Expression of Vascular Permeability Factor/Vascular Endothelial Growth Factor in Human Hepatocellular Carcinoma


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ABSTRACT

Vascular permeability factor (VPF)/vascular endothelial growth factor (VEGF) is unique in its ability to promote vascular permeability and endothelial cell growth, and its role in tumor development has received considerable attention. In this report, we describe the elevation of VPF/VEGF transcript expression in human hepatocellular carcinoma. Surgical samples of 23 patients with hepatocellular carcinoma were studied using reverse transcription-PCR analysis. The oligonucleotide primers were designed to amplify all four known splicing variants that could be expressed in the samples studied. Sixteen cases showed VPF/VEGF transcript expression in the tumor (16/23, 69.6%), whereas only 9 of the 23 patients showed it in the corresponding nontumoral part. There was no difference between the pattern of expression of VPF/VEGF isoforms in tumoral and nontumoral tissues. VPF/VEGF mRNA expression in the liver tumors was associated with fibrous capsule formation and septal formation (P < 0.05 respectively, Fisher's exact test). In situ hybridization confirmed the presence of VPF/VEGF mRNA expression in tumor cells and less intensely in hepatocytes of nontumoral liver. We also found that VPF/VEGF expression in the tumor cell was increased in the area adjacent to necrotic regions (presumably hypoxic regions). As a regulator of vascular permeability and endothelial cell growth factor, VPF/VEGF may play an important role in the development of hepatocellular carcinoma.

INTRODUCTION

VPF is a Mr 34,000–50,000 dimeric, disulfide-linked glycoprotein synthesized by several human and animal cell types, both normal and neoplastic (1–4). VPF was originally recognized for its ability to increase the permeability of the microvasculature to circulating macromolecules (i.e., plasma proteins; Ref. 3). More recently, VPF has also been shown to be a selective endothelial cell mitogen, and therefore has been alternatively called VEGF (5–8). By alternating splicing of mRNA, four different molecular species with 121, 165, 189, and 206 amino acids are determined (9), although the physiological significance of the multiple isoforms has not been fully established. VPF/VEGF has been reported to bind to at least two specific receptors found on endothelial cells, fit-1 and KDR (10, 11). VPF/VEGF was originally discovered as a tumor-secreted protein, and its role in tumor development has been investigated (5, 12). It is considered to play an important role in tumor biology in at least two ways: as a vascular permeability factor and/or endothelial growth factor. As a potent permeability factor, VPF/VEGF promotes extravasation of plasma fibrinogen, leading to the formation of a fibrin network which serves as a substrate for cell migration during angiogenesis. In addition, as an endothelial growth factor, VPF/VEGF stimulates endothelial cell proliferation and is likely to induce the formation of new blood vessels (13).

VPF/VEGF is synthesized and secreted by a variety of tumor cells in tissue culture and by several transplantable animal tumors in vivo (8, 14). Elevated expression of VPF/VEGF transcripts in human tumors was first demonstrated in tumors of the central nervous system (12, 15). Elevated expressions of VPF/VEGF have also been reported for tumors of the gastrointestinal tract, kidney, and breast in humans (1, 16–19). However, there has been only one report (20) on the expression of VPF/VEGF in HCC, which is well known for its hypervascularity.

In this study, we examined the VPF/VEGF expression in HCC using the RT-PCR method and compared it with the pathological features of HCC. We also examined for the first time the localization of VPF/VEGF transcripts in HCC by means of the in situ hybridization technique. The aims of our study were to determine whether VPF/VEGF is overexpressed in HCC compared with the nontumoral part of the liver and its role in the pathogenesis of HCC.

MATERIALS AND METHODS

Subjects and Tissue Samples. The subjects in this study were 23 patients with HCC who underwent surgical resections between June 1990 and September 1994 at Osaka University Hospital and Osaka Rosai Hospital. The study group comprised 18 men and 5 women ranging from 42 to 71 (mean age, 60.9 ± 7.6) years of age. None had apparent distant metastasis. None of the patients had been previously treated for HCC before surgical resection, such as with percutaneous ethanol injection or transcatheter arterial embolization. A tumor sample and a nontumoral part of the liver were obtained during the surgical resection. The samples were each divided into two pieces. One was subjected to fixation in formalin for histological examination, and the other was snap frozen in liquid nitrogen and stored at −80°C until use for VEGF/VPF expression analysis. Pathological features of the patients were obtained from the pathological reports and clinical records. Histological analysis of the tumors was done by experienced pathologists without knowledge of the analytical results. Tumors were graded as well differentiated, moderately differentiated, or poorly differentiated according to the grading system of the Liver Cancer Study Group of Japan (21). The differentiation grading was based on the predominant findings.

The tumors were also examined for size, number of additional nodules, fibrous capsule formation, existence of capsule invasion, septal formation, and portal vein invasion.

RT-PCR Analysis of the VEGF Transcript. Total cellular RNA was extracted from frozen samples using the single-step method (22), and 3 μg total RNA were dissolved in 8 μl RNase-free water. One μl of 0.01 absorbance 0.01 unit of random hexanucleotide primer, 1 μl of 200 units/μl Moloney murine leukemia virus-reverse transcriptase (Life Technologies, Inc., BRL, Gaithersburg, MD), 4 μl Moloney murine virus-reverse transcriptase buffer, 0.1 μl RNase inhibitor, 1 μl 0.1 mm DTT, and 5 μl of 5 mM dNTP mixture were added to each tube. RT was performed at 37°C for 60 min.
Five μl each of RT products were amplified by means of PCR. VEGF/VPF has been reported to have four isoforms arising from alternative splicing of its mRNA transcript (9). In this study, oligonucleotide primers flanking the insertion/deletion site of VEGF/VPF (codon 115) were used to amplify cDNA. The sense primer was 5'-CCGGAATTCACATTTGTTGTGCTGT-3' (1), and the antisense primer was 5'-CGAAGTGGTGAAGTTCATGGATG-3', and the anti-sense primer was 5'-TTCTGTATCAGTCTTTCCTGGT-3' (6, 9). cDNA was amplified by 0.5 units Taq DNA polymerase (Takara, Kyoto, Japan) in a 20-μl reaction mixture containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM deoxynucleotides, and 40 pm each primer. The PCR profile consisted of a 7-min initial denaturation at 94°C followed by 40 cycles of 1-min denaturation at 94°C, 1-min annealing at 60°C, 3-min extension at 72°C, and finally a 10-min extension at 72°C. Five μl each of the PCR reaction products were run on a 1% agarose gel and visualized using ethidium bromide staining.

Preparation of Single-Stranded RNA Probes for In Situ Hybridization. To amplify a VPF/VEGF cDNA fragment from mRNA, we used human lung poly(A)+ RNA (Toyobo, Osaka, Japan), which is abundant in VPF/VEGF (1). RT was performed, and the cDNA produced was amplified with PCR. We synthesized the following oligonucleotide primers with BamHI (sense primer) and EcoRI (antisense primer) restriction sites. The sense primer was 5'-CCGGAATTCACATTTGTTGTGCTGT-3' (1). PCR was performed with the following thermocycle parameters: a 7-min initial denaturation at 94°C followed by 40 cycles of 1-min denaturation at 94°C, 2-min annealing at 55°C, 3-min extension at 72°C, and finally a 10-min extension at 72°C. This amplified 204-bp VPF/VEGF cDNA fragment, which is common to all known VPF/VEGF splicing variants (9), was digested with BamHI and EcoRI and subcloned into the pBluescript II SK+ (Stratagene Inc., La Jolla, CA). The identity of the cloned insert was confirmed by DNA sequencing.

The pBluescript II SK+ construct with the VPF/VEGF insert was linearized with BamHI and transcribed in vitro from the T7 polymerase promoter to yield the antisense RNA probe. The same construct was linearized with EcoRI and transcribed from the T3 promoter to yield the sense (control) probe. The transcription reaction was performed in the presence of 0.35 mM digoxigenin-UTP (Boehringer Mannheim GmbH, Biochemica, Mannheim, Germany) to yield the digoxigenin-labeled RNA probe.

In Situ Hybridization. In situ hybridization was performed in three cases of HCC using a digoxigenin nucleic acid detection kit (Boehringer Mannheim GmbH, Biochemica) with some modifications (23). Frozen sections were cut at 8–10 μm, fixed for 20 min in 4% paraformaldehyde/0.1 M phosphate buffer, and rinsed in PBS (0.02 M phosphate buffer and 0.15 M NaCl). Specimens were then treated with 0.001% proteinase K, fixed in 4% paraformaldehyde/0.1 M phosphate buffer again, denatured in 0.2 N HCl, acetylated in 0.1 M triethanolamine and 0.25% acetic anhydride, and dehydrated through a graded ethanol series. Sections were prehybridized for 30 min at 50°C with 100 μl of hybridization fluid containing 50% formamide, 0.3 mM NaCl, 20 mM Tris-HCl (pH 8.0), 2 mM EDTA, 10% dextran sulfate, 0.2% sarcosyl, single-strength Denhardt's solution (0.2 g/liter BSA, 0.2 g/liter Ficoll, and 0.2 g/liter polyvinyl pyrrolidone), and 0.02% salmon sperm DNA in a humidified chamber. After the prehybridization, hybridization was performed in buffer containing hybridization fluid and 1.0 μg/ml of either antisense or sense probe at 50°C for 14 h. Next, sections were washed at 60°C for 20 min in 5X SSC (150 mM NaCl and 15 mM sodium citrate) and 30 min in 2X SSC/50% formamide, digested with RNase (10 μg/ml) at 37°C for 30 min, and washed again at 60°C for 30 min in 2X SSC/50% formamide. Then, blocking was performed in blocking buffer containing 1.5% BSA for 30 min. After the blocking buffer was removed, the solution containing 1.5 units/ml sheep anti-digoxigenin Fab fragment conjugated to alkaline phosphatase and 1.5% BSA was placed on each section. Sections were placed in a humidified chamber and incubated at room temperature for 1 h. After the sections were rinsed, a coloring reaction was performed using 230 μg/ml nitroblue tetrazolium salt and 180 μg/ml 5-bromo-4-chloro-3-indolyl-phosphate, and then sections were counterstained with methyl green.

Statistical Analysis. For comparison of the histopathological features between VEGF/VPF expression and nonexpression groups, qualitative variables were analyzed with the Fisher’s exact test; quantitative variables were evaluated with Student’s t test.

RESULTS

RT-PCR Analysis of VPF/VEGF Transcript in HCC Tissues and Normal Liver Tissues. RT-PCR analysis revealed that VPF/VEGF mRNA was expressed in the liver tumors of 16 of 23 patients examined (16/23, 69.6%). In 9 of 16 patients, the transcripts were also found in nontumoral liver, although the bands corresponding to VPF/VEGF in nontumoral liver were faint in 3 of these cases. In the remaining 7 patients, VPF/VEGF transcripts were detected only in tumor tissues. No VPF/VEGF transcript was found in either nontumoral or tumoral tissues of the livers in seven patients. Pathological features of HCC and the summary of VPF/VEGF analysis is shown in Table 1.

Mature VPF/VEGF transcripts are known to arise from alternative splicing of the primary gene transcript (9). The oligonucleotide primers were designed to amplify all four known splicing variants that

<p>| Table 1 A summary of pathological features and VPF/VEGF expression of patients with HCC |
|--------------------------------------|-------------------------------|----------------|----------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>Background</th>
<th>Tumor size (cm)</th>
<th>Additional nodules</th>
<th>Portal invasion</th>
<th>Capillary formation</th>
<th>Septal formation</th>
<th>Degree of differentiation</th>
<th>Vascularity in angiography</th>
<th>VEGF mRNA non-tumor</th>
<th>VEGF mRNA tumor</th>
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<tr>
<td>1</td>
<td>Cirrhosis</td>
<td>1.7</td>
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<td>+</td>
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<td>NI</td>
<td>NI</td>
<td>Poorly</td>
<td>Hypervascular</td>
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<td>+</td>
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<td>Hypervascular</td>
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<td>+</td>
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<td>Hypervascular</td>
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<td>+</td>
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<td>Hypervascular</td>
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<td>+</td>
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<td>Hypervascular</td>
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<td>+</td>
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<td>0</td>
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<td>Hypervascular</td>
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<td>+</td>
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<td>Hypervascular</td>
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<td>Hypervascular</td>
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<td>0</td>
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<td>+</td>
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<td>Hypervascular</td>
<td>-</td>
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<td>NI</td>
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<td>Hypervascular</td>
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<td>NI</td>
<td>Moderately</td>
<td>Hypervascular</td>
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<td>3</td>
<td>1</td>
<td>0</td>
<td>+</td>
<td>+</td>
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<td>Hypervascular</td>
<td>-</td>
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<td>0</td>
<td>+</td>
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<td>Moderately</td>
<td>Hypervascular</td>
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<td>+</td>
<td>Moderately</td>
<td>Hypervascular</td>
<td>-</td>
<td>-</td>
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<tr>
<td>23</td>
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<td>0</td>
<td>0</td>
<td>+</td>
<td>+</td>
<td>Moderately</td>
<td>Hypervascular</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* NI, not informative.  
* ND, not done.
could be expressed in the samples studied. Fig. 1 shows the results of
the electrophoretic analysis of PCR products in representative cases. In
patient 10 (Fig. 1, Lane 2, nontumoral part; Lane 3, tumoral part),
PCR products of VPF/VEGF transcripts were detected only in the
tumoral part. In patient 8 (Fig. 1, Lane 4, nontumoral part; Lane 5,
tumoral part) and patient 6 (Fig. 1, Lane 6, nontumoral part; Lane 7,
tumoral part), VPF/VEGF transcripts were detected in both the tu-
moral and nontumoral parts. In the majority of the cases, three
amplified bands of 403, 535, and 607 bp corresponding to 121, 165,
and 189 amino acid isoforms of VPF/VEGF (VPF/VEGF121 and VPF/VEGF165) were detected (Fig. 1, Lane 4). In the samples with faint bands, only two bands
(VPF/VEGF121 and VPF/VEGF165) were detected (Fig. 1, Lane 4).
VPF/VEGF levels in these cases with faint bands were designated as ± in Table 1. There seemed to be no difference between the
banding pattern in nontumoral and tumoral tissues. Notably, no sam-
ple was positive for the band corresponding to VPF/VEGF196.

Comparison of Pathological Features of Liver Tumors between
VPF/VEGF Expression and Nonexpression Groups. VPF/VEGF
mRNA expression in HCC was compared with the clinical and his-
tological features of the tumor. The comparison between VPF/VEGF
expression groups and nonexpression groups is shown in Table 2.
For comparison between VPF/VEGF expression and capsule invasion,
cases without capsule formation were eliminated from the analysis,
since capsule invasion cannot take place in the absence of capsule. It
is well known that vascularity is not homogeneous in the tumors of
HCC. HCC often shows in homogeneous enhancement, which is often
described as "mosaic-like," on enhanced CT (24). Therefore, we
evaluated the vascularity by a rather gross method, angiography. Tumors that demonstrated tumor staining were designated as hyper-
vascular. Twenty patients were found to have hypervascular tumors,
and VPF/VEGF transcript expression was observed in liver tumors of
14 of 20 patients. Only one patient (patient 13) was found to be
isovascular, whereas the liver tumor in this patient was found to the
express VPF/VEGF transcript. VPF/VEGF gene expression in liver
tumors was not related to increased vascularity of the tumors accord-
ing to Fisher's exact P test. However, because patient 13 had a rather
small tumor (2.8 cm in diameter), it might have been difficult to detect
tumor staining using angiography. In fact, in computed tomography,
this case exhibited enhancement by bolus injection of contrast
medium. Thus, this case should be regarded as hypervascular, al-
though the difference in vascularity between the tumor and the normal
part was not sufficient to be detected by angiography. Taken together,
all 21 cases subjected to angiography had hypervascular tumors, and
15 of them expressed VPF/VEGF transcript, whereas 6 cases did not.
Interestingly, VPF/VEGF mRNA expression in the liver tumors
was related to fibrous capsule formation and septal formation of the
tumor (P < 0.05, respectively, Fisher's exact P test). VPF/VEGF
mRNA expression in the liver tumors did not show any relationship
with other histopathological features of the tumors, namely, tumor
size, number of additional nodules, differentiation degree, capsule
invasion, and portal invasion.

VPF/VEGF mRNA expression in the nontumoral part of the liver was
also compared with hemoglobin levels, serum alanine aminotransferase
levels, and pathological diagnosis of the background liver disease. How-
ever, no significant relationships were found (data not shown).

In Situ Hybridization. By using the VPF/VEGF antisense probe,
we observed specific labeling in the tissue sections from HCC. In
general, VPF/VEGF mRNA was distributed homogeneously and in-
tensively throughout the tumor. Microscopic examination revealed
that VPF/VEGF is expressed by tumor cells (Fig. 2a). No specific eleva-
tion of hybridization was observed in the area adjacent to fibrotic
regions or in the vascular stroma in HCC. However, it is noteworthy
that, in the periphery of necrotic foci of the HCC, labeling with the
VPF/VEGF antisense probe was distinctly intensified in the tumor
cells (Fig. 2c). Hybridization of the antisense VPF/VEGF probe was
also evident, although less intensely, in the normal hepatocytes of
the nontumoral part of the liver. Distribution of VPF/VEGF mRNA in
nontumoral liver was also homogeneous (Fig. 2e). No significant
hybridization was noted in the vascular endothelial cells, either in the
tumoral tissue or in the nontumoral liver tissue. No specific cellular

Table 2 Comparison of the clinical and pathological features of HCC between VPF/
VEGF expression groups and nonexpression groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>With VPF/VEGF expression (n = 16)</th>
<th>Without VPF/VEGF expression (n = 7)</th>
<th>P</th>
</tr>
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<tr>
<td>Vascularity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Hypervascular</td>
<td>14</td>
<td>6</td>
<td>NS*</td>
</tr>
<tr>
<td>Isovascular</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hypovascular</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td>4.8 ± 4.3</td>
<td>3.5 ± 2.3</td>
<td>NS</td>
</tr>
<tr>
<td>No. of additional nodules</td>
<td>12</td>
<td>5</td>
<td>NS</td>
</tr>
<tr>
<td>Degree of differentiation</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Capsule formation</td>
<td>+</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>Capsule invasion</td>
<td>+</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Septal formation</td>
<td>+</td>
<td>6</td>
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<tr>
<td>Portal vein invasion</td>
<td>-</td>
<td>3</td>
<td>5</td>
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* Data expressed as mean ± SD.
labeling was seen with the sense control probes, and the nonspecific background was low (Fig. 2, b, d, and f).

**DISCUSSION**

In the literature, VPF/VEGF expression in normal liver has been reported, although the level of expression is not clarified. Berse et al. (1) found VPF/VEGF mRNA expression in a single case of normal human liver by means of Northern blot analysis and reported that VPF/VEGF transcripts were "readily detected" in human liver. On the other hand, Warren et al. (25) examined grossly normal human liver of those patients undergoing liver resection for colorectal cancer metastasis. They observed a detectable signal for VPF/VEGF in three of five livers using Northern blot analysis. Furthermore, the functions of VPF/VEGF in normal liver are poorly understood. In the present study, under the RT-PCR conditions used, VPF/VEGF mRNA was detected in nontumoral liver in only 9 of 23 patients. Therefore, the detection of the VPF/VEGF transcript in 16 of 23 HCCs suggests that VPF/VEGF mRNA is overexpressed in HCC compared with the nontumoral part of the liver. The in situ hybridization technique confirmed the presence of VPF/VEGF mRNA in the tumor cells and
less intensely in nontumoral hepatocytes. To our knowledge, this is the first report to reveal the localization of VPF/VEGF mRNA in tumor cells of HCC. Recently, Mise et al. (20) reported overexpression of VPF/VEGF in HCC by means of Northern blot analysis. However, they examined the localization of VPF/VEGF with immunohistochemistry. They found VPF/VEGF protein in HCC cells and the vascular endothelial cells, but they could not determine which cells synthesize VPF/VEGF. Taken their study into account, it seems that VPF/VEGF is synthesized by HCC cells and accumulates in the vascular endothelial cells, its target of action.

Three forms of VPF/VEGF mRNA have been shown to be expressed in HCC, with an abundance of VPF/VEGF_{121} and VPF/VEGF_{165}. Nontumoral liver shows a similar pattern of VPF/VEGF isoform expression, although the number of VPF/VEGF-expressing cases is small. In literature, central nervous system neoplasms and normal brain have been reported to express VPF/VEGF_{121}, VPF/VEGF_{165}, and VPF/VEGF_{189} (26). Our results are consistent with the studies on other human tumors, with no unique isoform to tumor tissue being observed. The VPF/VEGF_{106} isoform was not detected either in HCC or nontumoral liver in this study. To date, the VPF/VEGF_{106} transcript has been observed only in the human fetal liver cDNA library (9). Although HCC often produces embryonic protein such as α-fetoprotein, it appears that HCC does not express the fetal liver-type VPF/VEGF.

Solid tumors are composed of two distinct compartments, the malignant cells themselves and the vascular and connective tissue stroma that they induce and in which they are dispersed. The stroma provides the vascular supply that tumors require for obtaining nutrients, gas exchange, and waste disposal. Fibrin is considered to serve as a provisional stroma that is gradually replaced by granulation tissue and then by mature stroma (27). Tumor cells are not known to synthesize fibrinogen. Therefore, for fibrin to deposit at the extravascular site, the permeability of microvasculature must increase significantly. VPF/VEGF is thought to be responsible for the vascular hyperpermeability and consequent fibrin deposition in tumor (13). In this study, VPF/VEGF transcript expression in HCC was related to fibrous capsule formation and septal formation. The origin of capsule formation in HCC is unclear. It has been suggested that capsule formation is a result of compression and collageneization of the adjacent stroma (28, 29). However, this mechanism has been doubted, since the tumor size of HCC did not correlate with either incidence of capsule formation or thickness of the capsule (30, 31). These findings suggest that capsules might be formed by active fibrosis rather than by tumor compression on the adjacent stroma (30, 31). The origin of fibrous septa in a tumor also remains to be clarified. Nakashima et al. (29) suggested a possibility of fibrogenesis in the interface of two tumor nodules of different properties. If these fibrotic changes, namely, capsule formation and septal formation, are the result of active fibrosis, they require fibrin deposition in their initial stage (27).

In breast cancer, Brown et al. (19) have reported the strong expression of VPF/VEGF mRNA in infiltrating ductal carcinoma, which characteristically induces a desmoplastic stromal reaction. Their finding implies a correlation between increased expression of VPF/VEGF and the induction of a stromal response (19). Our finding offers additional support for the VPF/VEGF involvement in stroma formation.

To investigate the role for VPF/VEGF in angiogenesis, we compared VPF/VEGF expression with vascularity of HCC. All 21 cases subjected to angiography were revealed to have hypervascular tumors by angiography or computed tomography. Although VPF/VEGF expression was observed in 15 cases, it was not detected in 6 cases. This suggests that angiogenesis is not simply controlled by the presence of VPF/VEGF but is mediated by several angiogenic inducers. To date, many angiogenesis factors have been found, including basic fibroblast growth factor, transforming growth factor β, and tumor necrosis factor α (32). In fact, there has been a report on acidic and basic fibroblast growth factor expression in HCC (33). Therefore, it is likely that vascularization in some tumors is related to other angiogenesis factors.

The in situ hybridization technique revealed that VPF/VEGF mRNA was expressed in tumor cells and that the labeling was rather homogeneous in the tumors. Elevated VPF/VEGF expression was not evident in the area adjacent to the capsule or blood vessels, whereas VPF/VEGF is thought to be associated with stroma formation and increased vascularity. Our finding is consistent with other human tumor studies, including those on renal cell carcinoma (17), breast cancer (19), and adenocarcinoma of the gastrointestinal tract (16), which have reported that most tumor cells express VPF/VEGF mRNA. Specific overexpression of VPF/VEGF transcripts by tumor cells was not noted around the vessels in any of the studies. Alternatively, some studies observed elevated VPF/VEGF receptor (flt-1 and KDR) expression in the vascular endothelial cells (12, 16, 17, 19). Therefore, the distribution of fibrotic change or vascularization may be determined by VPF/VEGF receptor(s) expression, not by VPF/VEGF expression. Further study into the cellular distribution of VPF/VEGF receptors may be needed to clarify the role of VPF/VEGF in these pathological changes.

It is noteworthy that VPF/VEGF mRNA expression in the tumor cell was locally elevated in the area adjacent to tumor necrosis. This elevation around the necrotic area of the tumor has been reported in several other human cancers (12, 15–17, 19). Rapid cell proliferation in the center of a tumor can lead to increased interstitial pressure, which may lead to compression closure of capillaries and consecutive tumor necrosis (34). Tumor hypoxia has been reported to increase expression of VPF/VEGF mRNA in a variety of cultured tumor cells (15). Therefore, it is likely that, in the development of necrosis, hypoxia may increase the VPF/VEGF mRNA expression.

In this study, we observed VPF/VEGF mRNA expression in nontumoral liver in 9 of 23 patients. We sought to determine under what circumstances VPF/VEGF was expressed in nontumoral liver. However, VPF/VEGF expression in nontumoral liver was not related to hemoglobin levels, serum alanine aminotransferase levels, or pathological diagnosis of the background liver disease. Further study should clarify the mechanisms of VPF/VEGF transcripts expression in nontumoral liver.

We determined that VPF/VEGF is overexpressed in HCC, and that its expression in HCC was associated with capsule formation and septal formation and hypoxia of the tumor. As a regulator of vascular permeability and endothelial cell growth factor, VPF/VEGF may play an important role in the development of HCC.

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VPF/VEGF EXPRESSION IN HEPATOCELLULAR CARCINOMA


Expression of Vascular Permeability Factor/Vascular Endothelial Growth Factor in Human Hepatocellular Carcinoma

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