人類肝細胞癌侵襲、轉移的分子機轉

Molecular Mechanism of Invasion and Metastasis in Human Hepatocellular Carcinoma

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主持人： 李伯皇

執行機構：台大醫學院外科

e-mail: pohuang@ha.mc.ntu.edu.tw

中文摘要。肝癌為台灣癌症死因之首，angiogenesis 在腫瘤的轉移、成長及分化上扮演重要的角色，然而對腫瘤之再復發至今仍未完全清楚。因此我們收集了 17 個再復發性肝癌病人檢體檢測其 VEGF 及 CD31 的表現。82.4% 原發性及 88.2% 復發性肝癌檢體的肝癌部分與非肝癌部分 VEGF 的表現有明顯差異，而且 70.6% 復發性肝癌檢體 VEGF 的表現明顯高於原發性肝癌檢體。CD31 的檢測結果也與 VEGF 相似。此結果顯示復發性肝癌 angiogenesis 較嚴重，而且 VEGF 對肝癌的再復發扮演著重要的角色。

關鍵字：復發性肝癌、VEGF、CD31

I. ABSTRATE.

Hepatocellular carcinoma (HCC) is the leading cause of cancer deaths in Taiwan. Angiogenesis plays an important role in malignant transformation, and tumor growth and metastasis are angiogenesis-dependent. However, little is known about the recurrence of HCC up to date. In this study, we therefore examine the expression of VEGF and CD31 in 17 primary and recurrent HCCs cases. The expression of VEGF is significant difference in primary HCCs (82.4%, $P < 0.05$) and recurrent HCCs (88.2%, $P < 0.01$), respectively. And in recurrent HCCs, the expression of VEGF is stronger than in primary HCCs (70.6%, $P < 0.05$) also. The result of CD31 is as same as VEGF. These results show that the angiogenesis is more serious in recurrent HCCs, and suggest that VEGF may play an important role in liver tumor recurrence.

Key words: recurrent HCC, VEGF, CD31

II. INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world, especially in sub-Saharan Africa and Southeast Asia (1,2). Since 1984, it is the leading cause of cancer deaths in Taiwan. About 6000-8000 people died of this cancer every year in Taiwan (3). HCCs are characterized by the hypervascularity and have the propensities to invade portal veins, thus precluding many therapeutic strategies such as operation and transcatheter arterial embolization. It has been demonstrated that invasiveness of an HCC is the most crucial factor in determining the long-term outcome for the patient (4). Therefore, tumor angiogenesis, invasion and metastasis are the key points worthy investigation to further understand the tumor behavior and even therapy for HCCs.

Angiogenesis plays an important role in malignant transformation. Tumor growth and metastasis are angiogenesis-dependent (5). The angiogenesis process is regulated by angiogenic factors and angiogenic inhibitors (6). Among the angiogenic factors, vascular endothelial growth factor (VEGF) (also known as vascular permeability factor) is a secretory dimeric disulfide-bonded glycoprotein (7-10). Target cell specificity of VEGF is restricted to vascular endothelial cells (11). It has been demonstrated higher level of VEGF

expression can be found in 60-70% of human HCC tissues (12,13). The VEGF expression in the HCCs is associated with fibrous capsule formation and capsule invasion (14,15). However, whether the VEGF expression is correlated with tumor recurrence and prognosis is still unknown. In this study, we therefore examined the expression of VEGF protein and in situ expression of CD31in each human non-tumoral liver part and primary and recurrent HCCs.

III. METHOD

Tissue specimens. 17 patients were included in the current study. All patients had undergone curative hepatic resection twice for HCC between 1980 and 1997.

VEGF and specificity. HCC samples were cut into pieces and homogenized with lysis buffer for extracting proteins. The proteins were than separated by polyacrylamide gel electrophoresis containing. After electrophoresis the gel was removed and transferred to nitrocellulose membrane. The specificity of each VEGF in non-tumoral HCCs, primary HCCs and recurrent HCCs was confirmed by Western blot analysis. The densitometry value was assessed using computer-assisted image analysis for measuring the relative abundance of VEGF protein in non-tumoral HCCs, primary HCCs and recurrent HCCs.

Statistical analysis. All densitometry values were reported as mean ± SD. Statistical analysis was performed using t-test least significant difference, and P value <0.05 were considered significant.

Immunostaining. Each liver cyrosections (5 μm) were used immunohistochemistry stained for CD31, using the avidin-biotin-peroxidase complex method of Hsu et al., and counterstained with Hematoxylin.

IV. RESULT

VEGF protein expression in primary and recurrent from patients with HCC. The western blot analysis of VEGF was shown in Fig 1. Among the 17 HCCs, VEGF protein expressed in tumoral part was higher than in non-tumoral part in primary HCCs (82.4%) and recurrent HCCs (88.2%), respectively. And the VEGF protein expression in recurrent HCCs (70.6%) is higher than primary HCCs (Table 1). Our statically data showed that there are significant difference from in tumoral part and non-tumoral part in each primary HCCs ($P < 0.05$) and recurrent HCCs ($P < 0.01$), and that in recurrent HCCs and recurrent HCCs ($P < 0.05$) (Table 2 and Fig 1).

Expression of CD31. The immunohistochemistry staining result of CD31was shown in Fig 2. Our data showed the CD31expression are much highest in recurrent HCCs than in primary HCCs. CD31 was distributed in tumor vessel endothelium and accumulated in nearly blood vessel.

V. SELF-CRITICIZE

In this study, we execute the project completely and get good result. We examined the expression of VEGF protein and in situ expression of CD31 in each human non-tumoral liver part and primary and recurrent HCCs. And the results showed the over expression of VEGF and CD31 in recurrent HCCs, which indicated VEGF and CD31 may be used as a marker for liver tumor recurrence

VI. REFERENCE

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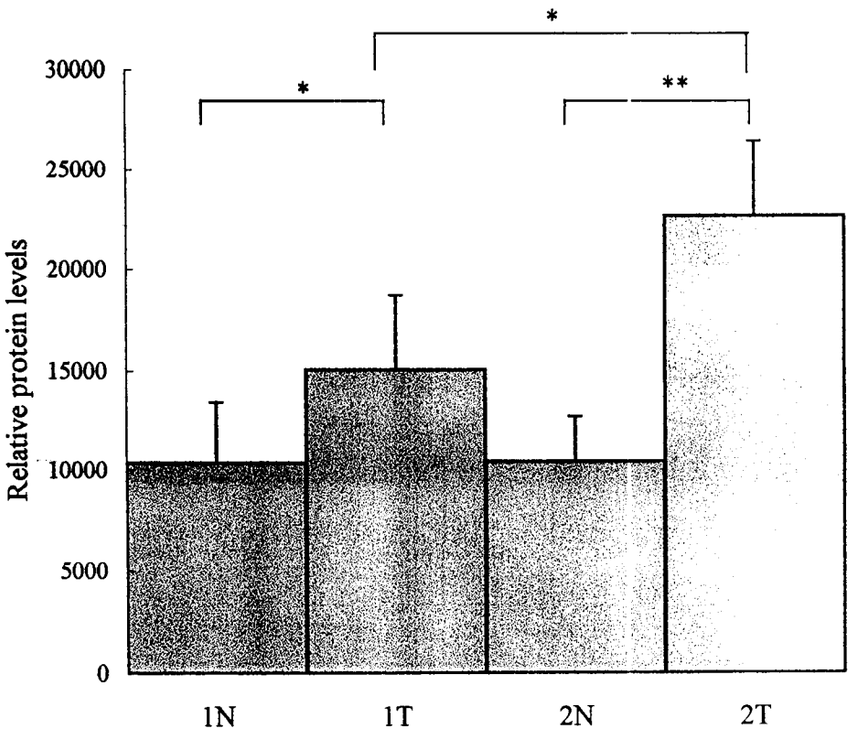

Fig 1. Relative abundance of VEGF protein in non-tumoral HCCs, primary and recurrent HCCs. (men ± SD of four separate experiments). *P<0.05, ** P< 0.01
(1N: non-tumoral part; 1T: primary HCCs; 2N: non-tumoral HCCs; 2T: recurrent HCCs)

Table 1. Percent of patients' cases of the expression of VEGF protein.

percent of patients' cases		
tumoral part > non-tumoral part		tumoral part
primary HCCs	recurrent HCCs	recurrent HCCs > primary HCCs
82.4 %	88.2 %	70.6 %

Table 2.Statyltical analysis of the relative abundance of VEGF protein in non-tumoral HCCs, primary HCCs and recurrent HCCs (mean ± SE).

	primary HCCs	recurrent HCCs
non-tumoral part	10362.76 ± 3063.64	10437 ± 2242.47
tumoral part	15048.53 ± 3654.19	22686.41 ± 3795.15

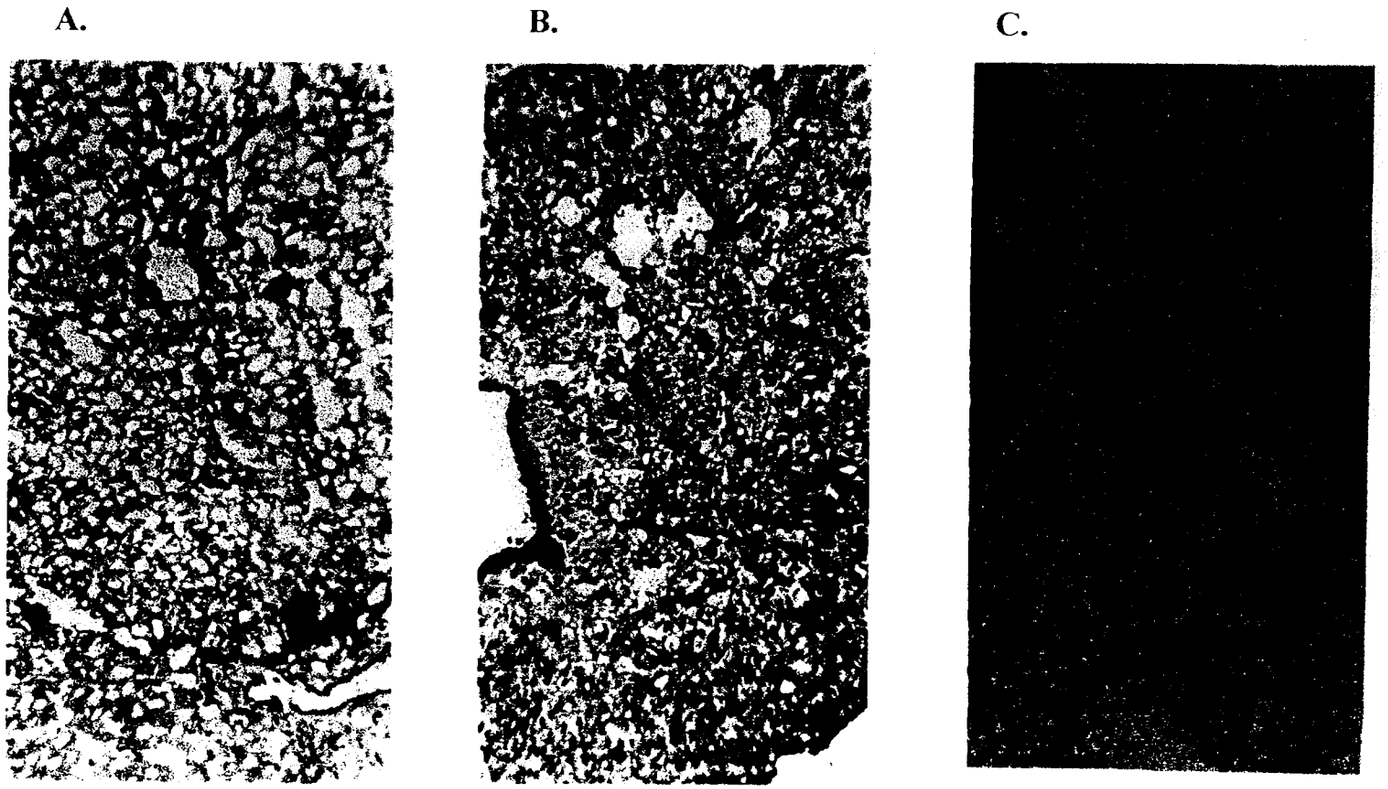

Fig 2. The immunohistochemistry staining for CD31 to investigate the angiogenesis of HCCs (A: primary HCC; B: recurrent HCC; C: non-tumoral parts)