mL vs >1 000 ng/mL), cirrhosis, Child-Pugh class of liver functional reserve (A vs B), tumor size (<3 cm vs 3 to 10 cm vs >10 cm), tumor encapsulation (complete vs incomplete or absent), presence of daughter nodules, vascular permeation (including vascular invasion and/or tumor thrombi in either the portal or hepatic vein), and cell differentiation grade (Edmondson and Steiner grades I to IV).

Detection of VEGF mRNA

Ethylenediamine tetraacetic acid (EDTA)-treated whole blood was centrifuged and the plasma fraction removed. The cellular fraction was enriched for mononuclear cells or possible tumor cells according to the method described by Oppenheim. Nucleated cells were isolated from peripheral blood using tetradecytrimethyl-ammonium bromide. Total cellular RNA was then extracted with PUREscript RNA Isolation Kits TRI-Zol (Life Technologies Inc., Gaithersburg, USA). cDNA was synthesized from 5 μg of the mRNA. The reverse transcription reaction solution contained 6 μL of 5×first strand buffer, 10 mmol/L dithiothreitol, 125 mmol/L each of dCTP, dATP, dGTP and dTTP, 0.3 μg of random hexamers, and 200 units of Superscriptase II Moloney murine leukemia virus reverse transcriptase (Life Technologies Inc.). The RNA solution was incubated at 95 ℃ for 10 min, quickly chilled on ice, then mixed with the reverse transcription reaction solution (total volume 20 μL), and incubated at 37 ℃ for 60 min. The sequences of the sense primers were 5'-AGTGTGTGCCCACTGAGGA-3' (VEGF) and 5'-AGTCAACGGATTTGGTCGTA-3' (GAPDH) and those of the antisense primers were 5'-AGTCAACGGATTTGGTCGTA-3' (VEGF) and 5'-GGAACATGTAAACCATGTAG-3' (GAPDH). The first polymerase chain reaction (RT-PCR) solution contained 5 μL of the synthesized cDNA solution, 10 μL of 10× polymerase reaction buffer, 500 mol/L each of dCTP, dATP, dGTP and dTTP, 15 pmol of each external primer (EX-sense and EX-antisense), 4 units of Thermus Brockiamus Prozyme DNA polymerase (PROtech Technology Ent. Co., Ltd. Taipei, Taiwan), and water. The PCR cycles were: denaturing at 94 ℃ for 1 min, annealing at 52 ℃ for 1 min, and primer extension at 72 ℃ for 1 min. The cycles were repeated 40 times. The PCR product was reamplified with internal primers for nested PCR to obtain a higher sensitivity. The first and second PCR components were the same, but for the primer pairs (IN-sense and IN-antisense), the final product was electrophoresed on 20g/L agarose gel and stained with ethidium bromide. Four different isoforms of human VEGF were identified, arising from alternative splicing of the primary transcript of a single gene. The majority were $VEGF_{121}$ (165 bp) and $VEGF_{165}$ (297 bp). The percentage intensity of the VEGF PCR fragment for each liver was relative to a GAPDH PCR fragment (122 bp). The intensity of bands was measured using Fujifilm Science Lab 98 (Image Gauge V3.12). The sensitivity of our assay was assessed using human hepatocytes.

A HepG2 (hepatoblastoma) cell line served as a positive control for VEGF mRNA expression. For negative controls, we used EDTA-treated water (filtered and vaporized).

Statistical analysis

A statistical software (SPSS for Windows, version 8.0, Chicago, Illinois) was employed, with Student's t-test used to analyze continuous variables and a chi-square or Fisher's exact test for categorical variables. Factors relating to the presence of postoperative hAFP mRNA in peripheral blood were analyzed by stepwise logistic regression. A Cox proportional hazards model was used for multivariate stepwise analysis to identify significant factors for predicting recurrence and mortality. Significance was taken as a P value <0.05.

RESULTS

RT-PCR analysis of VEGF transcript in peripheral blood

VEGF mRNA was expressed in the peripheral blood of 10 ($VEGF_{165}$ in 4 and $VEGF_{121}$ in 10) of 50 control patients (10/50, 20%). In the HCC group, isoform $VEGF_{165}$ was detected in 40 patients (80%) (with a concentration ranging from 0.198 to 0.7190) and isoform $VEGF_{121}$ in all 50 patients (100%) (concentration ranging from 0.2958 to 1.0356).

We did not detect isoforms $VEGF_{189}$ and/or $VEGF_{206}$ in either study or control patients

Table 1 Demographic, clinical and tumor variables of patients with HCC undergoing curative resection (n=50)

Variables		No. of patients (%)
Age (mean, years)		56.2±13
Male		31 (62)
Child- Pugh's class A		43 (86)
Serum AFP	<20 ng/mL	16 (32)
	20-10³ ng/mL	18 (36)
	>10³ ng/mL	14 (28)
HBsAg (+)		36 (72)
Anti-HCV (+)		13 (26)
Size of HCC	<3 cm	12 (24)
	3-10 cm	13(26)
	>10 cm	25(50)
Cirrhosis		40(80)
Edmondson-Steiner's	Grade I	4 (8)
	Grade II	12 (24)
	Grade III	18 (36)
	Grade IV	16 (32)
Complete capsule		19 (38)
Vascular permeation		29 (58)
Daughter nodules		31 (62)
Tumor necrosis		33(66)
Tumor hemorrhage		29(58)

AFP: serum alpha fetoprotein; HBsAg (+): positive hepatitis B surface antigen; Anti-HCV(+): positive hepatitis C virus antibody; Edmondson Steiner grade: differentiation grade.

Correlation of VEGF mRNA expression and tumor recurrence

Sixteen patients (32%) had clinically detectable recurrence during the follow up period (median 1.5 yr, range 1 to 2.5 yr), of whom 7 died. A high preoperative level of isoform $VEGF_{165}$ mRNA correlated significantly with tumor recurrence both univariately (P=0.021) and multivariately (P=0.050). Isoform $VEGF_{121}$ levels had no such correlation. By multivariate analysis, other significant predictors of recurrence included poor cellular differentiation (P=0.033), less encapsulation (P=0.020), more vascular permeation (P=0.018) and the presence of daughter nodules (P=0.006) (Table 2).

Correlation of VEGF mRNA expression and recurrence-related death

The preoperative level of isoform $VEGF_{165}$ in PB significantly correlated with death from recurrence both univariately

($P<0.001$) and multivariately ($P=0.048$). By multivariate analysis, a greater degree of vascular permeation significantly correlated with mortality ($P=0.045$), and poor cellular differentiation approached significance ($P=0.053$) (Table 3).

Table 2 Predictors of HCC recurrence

Variable	P values	
	UV	MV
Sex	0.895	-
Age	0.279	-
Size(<3 cm,>10 cm)	0.415	-
Liver cirrhosis	0.510	-
Child-Pugh class	0.528	-
Serum AFP	0.744	-
HBsAg (+)	0.280	-
Anti-HCV (+)	0.481	-
Edmondson Steiner grade	0.0005	0.033
Capsule	<0.0001	0.020
Vascular permeation	<0.0001	0.018
Daughter nodules	<0.0001	0.006
Tumor necrosis	0.344	-
Tumor hemorrhage	0.812	-
Serum VEGF$_{165}$ mRNA	0.0206	0.050
Serum VEGF$_{121}$ mRNA	0.520	-

UV: univariate analysis; MV: multivariate analysis; AFP: serum alpha fetoprotein; HbsAg(+): positive hepatitis B surface antigen; Anti-HCV(+): positive hepatitis C virus antibody; Edmondson Steiner grade: differentiation grade I, II *vs* III, IV; n.s: not significant.

Table 3 Correlation between clinical and tumor variables and recurrence-related mortality

Variables	P values	
	UV	MV
Sex	0.510	-
Age	0.440	-
Size (<3 cm, >10 cm)	0.519	-
Liver cirrhosis	0.510	-
Child-Pugh class	0.548	-
HBsAg (+)	0.351	-
Anti-HCV (+)	0.521	-
Edmondson Steiner grade	<0.001	0.053
Capsule	0.033	n.s.
Vascular permeation	<0.001	0.045
Daughter nodules	0.016	n.s.
Tumor necrosis	0.373	-
Tumor hemorrhage	0.306	-
Serum VEGF$_{165}$ mRNA	<0.001	0.048
Serum VEGF$_{121}$ mRNA	0.763	-

UV: univariate analysis; MV: multivariate analysis; AFP: serum alpha fetoprotein; HBsAg (+): positive hepatitis B surface antigen; Anti-HCV(+): positive hepatitis C virus antibody; Edmondson Steiner grade: differentiation grade I, II *vs* III, IV; n.s: not significant.

Correlation between VEGF mRNA expression and clinical and histopathologic features

There was no significant association between either isoform of VEGF mRNA and gender, age, serum AFP level, chronic HBV or HCV carriage, tumor size, coexisting cirrhosis, cellular differentiation, capsule, vascular permeation, daughter nodules, tumor necrosis, or tumor hemorrhage (all $P>0.05$).

DISCUSSION

Our study showed that a higher value of circulating VEGF mRNA isoform $_{165}$ before curative resection of HCC was significantly associated with an increased risk of postoperative recurrence and disease mortality. The presence of preoperative VEGF mRNA isoform $_{121}$ was not significantly predictive of outcomes.

The active form of VEGF was a homodimeric cytokine with molecular weight of 34-46 ku, the variation in size was due to alternative exon splicing which produces four different isoforms of 121, 165, 189 and 206 amino acids (monomeric size). The last three of those had heparin binding activity[15]. Different cancers have different expression of the isoforms. The majority of HCC could express an abundance of VEGF $_{121}$ and VEGF $_{165}$[4,7,8]. Further analysis by Ferrara indicated that VEGF$_{165}$ was the predominantly expressed form in human cDNA libraries as well as in most normal cells and tissues[8].

Several studies have revealed that the serum VEGF level is of value for predicting disease progression and prognosis in cancers of different origins, including the breast, gastrointestinal organs, kidney, urothelium, ovary, lung, and lymphoma[9-18]. However, the serum level of VEGF, which is what most other investigators have measured, might be influenced by other factors such as platelet number, associated liver cirrhosis, or coexisting infection[19-22]. For accurate measurement, citrated plasma processed within 1 h of venipuncture is better than serum.

Measuring VEGF in HCC tissue has the disadvantage of not being available until after a biopsy or resection has been done. The majority of angiogenic factors are soluble, diffusible peptides. Hence, the circulating level of angiogenic factors theoretically reflects the angiogenic activity of the tumor. Compared with expression in tumor tissue, the advantage of the measurement of circulating VEGF expression is that it can be performed without tissue specimens and repeated serially. Jinno *et al*, believed that circulating VEGF might be derived mainly from a large burden of tumor cells, but also partly from platelets activated by vascular invasion of HCC cells[20].

We chose to measure circulating mRNA expression of VEGF rather than the protein itself. According to El-Assal's study, the level of VEGF mRNA did not always correlate with the protein concentration[23]. Immunohistochemistry could not distinguish small amounts of protein, which may partly explain the discrepancy in protein and mRNA levels.

An additional question is whether the value of circulating VEGF (either protein or mRNA) corresponds with VEGF mRNA in HCC tissue. Tokunaga noted that the circulating VEGF mRNA isoform pattern in colon cancer was not always significantly correlated with the gene expression level. The release of VEGF mRNA might be influenced by some cells other than HCC cells[24]. Warren found VEGF mRNA in hepatocytes and some Kupffer cells[25]. According to Banks, the presence of mRNA for VEGF was also described in T lymphocytes, CD34* cells, and monocytes[19,21].

There are considerable discrepancies among reports about the clinical significance of VEGF expression in HCC[20,26-34]. The high recurrence rate after resection has been found to be the main determinant for the poor outcome of HCC[35-37]. Tumor invasiveness variables correlating with recurrence include high serum AFP, hepatitis, vascular permeation, the grade of cellular differentiation, infiltration or absence of capsule, tumor size, coexisting cirrhosis, the presence of daughter nodules, and multiple lesions. Therefore, a number of studies have been done to see if VEGF correlated with any or all of those factors.

Zhou showed that high VEGF expression in HCC was associated with portal vein tumor thrombosis[28]. Li reported that VEGF mRNA in HCC correlated significantly with portal

vein emboli, poorly encapsulated tumors, and microvascular density in HCC tissues[27]. Chow found that VEGF expression was significantly associated with the PCNA index and sonographic evidence of portal vein tumor thrombosis but not with the liver biochemical profile, tumor volume, gender, severity of liver disease, or tumor grading[26].

One possible explanation for the discrepancies may be the assessment of tumors of different sizes and etiologies. The number of study patients is another possible factor. Because most of the reported investigations were performed in small series, we used 50 patients which seemed an adequate sample size compared with other studies. It is also likely that the effect of VEGF on angiogenesis depends on not only tumor cell expression of VEGF, but also on the VEGF receptors in endothelial cells.

Some have reported a correlation between increased plasma VEGF protein level in HCC patients and tumor size, number, portal vein emboli, poorly encapsulated tumors, microscopic venous invasion, metastasis, and recurrence. Yamamoto found there was a positive correlation between the increment of intratumoral MVD and serum VEGF concentrations[38].

According to our study, a higher expression of circulating VEGF mRNA was significantly correlated with tumor recurrence and recurrence-related mortality but not with the other accepted measures of invasiveness. Circulating VEGF mRNA thus appears to be an independent risk factor of postoperative recurrence. There are several possible explanations for this dissociation.

Some studies have found that the histologic grade of HCC was associated with VEGF expression in noncancerous liver cells, suggesting a complex regulatory mechanism for circulating VEGF in liver disease. Coexisting liver cirrhosis might influence VEGF expression[22,23]. About 80% of our study patients had cirrhosis. Some investigators have found that VEGF expression was significantly higher in cirrhotic livers than in noncirrhotic livers. Furthermore, it has been shown that cirrhosis itself was associated with increased angiogenic activity. El-Assal et al observed that cirrhotic livers had significantly higher VEGF expressions than noncirrhotic livers, suggesting that VEGF might be associated with angiogenesis in cirrhosis[23]. In addition, some suggested a possible involvement of VEGF in angiogenesis of the cirrhotic liver but not in angiogenesis of HCC. Akiyoshi suggested that a low serum VEGF level in liver cirrhosis might reflect the degree of liver dysfunction and might be associated with the grade of hepatocyte regeneration[22], VEGF levels were decreased with the worsening of the Child-Pugh score. However, most of our patients were Child-Pugh class A, and their resectable lesions, were unlike those studied by Akyoshi.

The stage of cancer might influence the VEGF expression[38-41]. Chao showed that a lower range of circulating VEGF levels in patients with early-stage HCC overlapped considerably with those in normal controls or in patients with chronic hepatitis or cirrhosis[30]. Therefore, serum VEGF is probably not useful for early detection of HCC. In contrast, a large quantity of VEGF may be released by the large load of HCC cells in advanced diesease (stage VI B). Because plasma VEGF level is significantly higher in stage IVB than in stage IVA, other mechanisms may be responsible, such as agglutination and activation of platelets caused by vascular invasion and circulating tumor cells. It may thus be the platelets rather than or in addition to HCC cells that are responsible for the release of VEGF into circulation. Our patients did not have such an advanced disease.

The relation between tumor size and VEGF mRNA expression might be complex and dynamic because of different vascular growth patterns[5,34,42-44]. In small hypervascular HCCs, approximately 1.0 cm in diameter in those that grow with a pattern of vessel replacement, artery-like vessels are not well developed. Capillarization of the blood spaces is present but in an incomplete form, and portal tracts often appear within cancerous nodules. These HCCs are thought to receive a predominantly portal blood supply. As tumor size increases, portal tracts decrease in number, and artery-like vessels gradually increase in number and size. Well-differentiated HCCs measuring 1.0 to 1.5 cm in diameter are in a transitional stage from portal to arterial blood supply, with the reduction in portal flow preceding the increase in arterial flow. Therefore, blood flow in HCC at this point would be low. This may be the reason why many well-differentiated HCCs are not detected on angiography, with hypervascularity seen untill nodules become larger than 2 cm in diameter.

VEGF positivity may therefore gradually decrease with increasing tumor size. According to Yamaguchi, 36.8% of nodules larger than 3.0 cm were VEGF-negative[34]. El-Assal showed that, contrary to the usual angiographic findings, HCCs larger than 5 cm in diameter were not more vascular than smaller tumors and were less vascular than medium-sized lesions[23]. However, it has been reported that the intercapillary distance increased as the tumors size or weight increased, which may be caused by the significantly different rates of endothelial (50 to 60 h) and neoplastic cell (22 h) turnover.

These complicated changes in vascularity may account for the disparate results among reported studies. Suzuki reported that VEGF mRNA levels were not correlated with the vascularity of HCCs as seen on angiography[4]. On the contrary, Mise et al. showed that the degree of VEGF mRNA expression was significantly correlated with the intensity of tumor staining in angiograms ($P<0.01$)[5]. Some have reported higher VEGF expression in small-size and well-differentiated HCCs and suggested that VEGF played its most important role in a relatively early stage of angiogenesis. In general, advanced HCCs are mainly supplied with arterial blood, and arterial angiography may reveal hypervascularity. Serum VEGF concentrations have been reported to be significantly higher in advanced stage rather than in early stage of breast and gastric cancer[9,13,14].

Neovascularization appears to be one of the crucial steps in a tumor's transition from a small, harmless cluster of mutated cells to a large, malignant growth, capable of spreading to other organs throughout the body. Because of the complex nature of the angiogenic process, however, it seems that VEGF expression is not the sole contributor to angiogenesis in HCC. Other factors involved in this process may include TGF-β, TNF-α, IL-8, etc.

It has been reported recently that VEGF mRNA expression was readily induced by hypoxia or ischemia. This is why we excluded patients who received preoperative TACE, in order to avoid ischemia-induced changes in VEGF expression.

Although tumor necrosis was present in 66% of our study patients (Table 1), circulating VEGF mRNA did not statistically correlate with it. We also did not find any correlation with fibrous capsule or septum formation, in contrast to the findings of Inoue et al[12]. The origin of the capsule and fibrous septa in HCC is unclear. Nakashima et al suggested the possibility of fibrogenesis at the interface of two tumor nodules of different properties, a process requiring fibrin deposition in the initial stage[45]. Some authors stated that a capsule or septa was formed when the HCC nodule grew to 1.5 cm or larger. It has been suggested that capsule formation is a result of compression and collagenization of the adjacent stroma. However, this mechanism has been doubted, since the tumor size did not correlate with the thickness of the capsule or the incidence of its formation. This suggests that capsules are formed by active fibrosis rather than by tumor compression on the adjacent stroma.

According to our study, circulating VEGF mRNA did not significantly correlate with the grade of cellular differentiation.

We attributed this to the possibility of different histological grades coexisting in HCC tissues. Yamaguchi examined VEGF expression immunohistochemically in HCC of various histological grades and sizes[34]. In tumors composed of a single histological grade, VEGF expression was the highest in well-differentiated, followed by moderately differentiated, and then poorly differentiated HCC. In tumors consisting of cancerous tissues of two different histological grades, the expression was less intense in the higher-grade HCC component. VEGF was also expressed in the surrounding HCC tissues in which inflammatory cell infiltration was apparent. Based on these findings, VEGF expression in HCC tissues was thought to be partly related to the histological grade but other cytokines and growth factors could also cooperatively act to enhance or influence VEGF expressions in HCC.

Solid tumors such as HCC are composed of two distinct compartments, namely the malignant cells themselves and the vascular and connective tissue stroma. The stroma provides the vascular supply that tumors require for obtaining nutrients, gas exchange, and waste disposal. Fibrin serves as a provisional stroma that is gradually replaced by granulation tissue and then by mature stroma. Human HCC is commonly surrounded by a fibrous capsule with an abundant extracellular matrix, even in the early stage. It is possible that VEGF also plays a certain role in the stimulation of regional development of the stroma in HCC.

Angiogenesis also appears to be involved in the invasion of tumors into the surrounding tissues, because this invasion requires concomitant neovascularization through the sprouting of endothelial cells in the extracellular matrix. It has been reported that VEGF induced both urokinase-type and tissue-type plasmin in endothelial cells. These are the key proteases involved in the degradation of the extracellular matrix. Thus VEGF may promote the process of vascular invasion by HCC cells.

Some authors suggested that VEGF mRNA expression in PB, which correlates well with the shift of VEGF mRNA in liver tissue, was strongly related to the development of HCC, including the progression from preneoplastic to neoplastic tissue and the potential of post-resection recurrence, the invasiveness of HCC, and poor survival[42-44]. Some stated that serum VEGF was a predictor of invasion and metastasis of HCC and a potential biomarker of metastatic recurrence after curative resection[20].

Surgery remains the best potentially curative treatment for patients with HCC. High recurrence rate limits the long term survival. Examination of preoperative VEGF mRNA in PB expression may give us information about high risk of postoperative recurrence. Addition of neoadjuvant or antiangiogenic therapy before or after surgery may be considered for such patients. Furthermore, the serial measurement of circulating VEGF mRNA during postoperative follow-up to monitor the effect of therapy or the development of recurrence needs further investigation.

From this prospective study, we suggest that circulating VEGF mRNA expression, especially isoform $VEGF_{165}$, may play a significant role in the prediction of postresection recurrence of HCC.

ACKNOWLEDGEMENT

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Edited by Wang XL **Proofread by** Zhu LH