Abnormal Expression of Fibrinogen Gamma (FGG) and Plasma Level of Fibrinogen in Patients with Hepatocellular Carcinoma

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Abstract. Abnormal expression of genes has been related to progression of hepatocellular carcinoma (HCC). The present study investigated the mRNA expression of fibrinogen gamma (FGG) in HCC and levels of plasma fibrinogen of patients with HCC. Materials and Methods: Northern blot and semiquantitative RT-PCR were performed to determine the mRNA transcription of FGG. The plasma fibrinogen was measured quantitatively by the von Clauss method. Results: FGG was significantly up-regulated at the mRNA level in the SMMC-7721 and HepG2 HCC cell lines. FGG mRNA transcript was also up-regulated in HCC tissues when compared to non-cancerous adjacent tissues as control. The laboratory investigation of blood samples from 114 HCC patients showed significantly higher levels of plasma fibrinogen compared to healthy persons as control (3.75±1.41 vs. 2.90±0.46 g l⁻¹) (p<0.01). Moreover, plasma fibrinogen increased progressively with the tumor clinical stage of HCC patients. By multivariate logistic regression analysis, a positive level of plasma fibrinogen was found to have a significant correlation with the presence of tumor thrombosis. Conclusion: FGG mRNA was expressed abnormally in HCC and elevated plasma fibrinogen may serve as a useful predictor of clinical progression of HCC patients.

Fibrinogen, a protein precursor to the main clot structural protein of fibrin, has roles in processes such as blood clotting, fibrinolysis, cellular and matrix interactions, the inflammatory response and wound healing (1). Human plasma fibrinogen is produced by the liver. It is a dimer of three polypeptide chains termed α, β, and γ, whose expression is often up-regulated or down-regulated coincidentally (2). In addition, fibrinogen and its gamma chain containing many specific binding sites and can regulate cellular adhesion and invasion (3, 4). Recently, the correlation of fibrinogen and its degradation products with carcinogenesis was reported in tumors (5-7).

Hepatocellular carcinoma (HCC) is one of the most common human malignances in many countries and areas, particular in Asia and Africa. In China, around 110,000 people die from primary liver cancer each year, among which up to 95% patients were diagnosed as having HCC. It is becoming gradually evident that the development and progression of HCC is closely related to the abnormal expression of genes (8-9), although the molecular mechanism of hepatocarcinogenesis is still uncertain. In a previous study, we constructed a cDNA library which enriched cDNA sequences corresponding to mRNA species that might be specifically up-regulated in human HCC. The results implicated that some differential genes encoding plasma proteins possibly correlated with HCC (10). Here, we identified the mRNA expression of fibrinogen gamma (FGG) in HCC and investigated levels of plasma fibrinogen of HCC patients in order to explore a potential predictor of clinical progression of HCC patients.

Materials and Methods

Tissue specimens and cell lines used. HCC tissues and adjacent non-cancerous tissues were collected fresh from 37 surgical patients with HCC examined by pathology. All tissues were snap frozen in liquid nitrogen and stored at −80°C. The HCC cell lines SMMC-7721 (Institute of cell biology of CAS, Shanghai, P. R. China) and HepG2 (ATCC, Manassas, USA) were cultured in RPMI-1640 (Gibco BRL, Grand Island, USA) supplemented with 10% fetal bovine serum (Gibco BRL), 100 U/ml penicillin G and 100 μg/ml streptomycin. Cells were incubated at 37°C in a humidified atmosphere with 5% CO₂ in air.

Blood samples and measurement. The blood samples mixed with the anticoagulant sodium citrate (9:1) were collected from 114 HCC patients (92 male, 22 female, aged from 19 to 76 years, mean age 52.5 years) diagnosed histologically and 30 healthy volunteers served...
as controls (15 male, 15 female, mean age 41.0 years). All samples were centrifuged for 10 minutes at 3,000 rpm and the supernatants were stored at –80˚C. Fibrinogen levels were measured in all samples by COULTER ACL-200 automated coagulant analyzer. The details of clinical features of patients are given in Table I.

Clinical stage and tumor thrombosis. The Classification of Malignant Tumours (TNM) developed and maintained by the International Union Against Cancer (UICC) was referred to as our tumor clinical stage classifying criterion for 114 HCC patients: I: solitary tumor without vascular invasion; II: solitary tumor with vascular invasion, III: multiple tumors hepatic invasion and lymphoid metastasis. The presence of tumor thrombosis in a major branch of the portal or the hepatic vein was verified by computed tomography with or without angiography.

Northern blot and semiquantitative RT-PCR. The mRNA of SMMC-7721, HepG2 cells and normal hepatocytes were isolated and purified using an mRNA kit (Qiagen, Santa Clarita, USA) as described by the manufacturer. The detailed method of Northern blot was referred to in our previous work (11). Total RNA of tissues was isolated using RNeasy kit (Qiagen) as described by the manufacturer. The PCR primer of FGG was 5'-CTT AGA TGT AAG ATT CGG TAG-3' and 5'-TGT TCT GCG AGT AGG AG-3'. In parallel, human β-actin was amplified to serve as a control. All PCR products were analyzed on 1.0% agarose gel followed by quantitative analysis using densitometry (UVIPro analysis system; UVTec, Cambridge, UK).

Statistical analysis. The results of fibrinogen analysis are reported as mean values ± standard deviation. Comparisons of plasma fibrinogen in different clinical stages were evaluated by Student’s t-test. Relationships between categorical variables were compared using the chi-square test. Plasma level of fibrinogen was divided into two groups (positive and negative) according to the normal upper limit (3.4 g l–1). Odds ratio (OR) as a measure of the relative risk of tumor thrombosis was estimated by means of multivariate logistic regression analysis. Age, gender, HBsAg, Child-Pugh class and α-fetal protein (AFP) were included as covariates. P-values <0.05 were considered significant. All these tests were performed using SPSS 10 software (Chicago, USA).

Results

Analysis of FGG mRNA expression in HCC by Northern blot and RT-PCR. Northern blot analysis demonstrated that the FGG mRNA expression was up-regulated in SMMC-7721 and HepG2 HCC cell lines (Figure 1). By quantitative analysis on the RT-PCR products using densitometry, it was found that the expression of FGG mRNA was significantly higher in cancer tissues than in the adjacent non-cancerous tissues of patients with HCC (Figure 2 A and B).

Measurement of plasma fibrinogen levels in HCC patients. The mean plasma fibrinogen level in 30 healthy persons as the control was 2.90±0.46 g l–1 whereas that of 114 HCC patients was 3.75±1.41 g l –1. There was a significant difference between healthy control and HCC patients (p<0.01). Plasma fibrinogen levels had an increasing trend with the progression of clinical stage, in particular, the level of plasma fibrinogen was remarkably higher in clinical stage Ⅲ (4.07±1.07 g l–1) compared with stage Ⅰ (3.28±0.22 g l –1) and stage Ⅱ (3.60±0.78 g l –1) (Figure 3).

The correlation between plasma fibrinogen and tumor thrombosis. Positive fibrinogen (>3.4 g l –1) correlated strongly with the presence of tumor thrombosis (p<0.01) (Table II). Furthermore, we analyzed the thrombosis risk

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<th>Table I. Clinical features of HCC patients.</th>
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| Median age (range) | 49.7±2.1 | 51.9±1.8 | NS |%
| Gender (male/female) | 56/14 | 36/8 | NS |%
| HbsAg (negative/positive) | 20/50 | 14/30 | NS |%
| Child-Pugh class (%) | A: 54 | 20 | NS |%
| B: 12 | 18 | NS |%
| C: 4 | 6 | NS |%
| AFP (ng/ml) | 796±47 | 810±52 | NS |%
| Fibrinogen (g/l) | 3.21±0.43 | 4.61±0.12 | <0.001 |%

NS: Not significant; AFP: α-fetal protein.

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<th>Table II. Correlation between plasma fibrinogen levels and tumor thrombosis in HCC patients.</th>
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| Fibrinogen (#) | | | <0.01 |%
| Positive (%) | 22 (19.3) | 36 (31.6) | |
| Negative (%) | 48 (42.1) | 8 (7.0) | |

#Positivity indicates that plasma fibrinogen was greater than the normal limit value of 3.4 g l–1.
after adjustment for age, gender, HbsAg, Child-Pugh class and AFP. The unadjusted OR for the risk of tumor thrombosis in the fibrinogen positive group versus the negative group was 14.51 (95% confidence interval (CI), 4.05-41.37), and this risk remained significant after adjustment (OR 10.26, 95% CI, 2.13-58.42).

**Discussion**

Fibrinogen gamma was found to be overexpressed in pancreatic cancer and fibrinogen was taken as a potential tumor marker in pancreatic cancer (5). Our present study demonstrated that FGG mRNA was also up-regulated in both HCC cell lines and HCC cancer tissues. Thus, FGG expression could play an important role in cancer. Since FGG is one of three polypeptide chains of fibrinogen, it is possible that synthesis of fibrinogen might be increased while FGG expression was up-regulated. The investigation of blood samples from 114 HCC patients showed that the plasma level of fibrinogen was inhead significantly higher than that in healthy persons (\( p<0.01 \)). Moreover, fibrinogen progressively increased accompanying clinical stages I, II and III of HCC patients, which suggested that fibrinogen might be correlated with clinical progression of HCC patients.

![Figure 2. Semiquantitative RT-PCR analysis of FGG mRNA expression in HCC tissue. A, RT-PCR products (part results shown). Top panel: FGG mRNA; bottom panel: β-actin mRNA; lanes 1, 3, 5, 7, 9: cancer tissues; lanes 2, 4, 6, 8, 10: non-cancerous adjacent tissue as controls. B, Density value of RT-PCR products shown in A by densitometry.](image)

![Figure 3. Plasma fibrinogen levels of HCC patients in different clinical stages. \#p<0.05, compared with stage I; ##p<0.01, compared with stage I.](image)

HCC exhibits a high frequency of tumor invasion into the portal or hepatic vein (12). The exact mechanism of venous invasion in HCC remains unclear, but active neovascularization of the tumor is likely to play an important role. Our results showed a significant relationship between plasma fibrinogen and tumor thrombosis, and a positive
fibrinogen level was found to be significant for predicting the presence of tumor thrombosis by multivariate logistic regression analysis, which suggests that the determination of plasma fibrinogen levels in HCC patients might be useful for identifying tumor thrombus in the portal or the hepatic vein. Previous studies also showed that plasma fibrinogen was significantly increased in patients with colorectal carcinoma, which suggested that high plasma fibrinogen might represent a further risk factor for venous thrombus and was involved in the progression of the disease (6, 13). It was also found that preoperative plasma fibrinogen levels in gastric cancer patients correlated with the extent of tumor (14). Therefore, there is a close relationship between a high plasma fibrinogen and tumor progression. However, its exact function in the process of cancer progression is still to be further explored.

In conclusion, the present study demonstrated that FGG mRNA was expressed abnormally in HCC and an elevated level of plasma fibrinogen was related to clinical stage and tumor thrombosis, which suggests the latter to be a useful predictor of clinical progression of HCC patients.

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References


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