Relationship between the Expression of VEGF, Flk-1 and Flt-1 Proteins and Clinicopathology in Hepatocellular Carcinoma

Jihui Hao
Huikai Li
Yu Qin
Qiang Li
Dianchang Wang
Xishan Hao

Department of Abdominal Surgery, Tianjin Medical University Cancer Hospital, Tianjin 300060, China.

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OBJECTIVE To study the relationship between the expression of VEGF, Flk-1 and Flt-1 proteins and clinical pathology in hepatocellular carcinoma.

METHODS The expression of VEGF, Flk-1 and Flt-1 proteins in hepatocellular carcinomas from 60 patients was determined by immunohistochemistry (ABC method) and VEGF expression in relation to the clinicopathology evaluated.

RESULTS The positive rates of VEGF, Flk-1 and Flt-1 protein expression were 81.3%, 88.3%, 80.0% in tumor tissues, respectively, rates which were significantly higher than those in normal liver tissue ($P<0.05$). The expression of VEGF protein was correlated with the histologic grade and metastases of the tumors.

CONCLUSION The results showed that, in hepatocellular carcinoma, a higher expression of VEGF protein was associated with a higher degree of malignancy and a greater tendency for metastases. VEGF, Flk-1 and Flt-1 play an important role in tumourgenesis.

KEYWORDS: hepatocellular carcinoma (HCC), VEGF, Flk-1, Flt-1, Clinicopathology.

Vascular endothelial growth factor (VEGF), the most potent angiogenic factor so far detected, has been shown to be highly specific for endothelial cells in vitro and in vivo. It promotes endothelial cell proliferation and increases vascular permeability. VEGF is a 38-46 kDa heparin-binding, homodimeric glycoprotein that shares sequence homologies with platelet-derived growth factor. Four isoforms of VEGF, consisting of 206, 189, 165 and 121 amino acids through alternative splicing, have been characterized. VEGF acts upon binding to at least 2 tyrosine kinase receptors, c-fms-like tyrosine kinase or Flt-1 and fetal liver kinase-1 or Flk-1. The Flt-1 receptor occurs both as a soluble and membrane-bound receptor. In this study, we focused our attention to the distribution of VEGF, Flk-1 and Flt-1 expression in hepatocellular carcinoma, determined by immunohistochemical techniques (ABC method), especially to examine the relationship between expression of VEGF, Flk-1 and Flt-1 proteins and clinical pathology in hepatocellular carcinomas.

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MATERIALS AND METHODS

Clinical materials

The subjects of this study were 70 patients surgically treated at the Department of Abdominal Surgery, Tianjin Medical University Cancer Hospital, Tianjin, China between May 1999 and October 1999. The main tissue samples were obtained from 60 hepatocellular carcinoma patients. Their ages ranged from 35 to 72 with an average of 52.5 ± 2.3 years. They were all clinically and/or pathologically diagnosed. Another 10 specimens of normal hepatic tissue were selected as controls.

Methods

The specimens were fixed in 10% formalin, and embedded in paraffin. Sections were cut and mounted on silicane-coated glass slides.

Immunohistochemical staining for VEGF was performed, using labelled streptavidin-biotin. Paraffin-embedded tumor sections were deparaffinized in xylene and rehydrated in graded alcohol. The sections were immersed in methyl alcohol containing 3% H_2O_2 for 20 min and sequentially washed with PBS. Microwave antigen retrieval was done for 12 min. Blocking of nonspecific immunoreactivity was performed with normal goat serum at 37℃ for 15 min. The sections were then washed 3 times in PBS and rabbit antihuman VEGF, rabbit antihuman Flt-1, and rabbit antihuman Flk-1 (Zhongshan Biotechnology Inc., Beijing) were added and the sections incubated overnight at 4℃. After repeated washes with PBS, the tissue sections were incubated with biotinylated rabbit antihuman IgG for 1 h at room temperature, followed by 3 washes with PBS. Color was then developed with 0.03% diaminobenzidine (DAB) containing 0.01% H_2O_2. Sequential sections were counterstained in hematoxylin. Finally, slides were washed in distilled water, dipped in dilute ammonium hydroxide and mounted in a crystal mounting solution. PBS was used as a negative control and proven positive slices as a positive controls.

The immunohistochemical staining was assessed by 2 pathologists. A total of 2,000 cells were counted in 10 different microscopic fields at × 400 magnification (× 40 objective and × 10 ocular). If the number of positive cells was <10% it was considered to be negative, and if >10%, to be positive.

Statistical analysis

The statistical analysis was performed using SPSS 10.0 software. $P<0.05$ was considered significant.

RESULTS

Degree of VEGF expression in hepatocellular carcinoma tissue

The degree of VEGF expression in normal hepatocytes was 40.0%. In contrast, the extent of VEGF expression in the hepatocytes from hepatocellular carcinoma tissues was 81.3%, which was significantly higher compared to normal liver ($P<0.05$) (Fig.1,2).

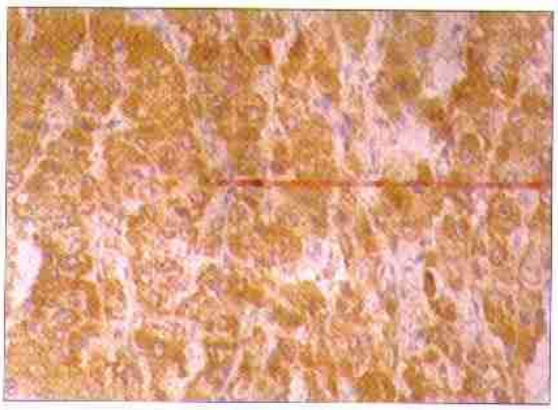

Fig.1. Immunostaining of human HCC with VEGF polyclonal antibody × 200.

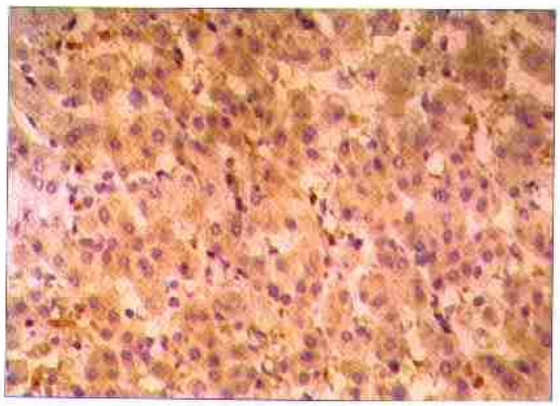

Fig.2. Immunostaining of human normal liver tissue with VEGF polyclonal antibody × 200.

Relationship between the level of VEGF expression in hepatocellular carcinoma and tumor grading

The level of VEGF expression in grade II hepatocellular carcinoma tissues was 72.2% (18 cases) and in grade III it was 72.0% (25 cases) in contrast to 100% in grade IV (17 cases). There was no difference in the extent of VEGF expression between grade II and III hepatocellular carcinomas, but the difference between grade II and III compared to grade IV was significant ($P<0.05$).

Relationship between the level of VEGF expression in hepatocellular carcinoma and invasion and metastasis

In this study, we used the nature of the tumor capsule, tumor embolus and tumor size to predict the potential for invasion and metastasis of the carcinoma. The level of VEGF expression in the hepatocytes from the tumors with a capsule was 60.0% (12 of the 20 cases with a capsule). In contrast, in tumors without a capsule it was 92.5% (37 of the 40 cases had no capsule). The extent of VEGF expression in tumor devoid of a capsule was significantly higher than those with capsule control ($P<0.01$). VEGF expression in the tumor embolus control group was 94.4% (34 of 36 cases) and in no tumor cell embolus control group was 62.5% (15 of 24 cases), the former being significanty higher than the latter ($P<0.01$). VEGF expression in tumors over 5 cm in diameter was 92.1% (35 of 38 cases) and for those under 5 cm was 63.6% (14 of 22 cases), with an obvious difference.

Extent of Flt-1 and Flk-1 expression in hepatocellular carcinoma tissue

The degree of Flk-1 expression in normal hepatocytes was 50.0% (5 cases), whereas in hepatocellular carcinomas it was 88.3%, a significant difference ($P<0.05$). Similarly, Flt-1 expression in normal hepatocytes was 40.0% in contrast to hepatocellular carcinoma tissues which was 80.0% (48 cases). Again the expression was higher in the tumor than that in normal tissues. ($P<0.05$) (Fig.3 to 6).

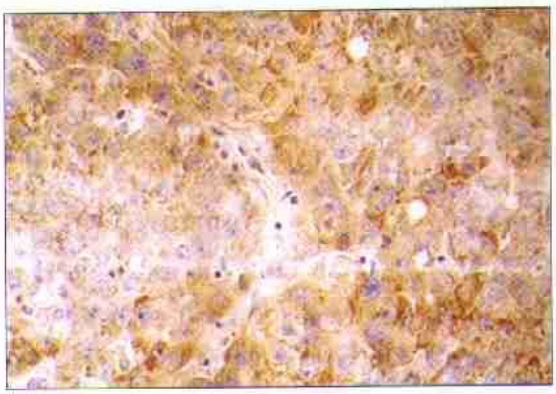

Fig.3. Immunostaining of human HCC with Flk-1 polyclonal antibody × 200.

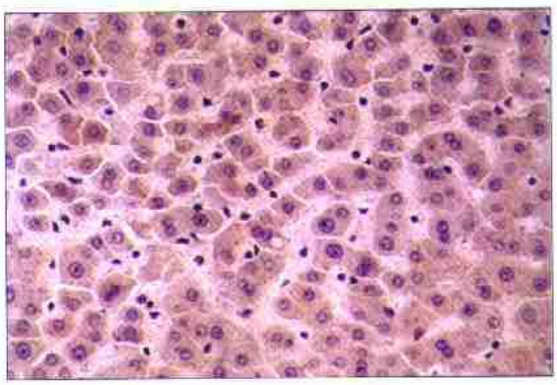

Fig.4. Immunostaining of human normal liver tissue with Flk-1 polyclonal antibody × 200.

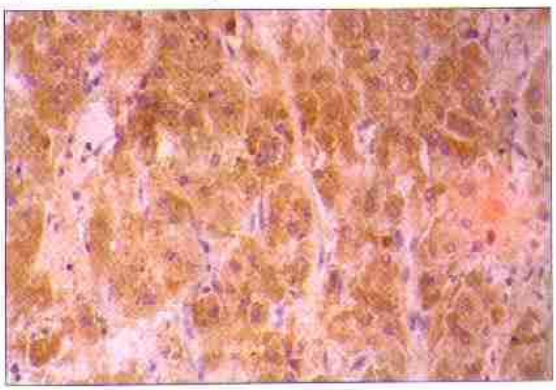

Fig5. Immunostaining of human HCC with Flt-1 polyclonal antibody × 200.

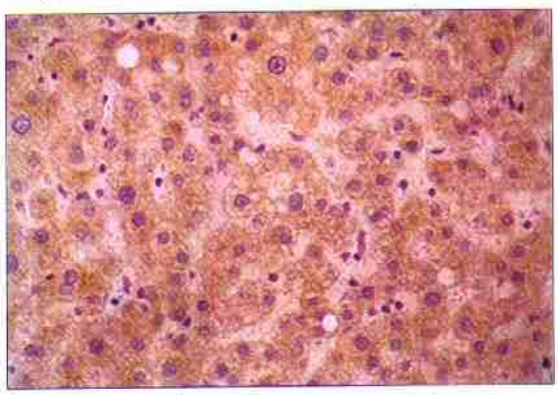

Fig.6. Immunostaining of human normal liver tissue with Flt-1 polyclonal antibody × 200.

Correlation among VEGF, Flt-1 and Flk-1 expression in hepatocellular carcinoma tissue

The respective level of VEGF, Flt-1 and Flk-1 expression in hepatocytes cancerous tissues was 81.3%, 88.3% and 80.0%. Our results showed that the expression of VEGF protein and its receptors displayed a positive correlation ($r=0.8155$, 0.7857, respectively $P<0.01$).

DISCUSSION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide, with an incidence of approximately 1 million new cases annually.[1] HCC is a hypervascular malignancy. In recent years, surgical resection or liver transplantation for HCC is the mainstay of treatment for curing the patients. However, the prognosis after resection of HCC has remained unsatisfactory because of a high incidence of postoperative recurrence. Angiogenesis, which is essential for tumor growth, invasion, and metastasis, is stimulated by VEGF, an important angiogenic factor.

VEGF exerts its actions on the microvasculature by interacting with specific endothelial cell receptors, and thus, contributes to angiogenesis and growth in many tumours.[2] Flt-1 (VEGF receptor-1) and KDR/Flk-1 (VEGF receptor-2) are high-affinity receptors for the angiogenesis factor. VEGF expression has been confirmed in human HCC, and VEGF is thought to be involved in the angiogenesis within HCC tissues.[3] It was shown that ll cultured hepatocellular carcinoma cells exhibited a high level of VEGF mRNA and hepatocellular carcinoma cells over-expressed the VEGF gene and protein.[4] Higher levels of VEGF mRNA were observed in HCC and metastatic liver tumors compared to corresponding nontumorous tissues. Mise et al. reported that the degree of VEGF mRNA expression was significantly correlated with the intensity of tumor growth based on angiograms.[5]

We knew that tumor capsule, tumor emboli and tumor size were significant prognostic factors. In our study, we found that increased tumor expression of VEGF was associated with adverse clinicopathologic features in HCC patients, such as the presence of a tumor capsule, a tumor embolus and tumor size of more than 5 cm. So we can conclude that overexpression of VEGF is correlated with an adverse prognosis in HCC patients.

In this study, we also found that Flt-1 and Flk-1 expression in HCC tissues was higher than that in normal liver. There was a positive correlation among VEGF, Flt-1 and Flk-1 expression in HCC tissue. Similar conclusions[6] have been reported.

The role of VEGF in developmental angiogenesis is emphasized by the finding that loss of a single VEGF allele results in defective vascularization and early embryonic lethality.[7] HCC is a highly vascular tumor. If VEGF is predictive of outcome in HCC patients, it may be reasonable to suggest that angiogenesis may be important in the pathogenesis or potential therapy of HCC.[8] A large number and great diversity of antiangiogenic agents are being evaluated in current phase I, II, and III clinical trials, with some encouraging results.[9,10]

REFERENCES

1 Simonetti RG, Camma C, Fiorello F, et al. Hepatocellular carcinoma. A worldwide problem and the major risk factors. Dig Dis Sci. 1991;36:962–972.

2 Shimamura T, Saito S, Morita K. Detection of vascular endothelial growth factor and its receptor expression in human hepatocellular carcinoma biopsy specimens. Gastroenterol Hepatol. 2000;5:640–646.

3 Yamaguchi R, Yano H, Nakashima Y. Expression and localization of vascular endothelial growth factor receptors in human hepatocellular carcinoma and non-HCC tissues. Oncol Rep. 2000;7:725–729.

4 Minra H, Miyazaki T, Kuroda M, et al. Increased expression of vascular endothelial growth factor in human hepatocellular carcinoma. J Hepatol. 1997;27:854–861.

5 Mise M, Arii S, Higashituji H, et al. Clinical significance of vascular endothelial growth factor and basic fibroblast growth factor gene expression in liver tumor. Hepatology. 1996;23:455–464.

6 Suzuki H, Seto K. Paracrine upregulation of VEGF receptor mRNA in endothelial cells by hypoxia –exposed hep G_2 cells. Am J Physiol. 1999;276:G92–97.

7 Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocrine Reviews. 2004;25:581–611.

8 Chao Y, Li CP, Chau GY, et al. Prognostic significance of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin in patients with resectable hepatocellular carcinoma after surgery. Ann Surg Oncol. 2003;10:355–362.

9 Kerbel RS. Clinical trials of antiangiogenic drugs: opportunities, problems, and assessment of initial results. J Clin Oncol. 2001;19:45S–51S.

10 Rosen L. Antiangiogenic strategies and agents in clinical trials. Oncologist. 2000;5(Suppl 1):20–27.