Is the vascular endothelial growth factor messenger RNA expression in resectable hepatocellular carcinoma of prognostic value after resection?

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INTRODUCTION
Angiogenesis, the establishment of a neovascular blood supply from preexisting blood vessels, is a complex multistep process. The process may include the extravascular matrix remodeling and the 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 and developing into a vascular network[9,10].

One of the most potent, direct acting, and specific factors with angiogenic activity is vascular endothelial growth factor (VEGF)[11,12].

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 suggested that VEGF may be a marker for metastasis in HCC because they found markedly elevated VEGF protein levels in HCC patients with remote metastases compared with those without metastasis[13-15]. However, most such studies determined VEGF protein concentrations by enzyme immunoassay. To our knowledge, in the prediction of postresection recurrence, little is known about the prognostic significance of VEGF mRNA expression in tumor tissues. We conducted this prospective study to investigate the correlation between VEGF mRNA expression in HCC tissues and postoperative recurrence of HCC.

MATERIALS AND METHODS

Study population
Fifty 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 from July 2001 to April 2003, 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. 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 subsegmentectomies, and 1 wedge resection). HCC tissues were obtained from all 50 patients after resection. A control group including 10 healthy volunteers without liver disease (5 men, 5 women, mean age 40 yr) and 20 patients with chronic liver disease but without evidence of HCC also received liver biopsy during laparotomy on them for other reasons. All these HCC tissues and liver biopsy tissues (from control group patients) were examined for VEGF mRNA.

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 clinical recurrence.

Clinicopathological variables analyzed included age, sex (male vs female), the presence of liver cirrhosis, Child-Pugh class of liver functional reserve (A vs B), 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), 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
It included extraction of RNA, reverse transcription and amplification of cDNA of VEGF and GAPDH by PCR.

VEGF mRNA of liver tissue
Extraction of RNA
We homogenized resected tissues completely in 1 mL of RNA–bee3M, and added 0.2 mL chloroform and shocked vigorously for 15-30 s. We stored the sample on ice for 5 min and centrifuged it at 12 000 g for 15 min. We transferred the supernatant to a new 1.5 mL eppendorf tube and precipitated it with 0.5 mL of isopropanol. Precipitation could be as short as 5 min at 4 °C. We incubated it at 70 °C for 2 mimutes, chilled it to 23 °C, and carefully removed ethanol. We removed the supernate and dissolved RNA in DEPC-H2O (usally between 50-100 µL) and store at -80 °C.

Reverse transcription
We heated the RNA sample at 55 °C for 10 minutes and chilled it on ice. We added the following components: (1) 4 µL 5×RT butter containing 50 mmol/L Tris-HCl (pH 8.3), 75 mmol/L KCl, 3 mmol/L MgCl2, and 10 mmol/L DTT (dithiothreitol), (2) 3 µL 10 mmol/L dNTP, (3) 1.6 µL Oligo-d(T)n, and 0.4 µL random hexamers (N6) (1 µg/µL), (4) 0.5 µL RNase inhibitor (40 units/µL), (5) 3 µL 25 mmol/L MnCl2, (6) 6 µL RNA in DEPC-H2O, (7) 0.5 µL DEPC-H2O. We incubated it at 70 °C for 2 minutes, chilled it to 23 °C to anneal primers to RNA. We added 1 µL of M-MLV RTase (moleny murine leukemia virus reverse transcriptase, 200 units/µL, Promega). We incubated it at 80 min at 40 °C. We heated the reaction at 94 °C for 5 min, chilled it on ice and stored cDNA at -20 °C.

Amplification of cDNA of VEGF and GAPDH by PCR
The sequences of the sense primers were 5'-AGTGTGTGCCCA CTGAGGA-3' (VEGF) and 5'-AGTCAACGGATTTGGTCGTA-3' (GAPDH) and those of the antisense primers were 5'-AGTCAACGGATTTGGTCGTA-3' (VEGF) and 5'-GGAACATGTCAAACATCGTAG-3' (GAPDH). The first polymerase chain reaction (RT-PCR) solution contained 5 µL of the synthesized cDNA solution, 10 µL of 10x 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 °C for 1 min, annealing at 52 °C for 1 min, and primer extension at 72 °C 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 20% 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 VEGF121 (165 bp) and VEGF165 (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 hepatoblastoma cell line (HepG2) 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. Parameters 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 the significant variables for predicting recurrence and mortality. Significance was taken as a P value <0.05.

RESULTS
RT-PCR analysis of VEGF transcript in liver tissues
VEGF mRNA was expressed in the liver tissues of 10 (VEGF165 in 10 and VEGF121 in 6) out of 30 control patients. In the HCC group, isoform VEGF165 was detected in all the 50 patients (100%) (with a concentration ranging from 0.1860 to 0.7240) and isoform VEGF125 in 40 patients (80%) (with a concentration ranging from 0.2549 to 1.0298).

We did not detect isoforms VEGF125 in HCC tissues but VEGF206 was detected in both HCC tissues or control liver tissues.

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

| Variables       | No. of patients (%) |
|-----------------|---------------------|
| Age             | 56.2±13             |
| Male            | 31 (62)             |
| Cirrhosis       | 40 (80)             |
| Child- Pugh’s dA | 43 (86)             |
| Serum AFP       |                     |
| <20 ng/mL       | 16 (32)             |
| 20-100 ng/mL    | 18 (36)             |
| >100 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)             |
| Edmondson-Stener’s grade I | 4 (8) |
| Grade II        | 12 (24)             |
| Grade III       | 18 (36)             |
| Grade IV        | 16 (32)             |
| Absent or incomplete capsule | 31 (62) |
| 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 clinical recurrence

During the follow up period (median 1.5 yr, range 1 to 2.5 yr), 16 patients (32%) had clinically detectable recurrence, of whom 7 died. A higher level of isoform VEGF<sub>165</sub> mRNA in HCC tissue correlated significantly with clinical recurrence both univariately (P=0.022) and multivariately (P=0.038). Isoform VEGF<sub>165</sub> 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).

### Table 2 Predictors of HCC recurrence

| Variable                        | 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  | -      |
| HBAg (+)                        | 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  | -      |
| Tissue VEGF<sub>165</sub> mRNA | 0.022  | 0.038  |
| Tissue VEGF<sub>121</sub> mRNA  | 0.622  | -      |

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

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

| Parameters                        | UV     | MV     |
|-----------------------------------|--------|--------|
| Sex                               | 0.510  | -      |
| Age                               | 0.440  | -      |
| Size (<3 cm, >10 cm)              | 0.519  | -      |
| Liver cirrhosis                   | 0.510  | -      |
| Child-Pugh class                  | 0.548  | -      |
| HBAg (+)                          | 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  | -      |
| Tissue VEGF<sub>165</sub> mRNA   | 0.018  | 0.045  |
| Tissue VEGF<sub>121</sub> mRNA    | 0.744  | -      |

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

Correlation of VEGF mRNA expression and recurrence-related death

The level of isoform VEGF<sub>165</sub> in HCC tissue significantly correlated with death due to recurrence both univariately (P=0.018) and multivariately (P=0.045). 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).

Correlation between VEGF mRNA expression in HCC tissues and clinical and histopathologic features

There was no significant association between 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 (P>0.05).

**DISCUSSION**

Our study revealed that a higher value of VEGF mRNA isoform VEGF<sub>165</sub> in resected HCC tissues was significantly associated with an increased risk of postoperative recurrence and disease mortality. The value of VEGF mRNA isoform VEGF<sub>121</sub> in HCC tissues was not significantly predictive of the outcome.

VEGF is also known as a vascular permeability factor and vascular endothelial growth factor. Its active form is a homodimeric cytokine with molecular weight 34-46 ku. The variation in size due to alternative exon splicing might produce four different isoforms of 121, 165, 189 and 206 amino acids (monomeric size). The last had heparin binding activity. Different cancers might have different expression of the isoforms. The majority of HCC expressed an abundance of VEGF<sub>121</sub> and VEGF<sub>165</sub>. According to Ferrara’s finding, VEGF<sub>165</sub> was the predominantly expressed form in human cDNA libraries as well as in most normal cells and tissues.

Some authors have shown that the VEGF level in serum or in tissue is of value for predicting disease progression and prognosis in different cancers, such as the gastrointestinal origins, breast, lung, urothelium, ovary, and lymphoma. Compared with expression in tumor tissue, the advantage of measurement of serum VEGF level is that it can be performed without tissue specimens and repeated, but it may be influenced by some factors such as coexisting liver cirrhosis, associated infection and platelet activation.

In addition, the expression of VEGF mRNA in serum might not always correlate significantly with the gene expression level of tumors. Therefore, we used liver tissue instead of serum in this study. Warren found VEGF mRNA in hepatocytes and in some Kupffer cells. However, release of VEGF mRNA might also be influenced by some cells other than HCC cells. The presence of mRNA for VEGF has also been described in T lymphocytes, CD34<sup>+</sup> cells, and monocytes.

For more accuracy, we chose to measure mRNA expression of VEGF in liver tissue rather than the protein itself. The level of VEGF mRNA did not always correlate with the protein concentration. Immunohistochemistry could not distinguish small amounts of protein, which may partly explain the discrepancy in protein and mRNA levels.

The high recurrence rate after resection is the main determinant for the poor outcome of HCC. Tumor invasiveness variables correlated with recurrence include high serum AFP, hepatitis, vascular permeation, grade of cellular differentiation, infiltration or absence of capsule, tumor size, coexisting cirrhosis, 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.

Among reports about the clinical significance of VEGF...
Coexisting liver cirrhosis may influence VEGF expression. Overlapped considerably with those of normal controls or patients with chronic hepatitis or cirrhosis, lower range of VEGF levels in patients with early-stage HCC be significantly higher in advanced rather than early stages of expression. The stage of cancer might also influence VEGF. TGF-β, TNF-α, IL-8, etc.

About 80% of our study patients had cirrhosis. Some investigators have found that VEGF expression was significantly higher in cirrhotic liver than in noncirrhotic liver. Furthermore, it has been shown that cirrhosis itself was associated with increased angiogenic activity. According to El-Assal, cirrhotic livers had significantly higher VEGF expressions than noncirrhotic livers[32]. In addition, some suggested a possible involvement of VEGF in angiogenesis of cirrhotic liver but not in angiogenesis of HCC[31,32]. Akiyoshi suggested that a low serum VEGF level in liver cirrhosis might reflect the degree of liver dysfunction and be associated with the grade of hepatocyte regeneration and VEGF levels decreased with the worsening of Child-Pugh score[31]. Whereas, most of our patients belonged to Child-Pugh class A, with resectable lesions, unlike those studied by Akiyoshi.

According to the cell differentiation, the regulation of VEGF may be complex. In our study, VEGF mRNA did not significantly correlate with the grade of cell differentiation. We attribute this to the possibility of different histological grades coexisting in some HCC tissues. Yamaguchi examined VEGF expression immunohistochemically in HCC with various histological grades and sizes[49]. 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.

We also found no correlation between VEGF and the absence or presence of fibrous capsule or septum formation, which was in contrast to the findings of Suzuki et al.[31]. 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 with different properties, a process requiring fibrin deposition in the initial stage when the HCC nodule grows to 1.5 cm or larger[52]. 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.

In our study, a higher level of VEGF mRNA in tumor tissue correlated with more postresection recurrences. We attribute it to two possible mechanisms. One is that the higher angiogenesis may have more invasive nature of cancer to spread into the surrounding tissues. This invasion requires concomitant neovascularization through the sprouting of endothelial cells and neoplastic cells. 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[7,13]. On the contrary, Mise et al. showed that the degree of VEGF mRNA expression was significantly correlated with the intratumor staining in angiograms (P<0.01)[14]. 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.

The stage of cancer might also influence VEGF expression[13,35]. VEGF concentrations have been reported to be significantly higher in advanced rather than early stages of breast, colon and gastric cancer[16-18,21]. Chao showed that a lower range of VEGF levels in patients with early-stage HCC overlapped considerably with those of normal controls or patients with chronic hepatitis or cirrhosis[49].

Coexisting liver cirrhosis may influence VEGF expression.
VEGF, in HCC tissues may play a significant role in the prediction of postresection recurrence of HCC.

ACKNOWLEDGEMENT
This study was supported by grants from the Department of Medical Research, Mackay Memorial Hospital, Taiwan (MMH 9237).

REFERENCES

1. Zetter BR. Angiogenesis and tumor metastasis. Ann Rev Med 1998; 49: 407-424
2. Skobe M, Rockwell P, Goldstein N, Vosseler S, Fusieng NE. Halting angiogenesis suppresses carcinoma cell invasion. Nat Med 1997; 3: 1222-1227
3. Marme D. Tumor angiogenesis: the pivotal role of vascular endothelial growth factor. World J Urol 1996; 14: 166-174
4. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med 1995; 1: 27-31
5. Folkman J. Endothelial cells and angiogenic growth factors in cancer growth and metastasis. Cancer Metastasis Rev 1990; 9: 171-174
6. Liotta LF, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. Cell 1991; 64: 327-336
7. Fidler IJ, Ellis LM. The implications of angiogenesis for the biology and therapy of cancer metastasis. Cell 1994; 79: 185-188
8. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell 1996; 86: 353-364
9. Fox SB, Gatter KC, Harris A. Tumour angiogenesis. J Pathol 1996; 179: 232-237
10. Dvorak HF, Brown LF, Detmar M, Dvorak AM. Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. Am J Pathol 1995; 146: 1029-1039
11. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: identification of a fourth molecular species and characterization molecular species and characterization of alternative splicing of RNA. Mol Endocrinol 1991; 5: 1806-1814
12. Ferrara N, Houck K, Jakeman L, Leung DW. Molecular and biological properties of the vascular endothelial growth factor family of proteins. Endocrinol Rev 1992; 13: 18-32
13. Suzuki K, Hayashi N, Miyamoto Y, Yamamoto M, Ohkawa K, Ito Y, Sasaki Y, Yamauchi Y, Nakase K, Noda K, Enomoto M, Araki K, Yamada Y, Yoshihara H, Tujimura T, Kawano K, Yoshikawa K, Kamada T. Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res 1996; 56: 3004-3009
14. Mise M, Arii S, Higashitani H, Furutani M, Niwano M, Harada T, Ishigami T, Toda Y, Nakayama H, Fukumoto M, Fujita J, Imanuma M. Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. Hepatology 1996; 23: 455-461
15. Miura H, Miyazaki T, Kuroda M, Oka T, Machimani R, Kodama K, Ishibuya M, Makuuchi M, Yamauchi T, Yamanaka K, Kato K, Enomoto M, Araki K, Yamada Y, Yoshihara H, Tujimura T, Kawano K, Yoshikawa K, Kamada T. Expression of vascular permeability factor/vascular endothelial growth factor in human hepatocellular carcinoma. Cancer Res 1996; 56: 3004-3009
16. Brown LF, Berse B, Jackman RW, Tognazzi K, Guidi A, Dvorak HF, Sanger DR, Connolly JL, Schnitt SJ. Expression of vascular endothelial permeability factor (vascular endothelial growth factor) and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995; 55: 3964-3968
17. Takahashi Y, Kitada Y, Bucan CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. Cancer Res 1995; 55: 3964-3968
18. Inoue K, Ozeki Y, Suganuma T, Sugiyama Y, Tanaka S. Vascular endothelial growth factor expression in primary esophageal squamous cell carcinoma: association with angiogenesis and tumor progression. Cancer 1997; 79: 206-213
19. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, Dvorak HF. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptor in adenocarcinomas of the gastrointestinal tract. Cancer Res 1993; 53: 4772-4779
20. Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Sowa M. Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. Cancer 1996; 77: 858-863
21. Imoto H, Osaki T, Taka S, Ogami A, Ichiyoshi Y, Yasumoto K. Vascular endothelial growth factor expression in non-small-cell lung cancer: prognostic significance in squamous cell carcinoma. J Thorac Cardiovasc Surg 1995; 111: 1007-1014
22. Salven P, Ruotsalainen T, Mattsson K, Joensuu H. High pre-treatment serum level of vascular endothelial growth factor (VEGF) is associated with poor outcome in small-cell lung cancer. Int J Cancer 1996; 79: 314-317
23. Miyake H, Haru I, Yamana K, Gohi K, Arakawa S, Kamidono S. Evaluation of serum level of vascular endothelial growth factor as a new predictor of recurrence and disease progression in patients with superficial urothelial cancer. Urology 1999; 53: 302-307
24. Tempfer C, Obrmair A, Hefler L, Haeusler G, Giltsch G, Kaicz V. Vascular endothelial growth factor serum concentrations in ova-rin cancer. Ostl Genyrol 1998; 90: 360-363
25. Salven P, Aihinen H. A high pretreatment serum vascular endothelial growth factor concentration is associated with poor outcome in non-Hodgkin's lymphoma. Blood 1999; 90: 3167-3172
26. Banks RE, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, Selby PJ. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: bearing human hepatocellular carcinoma. Br J Cancer 1998; 77: 956-964
27. Jinno K, Taniimizu M, Hyodo I, Nishikawa Y, Hosokawa Y, Doi T, Endo H, Yamashita T, Okada Y. Circulating vascular endothelial growth factor (VEGF) is a possible tumor marker for metastasis in human hepatocellular carcinoma. J Gastroenterol 1998; 33: 376-382
28. Wartiovaara U, Salven P, Mikkola H, Lassila R, Kaakkoenen J, Joukov V, Orpana A, Ristimaki A, Heikkinheimo M, Joensuu H, Altaloto K, Palotie A. Peripheral blood platelet levels express VEGF-C and VEGF which are released during platelet activation. Thromb Haemost 1998; 80: 171-175
29. Banks RE, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, Selby PJ. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. Br J Cancer 1998; 77: 956-964
30. Akiyoshi F, Sata M, Suzuki H, Uchimura Y, Mitsuayama K, Matsuo K, Tanikawa K. Serum vascular endothelial growth factor levels in various liver diseases. Dig Dis Sci 1998; 43: 41-45
31. El-Assad ON, Yamano A, Suda Y, Yamaguchi M, Igarashi M, Yamamoto A, Nakiba T, Nagasue N. Clinical significance of microvesseled density and vascular endothelial growth factor expression in hepatocellular carcinoma and surrounding liver: possible involvement of vascular endothelial growth factor in the angiogenesis of chrothic liver. Hepatology 1998; 27: 1554-1562
32. Tokunaga T, Oshika Y, Abe Y, Ozeki Y, Sadehoro S, Kijima H, Tsuchida T, Yamazaki H, Ueyama Y, Tamaoki N, Nakamura M. Vascular endothelial growth factor (VEGF) mRNA isoform expression pattern is correlated with liver metastasis and poor prognosis in colon cancer. Br J Cancer 1997; 76: 1007-1011
33. Warren RS, Yuan H, Matti MR, Gillett NA, Ferrara N. Regulation by vascular endothelial growth factor of human colon cancer tumorigenesis in a mouse model of experimental liver metastasis. J Clin Invest 1995; 95: 1789-1797
34. Poon RT, Fan ST, Lo CM, Liu CL, Wong J. Intrahepatic recurrence after curative resection of hepatocellular carcinoma. Long-term results of treatment and prognostic factors. Ann Surg 1999; 229: 216-222
35. Jeng KS, Chen BF, Lin HJ. En bloc resection for extensive hepatocellular carcinoma. Is it advisable? World J Surg 1994; 18: 834-849
36. Jeng KS, Sheen IS, Chen BF, Wu YJ. Is the p53 gene mutation of prognostic value in hepatocellular carcinoma after resection? Arch Surg 2000; 135: 1329-1333
37. Yamamoto J, Kosuge T, Takayama T, Shimada K, Yamazaki S,
Ozaki H, Yamaguchi N, Makuuchi M. Recurrence of hepatocellular carcinoma after surgery. Br J Surg 1996; 83: 1219-1222

Jeng JS, Sheen IS, Tsai YC. Gamma glutamyl transpeptidase messenger RNA may serve as a diagnostic aid in hepatocellular carcinoma. Br J Surg 1996; 83: 1219-1222

Ng IO, Lai EC, Fan ST, Ng MM, So MK. Prognostic significance of pathologic features of hepatocellular carcinoma. A multivariate analysis of 278 patients. Cancer 1995; 76: 2443-2448

Chow NH, Hsu PI, Lin XZ, Yang HB, Chan SH, Cheng KS, Huang SM, Su IJ. Expression of vascular endothelial growth factor in normal liver and hepatocellular carcinoma: an immunohistochemical study. Hum Pathol 1997; 28: 698-703

Qin LX, Tang ZY. The prognostic molecular markers in hepatocellular carcinoma. World J Gastroenterol 2002; 8: 385-392

Chao Y, Li CP, Chau GY, Chen CP, King KL, Lui WY, Yen SH, Chang FY, Chan WK, Lee SD. Prognostic significance of vascular endothelial growth factor mRNA expression in invasion and metastasis of hepatocellular carcinoma. J Exp Clin Cancer Res 1998; 17: 13-17

Zhou J, Tang ZY, Fan J, Wu QZ, Li XM, Liu YK, Liu F, Sun HC, Ye SL. Expression of platelet-derived endothelial cell growth factor and vascular endothelial growth factor in hepatocellular carcinoma and portal vein tumor thrombus. J Cancer Res Clin Oncol 2000; 126: 57-61

Terada T, Nakamura Y. Arterial elements and perisinusoidal cells in borderline hepatocellular nodules and small hepatocellular carcinomas. Histopathology 1995; 27: 333-339

Nakashima O. Pathological diagnosis of hepatocellular carcinoma. Nippon Rinsho 2003; 59(Suppl 6): 333-342

Denekamp J. Angiogenesis, neovascular proliferation and vascular pathophysiology as targets for cancer therapy. Br J Radiol 1993; 66: 186-196

Edited by Wang XL  Proofread by Zhu LH