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# Placenta growth factor not vascular endothelial growth factor A or C can predict the early recurrence after radical resection of hepatocellular carcinoma

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**Abstract**

The purpose of this study was to evaluate the relationship between the expression of PlGF in tumor tissue and clinical outcomes in HCC patients. Tumor PlGF and vascular endothelial growth factor (VEGF)-A and VEGF-C mRNA were analyzed. Results demonstrated that patients with PlGF expression levels higher than median tended to have early recurrence compared to patients with PlGF expression lower than median ($P = .031$). In patients with AJCC stage II–III disease, this difference was even more significant ($P = .002$). In contrast, VEGF-A and VEGF-C could not predict early recurrence-free survival. Since PlGF expression correlated with early recurrence of HCC, PlGF may be an important prognostic indicator in HCC.


*Keywords:* Vascular endothelial growth factor; Placenta growth factor; Angiogenesis; Hepatocellular carcinoma; Recurrence

## 1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. HCC is a hypervascular tumor mainly supplied by hepatic arteries [1] and characterized by a propensity for vascular invasion. HCC is associated with a high metastatic potential. Early postoperative recurrence in the liver remnant or growth of tumor at distant sites as a result of metastasis is a common phenomenon following resection of HCC [2]. Angiogenesis is now recognized as playing a pivotal role in hepatocarcinogenesis and regarded as one of the marker for invasiveness and metastasis in HCC.

Tumor angiogenesis is regulated by a variety of pro- and anti-angiogenic factors. Among them, vascular endothelial growth factor A (VEGF-A) has been studied most extensively and is considered to

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be the most important proangiogenic factor in HCC [3,4]. Similarly, vascular endothelial growth factor C (VEGF-C), which may play a role in the maintenance of lymphatic endothelium and/or in lymphangiogenesis, is also reported to be an angiogenic factor in some human cancers [5–7]. VEGF-C expression in HCC has also been reported [8]. The correlation between VEGF expression and the prognosis of HCC, however, remains unclear. Li et al. [9] and Ng et al. [3] both reported that expression of VEGF is related to invasion and metastasis of HCC [3,9]. Yamaguchi et al. [1], however, found that VEGF expression was lower in advanced HCC with increased tumor vasculatures [1]. These observations suggest that other angiogenic factors may also contribute to angiogenesis stimulation in HCC.

Placenta growth factor (PlGF) is a dimeric glycoprotein, structurally and functionally related to VEGF [10]. PlGF appears to be crucial for pathological angiogenesis in adults [11] and is not produced by the majority of normal human tissues [12]. A prognostic implication of tumor PlGF expression level independent of conventional pathologic prognosticators has been demonstrated in a variety of cancers. For example, in human gastric cancer, PlGF expression level was significantly correlated with serosal invasion, positive lymph node metastases, tumor stages, and patient survival [13]. PlGF levels were also significantly associated with histological grade, total tumor vascularity, and prognosis in patients with renal cancer [14]. Exposure of melanoma cells to PlGF resulted in a specific proliferative response [15]. PlGF expression was also shown to correlate with disease progression and patient survival in colorectal cancer [16].

To date, PlGF expression in HCC and any correlation between PlGF and prognosis remains unknown. Therefore, the aim of this study was to evaluate the expression of PlGF in postoperative HCC patients following primary resection of the liver to determine if a correlation between PlGF expression and early postoperative recurrence of HCC existed.

## 2. Materials and methods

### 2.1. Patients

Seventy-one consecutive patients who underwent primary liver resection for HCC at the National Taiwan University Hospital were included in this study. Patients who had received preoperative chemotherapy or embolization were excluded. All patients received routine chest X-ray, abdominal ultrasound (US), computed tomography (CT) and hepatic angiography prior to the liver resection. Intra-operative US was routinely performed in all the patients. Tumor complete resection (defined as microscopically free of tumor at the section margin, and no evidence of space occupying lesions in the remaining liver as detected by image studies) were successfully performed in all 71 patients. Neither preoperative therapy nor postoperative adjuvant chemotherapy was administered. The tumor tissue and 62 patient-matched non-tumor tissue samples were collected and snap frozen in liquid nitrogen immediately after excision from the patients under the approval of the Institutional Review Boards and each patient's written informed consent. Clinical data were obtained from the medical records. Tumor differentiation, venous invasion, maximum tumor diameter, and non-tumor part of the excised liver were all examined by an experienced pathologist. The pathologist was blinded to all clinical information.

The patients were followed up at least once every 3 months. Serum alpha fetoprotein (AFP) level and abdominal US were employed to screen for tumor recurrence. Abdominal CT and/or magnetic resonance image (MRI) were performed if AFP levels elevated or a new space occupying lesion was detected by US. Hepatic angiography was performed in the even that recurrent HCC (i.e., new hypervascular lesion found during the arterial phase of hepatic angiography) was suspected. Patients that developed HCC nodules that developed within 1 year of the primary surgery, near the site of the original tumor or if nodules shared the same portal blood supply as the primary tumor, were considered as having early intrahepatic recurrence [17,18].

### 2.2. Quantitative real time reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA of the tumor and non-tumor tissue samples were extracted by Trizol Reagent (Invitrogen, USA) and an RNeasy Mini Kit (Qiagen, Germany). The isolated RNA was quantified by OD260nm and qualitated by a Bioanalyzer (Agilent Technology, USA). RNA concentration was determined by a spectrophotometer (GeneQuant II, Pharmacia Biotech, England) and an equal quantity of total RNA was used from different samples for RT-PCR. Real-time RT-PCR was performed with SuperScript II Reverse Transcriptase (Invitrogen). In brief, 1 μg total RNA were mixed with 1 μL dNTP (Viogene, Taiwan) and 1 μL oligo-dT primer (MDBio, Inc.) for 5 min at 65 °C. Then, RNA was reverse-transcribed following the manufacturer's protocol. Real-time PCR was performed using the iCycler iQ Real-Time detection system (Bio-Rad, Hercules, CA) with SYBR-Green I (stock solution