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The Diagnosis value of Fucosylated GP73 in Hepatocellular carcinoma

Lianhua Cui*, Chongchong Jiao, Yuncai Liu, Hongzong Si
Department of Public Health, Qingdao University Medical College, Qingdao, China

Abstract: **Objective: To analyze the application and clinical values of fucosylated Golgi protein 73 (Fuc-gp73) in diagnosing hepatocellular carcinoma.Methods: A total of 481 serum samples were collected, including 218 cases of HCC, 61 cases of chronic hepatitis B patients, 96 cases of liver cirrhosis, 106 cases of healthy controls, from Sep. 2011 to Apr. 2013 in Affiliated Hospital of Qingdao University Medical College. Fuc-gp73 was isolated by using microcentrifugalcolumn conjugated with Lentil Lectin (LCA), LCA affinity ELISA was used to detect the serum level of Fuc-gp73. Chemiluminescent immunoassay was used to detect the serum level of AFP. Results: The serum level of Fuc-gp73 was uncorrelated with age, gender, tumor TNM stage, tumor size and serum AFP level. Serum Fuc-gp73 level in patients with lymph node metastasis was significantly higher than that non-metastasis($P<0.05$). Fuc-gp73 level in HCC group was significantly higher than liver cirrhosis group, chronic hepatitis group and healthy control group. Fuc-gp73 level in liver cirrhosis group was significantly higher than chronic hepatitis B and healthy control groups. The area under the curve (AUC) of Fuc-gp73 and AFP in single detection for the diagnosis of HCC was 0.939 and 0.818, respectively. Fuc-gp73 had a sensitivity of 83% and a specificity of 95.1%,when the put 8.24ng/ml as the optimal cut-off point. Conclusion: Serum Fuc-gp73 was highly expressed in the patients with HCC, and its level correlated with lymph node metastasis of HCC. The diagnosis accuracy on HCC of serum Fuc-gp73 was better than serum AFP.**

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* Corresponding Author: Lianhua Cui, qdlhcui@163.com

1. Introduction

Hepatocellular carcinoma (HCC) is one of the common malignant tumors worldwide and its incidence and mortality have an upward trend year by year [1]. The majority of HCC cases are detected at advanced stages of the disease, and the prognosis of HCC is generally poor. Consequently, early diagnosis and treatment are the keys for effective treatment of patients with HCC. Nowadays, the most commonly used monitoring method is regular monitoring of plasma fetoprotein (alpha fetoprotein, AFP) level and supersonic inspection for the high-risk groups of HCC. However, the sensitivity and specificity of AFP are far from satisfactory[2]. Therefore, to seek the new tumor markers with higher sensitivity and specificity has important meaning for improving the diagnosis accuracy of HCC and making effective treatment strategies.

In recent years, with the rapid development of genomics and proteomics research, some potential new tumor markers have been found. The Golgi membrane protein 73 (Golgi protein 73, GP73) possibly could serve as a better the serum markers in the diagnosis of HCC. GP73 is a resident Golgi-specific membrane protein expressed by biliary epithelial cells in normal liver, and its expression is increased markedly in chronic liver diseases, especially in HCC cells [7]. Glycosylation segments of GP73 is the GP73 heterogeneity, also known as fucosylated golgi protein 73 (Fuc - GP73), it has a great potential value in the early diagnosis of HCC [3]. Therefore, this study was to investigate the expression and clinical significance of serum Fuc-gp73 in patients with chronic hepatitis, cirrhosis and liver cancer patients and normal group to evaluate the detection value of Fuc-gp73 in the diagnosis of HCC.

2. Methods

2.1. Study Subjects

This study subjects included 218 cases of patients with HCC, 96 patients with chronic hepatitis, 61 cases of patients with cirrhosis and 106 cases of healthy controls, which were obtained from September 2011 and April 2013 in Qingdao University medical college affiliated hospital. 218 cases of HCC patients were diagnosed by histopathology or conformed to the 2010 NCCN guidelines. All patients have never received operation, chemotherapy, interventional, or molecular targeted therapy. The stage of HCC is according to the TNM stage of NCNN. Hepatic cirrhosis was diagnosed by histological examination or conformed to hepatic cirrhosis diagnostic criteria of the prevention guide of chronic hepatitis B. Chronic hepatitis conformed to the guide of chronic hepatitis diagnostic criteria of the prevention guide of chronic hepatitis B. This study was

approved by Qingdao university medical college ethics committee and informed consents were obtained from all patients. We collected demographic information, clinical information and peripheral venous blood from every subject.

2.2. Specimen Collection

A blood sample was collected from each subject, the supernatant was obtained after high speed centrifugation, and the serum stored at - 80 ℃ until testing.

2.3. The Purification Steps of the GP73 variant

According to the manufacturer's instructions (Hotgen Biotechnology Co., Ltd., Beijing, P.R. China) [4], the purification of the GP73 variant was carried out. The Purification Steps of the GP73 variant were as follows:

(1) To centrifugal the serum samples completely, take out the centrifugal column which has been saved at 4 ℃ and refuse to the lower liquid of collection tube.
(2) Blood dilution: draw 250μl serum in vitro, add it to 350μl cleaning fluid to dilute and mix by gently swirling.
(3) Add the sample: draw 450μl diluted samples to join the upper part of the centrifugal tube, which was then put in an incubator chamber at 37 degrees. This step need not cover the centrifugal tube cover, waiting for the diluent flows into the lower of collection tube, it need about 20 minutes.
(4) Abandon the liquids in the lower of collection tube.
(5) Add 600μl cleaning fluid into the upwards of centrifuge tube, waiting for all cleaning fluid flow into the bottom of collecting tube (about 5 minutes), covering the centrifugal tube cover, 3000 turn centrifugal 1 min at room temperature.
(6) Abandon the liquids in the lower of collection tube.
(7) Repeat step (5) (6) again.
(8) Add 450μl eluent into the upwards of centrifugal tube, waiting for all eluent flow into the bottom of collecting tube, covering the centrifugal tube cover, incubate for 30 minutes at 37 ℃ constant temperature box.
(9) Put out of the centrifuge tube, 3000 turn centrifugal
(10) 1 min at room temperature.
(11) Collected the liquid into the lower collection Tube (containing GP73 variant), set aside.
(12) To detect the content of GP73 variant in collecting Liquid.

2.4. Measurement of Serum Fuc-gp73 and AFP

Serum Fuc-gp73 was isolated by using microcentrifugal column conjugated with lens culinaris agglutinin (LCA), LCA affinity ELISA was used to detect the serum level of Fuc-gp73 quantitative assay according to the manufacturer's instructions (Hotgen Biotechnology Co., Ltd., Beijing, P.R. China) [4]. Serum AFP levels were measured using a Chemiluminescent immunoassay.

2.5. Statistical Analysis

All analyses were performed using the Statistical Package for the social Sciences software (ver.13.0; SPSS, Chicago,IL,USA). The relationship between the Fuc-gp73 and clinical features of HCC patients were analyzed using the chi-square test.the serum Fuc-gp73 levels are presented as the median and were analyzed with a Kruskal-Wallis H test. The best cutoff value of the serum Fuc-gp73 and AFP in the diagnosis of HCC were determined by using the receiver-operating characteristic curve (ROC curve). We also evaluated the value of Fuc-gp73 and AFP value for early diagnosis of HCC by using ROC curve.

3. Results

3.1. Study Characteristics

A total of 481 samples, including 218 patients with liver cancer, 61 patients with liver cirrhosis, 96 patients with chronic hepatitis, 106 healthy control group. There

Table 1 Relationship between Fuc-gp73 and clinical characteristics in HCC

Characteristics	Number	Fuc-gp73 Positive N(%)	χ^2 value	*P* value
Gender				
Male	168	90（53.6）	0.90	＞0.05
Female	50	29（58.0）		
Age (years)				
＜50	72	43（59.7）	0.71	＞0.05
≥50	146	102（69.8）		
TNM stage				
Ⅰ＋Ⅱ	145	58（40.0）	0.39	＞0.05
Ⅲ+Ⅳ	73	36（49.3）		
Tumor size(cm)				
<5cm	103	66（64.1）	0.92	＞0.05
≥5cm	115	64（55.7）		
Lymph node metastasis				
Yes	107	77（72.0）	11.36	
No	111	41（36.9）		＜0.05
HBsAg				
Negative	52	30（57.7）	1.07	＞0.05
Positive	166	87（52.4）		
AFP (ng/ml)				
<400	142	90（63.4）	0.44	＞0.05
≥400	76	45（59.2）		

was no statistically significant difference on the age and gender among the four groups (P > 0.05). The number of HBsAg positive in liver cancer, liver cirrhosis, hepatitis and healthy control group are 166, 21, 67 and 19, respectively.

3.2. Relationships Between Serum Fuc-gp73 and Clinical Features

The relationship between serum fuc-gp73 and clinical features are shown in table 1. The serum levels of Fuc-gp73 were uncorrelated with age, gender, tumor TNM stage, tumor size and serum AFP level. Serum Fuc-gp73 levels in HCC patients with lymph node metastasis was significantly higher than that patients with non-metastasis (P＜0.05)

3.3. Distribution of Serum Fuc-gp73 in Different Groups

Table 2 shows the distribution of serum fuc-gp73 in different groups. The serum Fuc-gp73 levels of liver cancer group was significant higher than that in liver cirrhosis group, chronic hepatitis group and healthy control group (P<0.01). Fuc-gp73 level in liver cirrhosis group was significantly higher than chronic hepatitis B and healthy control groups (*P*＜0.01). There was a significant statistical difference of Fuc-gp73 level between chronic hepatitis B group and healthy control group (*P*＜0.01).

Table 2 Serum Fuc-gp73 levels in different group.

Group	Number	Fuc-gp73（ng/mL） M(95% CI)
Liver cancer	218	20.08(2.62-97.30)
Liver cirrhosis	61	4.35(0.38-11.09) [a]
Chronic hepatitis	96	1.85(0.00-6.79) [a b]
Healthy controls	106	1.04 (0.00-5.28) [a b c]

a : Compared with liver cancer group, P＜0.01;

b : Compared with liver cirrhosis group, P＜0.01;

c: Compared with chronic hepatitis group, P＜0.01.

3.4. The Role of Fuc-gp73 and AFP in the Diagnosis of HCC

ROC curves showed that the area under the curve (AUC) of Fuc-gp73 and AFP in single detection for the diagnosis of HCC was 0.939 and 0.818, respectively. Fuc-gp73 had a sensitivity of 83% and a specificity of 95.1%,when the put 8.24ng/ml as the optimal cut-off point. Fuc-gp73 level had a significantly higher sensitivity and specificity than AFP in diagnosis of HCC (P <0.01).

4. Discussion

The present study demonstrated that patients with HCC exhibit markedly higher levels of Fuc-gp73 in the serum compared with patients with chronic hepatitis, liver cirrhosis and healthy controls, and its level correlated with lymph node metastasis of HCC. Serum Fuc-gp73 on the diagnosis accuracy of HCC was better than serum AFP, and could make up the false dismissal rate of AFP.

Although serological α-fetoprotein (AFP) is widely used for the diagnosis of HCC, it was not a optimal serological marker due to its low sensitivity and specificity. 30% to 40% of HCC patients were low or negative serum level of AFP, and AFP will also increase in the chronic liver diseases and other malignant tumors [5-7].

Glycosylation is one of the common ways in the post-translational modification of proteins and about 50% of the protein has been glycosylation modification [8]. Researches show that degree of glycosylation of glycoprotein has changed in the process of the occurrence and development of many diseases [9]. Among them, core fucosylation pays an important role in the development of tumor. Core fucosylation is a type of N-linked glycosylation in which an α1, 6-linked fucose is added to the innermost N-acetylglucosamine (GlcNAc) residue. It has been demonstrated that core fucosylation is involved in regulating biological processes in mammals [10, 11]. Additionally, abnormal core fucosylation has been observed in human pathological processes, including oncogenesis and metastasis [12, 13]. Previous study indicate that the expression of the core fucosylation significantly changes in the hepatocarcinogenesis [14], and GP73 will also have core fucosylations along with liver carcinogenesis, which .may be more closely with the incidence of HCC [15]. In 2008, Norton [3] et al. confirmed that the GP73 was secreted by HCC cells, which was a kind of fructose by traditional affinity lectin chromatography, and the further study found that 72% of GP73 secreted by HCC cells have core fucosylation. While the study regarding on Fuc-gp73 in the early diagnosis of HCC has just started, the related research reported rarely. In a study included 80 patients with liver cancer, sensitivity and specificity of Fuc-gp73 in the diagnosis of HCC were 90% and 100% by mass spectrometry analysis, respectively[16]. But it was not suitable to the clinical application because of complexity and high cost of mass spectrometry.

As for Fuc-gp73 diagnosis value of HCC, the detection of Fuc-gp73 and AFP were determined by ROC curves. The ROC-area of the detection of Fuc-gp73 is 0.939 when it is used in the diagnosis of liver cancer, the optimal cutoff value of 8.24 ng/ml, the sensitivity and specificity were 0.830 and 0.951, respectively. Compared with the research by Drake [16] et al, the sensibility and specificity of our research were

decreased. It may be related with different sources of study population, sample sizes and detection means. We also found that the expression of serum Fuc-gp73 is not significantly related with clinical index of age, gender, tumor size, stage of TNM and AFP. While serum Fuc-gp73 level in patients with lymph node metastasis was significantly higher than that non-metastasis. Compared with serum AFP, the detection of serum Fuc-gp73 level had a significantly higher sensitivity and specificity than AFP in diagnosis of HCC.

Fuc-gp73 may become a better marker than AFP in the diagnosis of HCC, but there are still some limitations. First of all, this is just the first phase of the biological markers study, which still need more verification work before clinical application; Secondly, because of the limitation of study population and sample sizes, current researches can not confirm the relationship between the expression of new markers and the occurrence and development of liver diseases; Thirdly, because of current Fuc-gp73detection method is complicated, time-consuming and exhausting. Fuc-gp73 is not suitable for high-throughput analysis of multicenter, large sample research.

5. Conclusion

In conclusion, this research made a preliminary evaluation about the diagnostic value of Fuc-gp73's in HCC and the result show that Fuc-gp73 has higher sensibility and specificity than AFP. Whether Fuc-gp73 can be a marker for early diagnosis of HCC, it requires a powerful clinical data to support.

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