Fibrinogen Like Protein 1 as a Potential Biomarker for Hepatitis B Virus Related Hepatocellular Carcinoma

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Research Article

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Abstract

Background

There is an urgent need for new serum biomarkers for early screening of HBV-related hepatocellular carcinoma (HCC). Fibrinogen like protein 1 (FGL1) may develop the potential diagnostic value of alpha fetoprotein (AFP) in HBV-related HCC.

Methods

The TCGA database was used to screen out genes related to liver cancer and perform differential expression analysis. Enzyme-linked immunosorbent assay and chemiluminescence immunoassay were used to detect concentrations of FGL1 and AFP. Using immunofluorescence semi-quantitative method to detect the mean fluorescence intensity of FGL1.

Result

FGL1 is lower in tumor tissues than in normal tissues. The serum levels of FGL1 and AFP in patients with HBV-related HCC are significantly higher than others for each group. Compared with other groups, the area under the receiver operating curve (AUC) of FGL1 is higher than that of AFP when compared with the normal group, and the AUC of other groups is lower than that of AFP. The combination of the two can increase the AUC to 0.862 (95%CI, 0.786 ~ 0.918) in distinguishing benign liver disease from HBV-related HCC. The specificity of FGL1 and AFP in the diagnosis of HBV-related HCC is 98.39% and 70.97%, respectively. The specificity of the combination was 93.55%. In distinguishing the A and B stages in the BCLC staging, the combination of the two increased the AUC from 0.584 to 0.647. When distinguishing benign liver disease from HBV-related HCC, the AUC of FGL1 reached 0.849, with a specificity of 100%.

Conclusion

FGL1 can be used as a non-invasive biomarker for HCC. When combined with AFP, the diagnostic efficiency and specificity were improved.

1. Background

Globally, primary hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer death, with approximately 906,000 new cases and 830,000 deaths ^[1]. Hepatitis B virus (HBV) accounts for half of the global viral hepatitis deaths, half of all liver cancer deaths and one third of all liver cirrhosis deaths ^[2]. In China, HBV infection accounts for 63% of the total deaths from cirrhosis and other chronic liver diseases, and 53% of the total deaths from liver cancer. China is the country with the largest number of hepatitis B and C virus infections in the world ^[3]. Although China vigorously popularizes hepatitis B vaccine injection, it is estimated that there are still 77 to 97 million chronic HBV infections, of which more than 20 to 30 million are in active liver disease ^[4], and about 7 million develop cirrhosis or liver cells Cancer ^[5]. Although the diagnosis and treatment of HCC have made great progress in recent years, it still cannot change the fact that most of the confirmed cases of HBV-related HCC are in the middle and late stages, and the metastasis and recurrence of HCC have led to no significant improvement in the five-year survival rate of patients. So far, Alpha fetoprotein (AFP) has been widely used in the laboratory for early screening of HBV-related HCC, but its diagnostic value still needs to be improved. Some HCC patients express low level AFP and cause AFP negative. The commonly used positive cut-off value is $AFP \geq 20 \text{ ng / ml}$ ^[6]. Therefore, there is an urgent need for new serum biomarkers for early screening of HBV-related HCC.

Advances in bioinformatics and the development of high-throughput sequencing have surfaced some tumor markers. For example, it found that GBP5 gene is highly correlated with immune infiltration ^[7]. DPP9 plays an important role in the pathogenesis of human HCC ^[8]. The appearance of these tumor markers is helpful for the diagnosis of tumors. In this study, immune-related biomarkers were screened in order to find markers related to HCC. FGL1 contains a fibrinogen related domain in its C-terminal portion, which is similar to tenascins, fibroleukin, angiopoietins, fibrinogen β and γ chains ^[9]. The difference is that FGL1 lack of fibrinogen's three functional domains, such as the platelet binding-site, the cross-linking region and the thrombin-sensitive site. It is regarded as an acute phase reactant which is secreted by liver ^[10].

2. Materials And Methods

2.1 OncoLnc Database Analysis

OncoLnc (<http://www.oncolnc.org/>) contains survival data from 8,647 patients and 21 cancer studies conducted by the Cancer Genome Atlas (TCGA), as well as mRNA and miRNA RNA-SEQ expression from TCGA, and from the lncRNA expression of MiTranscriptome beta. Use OncoLnc for hepatocellular carcinoma-related gene screening.

2.2 GEPIA Database Analysis

GEPIA (Gene Expression Profiling Interactive Analysis) (<http://www.oncolnc.org/>) has always been a valuable and highly cited resource for gene expression analysis based on tumor and normal samples from TCGA and GTEx databases. Contains RNA sequencing data based on 9,736 tumor samples and 8,587 normal samples from TCGA and GTEx databases. We use Plotly to organize and plot GEPIA data.

2.3 Research Object Selection

The proportion of males in HCC patients in China is higher than that of female patients, so this part of study only included male patients. On the other hand, it also controlled for the interfering factors brought by gender variables. All patients were recruited in the Department of Oncology, Department of Infectious Diseases and Physical Examination Center, Renmin Hospital of Wuhan University, Hubei Province, China from April 2021 to June 2021. According to the guidelines of the Chinese Society of Hepatology, the Chinese Society of Infectious Diseases and the Chinese Medical Association^[11], all 153 patients and normal controls were divided into HBV-related HCC group (55 cases), HBV-infected liver cirrhosis (LC) group (31 cases), and chronic hepatitis B (CHB) group (31 cases) and the control group of healthy people (HC) (36 cases). The pathological tissues of liver cancer patients in the Department of Oncology, Renmin Hospital of Wuhan University, Hubei Province, China from April 2021 to June 2021 were collected. According to relevant guidelines, they were divided into HCC group (4 cases) and normal population control group (3 cases). All HCC patients were confirmed by liver pathological examination, X-ray examination or MRI examination. Exclusion criteria for included cases: (1) Exclude malignant tumors other than primary liver cancer. (2) Exclude other hepatitis virus infections other than HBV. (3) Exclude diseases such as severe diabetes, hyperthyroidism, and cardiovascular disease. (4) Exclude pregnant patients. All normal control populations tested negative for HBV, HCV, syphilis and HIV as well as all biochemical tests were normal.

This study has been reviewed and approved by the Medical Ethics Review Committee of Renmin Hospital, Wuhan University. According to the requirements of the Medical Ethics Review Committee of Renmin Hospital, Wuhan University, all the included persons agreed and signed the informed consent form.

2.4 Sample Collection

Venous blood was collected from patients who had been fasted for more than 8 hours in the morning, centrifuged at 3500/rpm for 15 minutes at room temperature, and transferred the serum from the collection tube with separating gel to the cryotube, and stored it at -80°C for further use. Blood was collected using an anticoagulant tube, immediately reversed and mixed, centrifuged after several minutes, and plasma was collected. We use 10% paraformaldehyde to fix the fresh tissue, use gradient concentration of alcohol to dehydrate the tissue block, and then immerse it in xylene. After the tissue block is transparent, it is immersed in paraffin for embedding and sectioning, and stored at room temperature.

2.5 Laboratory Analysis

Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ -glutamyl transpeptidase (γ -GT), albumin (ALB), total bilirubin (TBIL), hypersensitive C-reactive protein (hs-CRP) and direct bilirubin (DBIL) was analyzed by enzymatic method using the fully automatic biochemical analyzer ADVIA 2400 (Siemens, Germany). SysmexCA-7000 (Kobe, Japan) was used to detect fibrinogen (FIB) and d-dimer. We use ABI ViiA7 real-time fluorescent quantitative polymerase chain reaction system (ABI, America) to detect HBV DNA. AFP was detected using Siemens ADVIA Centaur XP (Siemens, Germany).

We use a commercial enzyme-linked immunosorbent assay (ELISA) kit (product number JL47875) produced by Jianglai Biological Company to detect FGL1. The kit uses a double-antibody one-step sandwich method for enzyme-linked immunosorbent assay. The detection range is 46.875pg/ml~1500pg/ml, and the specimens were diluted 100 times for testing, and the auxiliary hole is set to ensure the detection accuracy.

2.6 Immunofluorescence Quantification

Purchase the FGL1 antibody (Cat. No.: 67391-1-Ig) produced by Proteintech Group, and entrust Siwega Biotechnology (Wuhan) to perform immunofluorescence quantitative experiments.

2.7 Data Analysis

The experimental data was analyzed using SPSS version 20.0 (IBM Corp, NY) and MedCal 15.2.2 (Ostend, Belgium), GraphPad Prism 6.0 (GraphPad Software, Inc, La Jolla, CA) was used for image rendering, and Image J 1.53J (National Institutes of Health, Bethesda) for immunofluorescence quantitative analysis. The measurement data uses the single-sample Kolmogorov-Smirnov method to test whether the data of each group conforms to normality, the normal distribution data is represented by $\bar{x} \pm s$, the comparison between multiple groups is performed by analysis of variance, and the further pairwise comparison is performed by LSD-*t* test. The skew distribution data is represented by $M(P_{25} \sim P_{75})$, the comparison between multiple groups uses the Kruskal-Wallis H test, the comparison between the two groups uses the Mann-Whitney U test, and the pairwise comparison uses the Bonferroni adjustment test level method. Perform binary logistic regression analysis to calculate the predictor variables, and draw the ROC curve of AFP, FGL1 and joint detection, and calculate the area under the curve, sensitivity, and specificity. $P < 0.05$ indicates that the difference is statistically significant.

3. Results

3.1 Gene Expression

Five hepatocellular carcinoma related genes were screened by OncoLnc, namely fibrinogen like 1 (FGL1), growth differentiation factor 15 (GDF15), glucose-6-phosphate dehydrogenase (G6PD), junctional adhesion molecule 2 (JAM2), hyaluronan mediated motility receptor (HMMR). We expand the scope to analyze the expression of these five genes in LIHC, LGG, LUAD, PAAD and ESCA. It is found that FGL1 is highly expressed in hepatocellular carcinoma than other cancers. Analysis of the FGL1 mRNA expression level in tumor and normal tissues shows that the expression of FGL1 in tumor tissues is significantly lower than that in normal tissues, and the difference is statistically significant. ($P<0.05$) The results are shown in the Figure1 and Table 1.

Table 1. Gene expression

	LIHC	LGG	LUAD	PAAD	ESCA
FGL1	9.5	0.4	1.8	1.6	0
GDF15	5.5	0.7	5.9	5.1	3.8
G6PD	3.4	4.8	5.8	4.8	6.2
JAM2	1.3	5.8	2.2	2.8	1.7
HMMR	1.8	1.0	2.6	2.1	3.9

3.2 Characteristics of The Study Population

There was no significant difference in age between the groups ($P>0.05$). Through the analysis of liver biochemical indicators, ALT, AST, ALP, GGT, ALB, TBIL, DBIL, hs-CRP, the differences in the control group, CHB group, LC group and HCC group were statistically significant ($P<0.05$, $P<0.001$). The differences in the expression of blood coagulation indexes D-dimer and FIB in each group were also statistically significant ($P<0.001$). The HBV DNA content in the CHB group was significantly higher than the other three groups ($P<0.001$). The disparity in the expression of AFP and FGL1 in each group was statistically significant ($P<0.001$). The basic information of all the population included in the study in Table 2.

Table 2. Characteristics of the study population

Characteristics	Controls (n = 36)	CHB (n = 31)	LC (n = 31)	HCC (n = 55)	P
Age (years)	55.61±4.85	55.10±5.20	55.35±5.72	54.56±6.76	>0.05
ALT (U/L)	19.00(14.25~26.75)	59.00(29.00~169.00)	49.00(28.00~88.00)	31.00(26.00~48.50)	<0.001
AST (U/L)	21.00(17.00~23.00)	43.00(25.00~108.00)	49.00(35.00~108.00)	38.50(29.00~59.50)	<0.001
ALP (U/L)	67.20(61.58~80.38)	87.80(69.70~131.80)	98.60(75.00~148.20)	114.00(81.00~185.15)	<0.001
GGT (U/L)	14.00(17.50~25.75)	22.00(42.00~150.00)	70.00(32.00~105.00)	77.00(39.50~180.50)	<0.001
ALB (g/L)	43.75(41.90~45.08)	43.00(38.60~44.50)	31.50(28.40~35.30)	39.20(35.60~41.95)	<0.05
TBIL (μmol/L)	12.15(9.20~14.75)	20.70(13.50~30.60)	21.10(15.00~30.40)	19.00(11.95~26.85)	<0.001
DBIL (μmol/L)	3.90(3.20~4.93)	6.50(4.00~10.60)	15.1(5.70~45.40)	6.20(4.20~10.00)	<0.001
D-dimer (mg/L)	0.32(0.15~0.65)	0.31(0.18~0.74)	1.70(0.27~4.75)	0.69(0.30~2.27)	<0.001
FIB(g/L)	2.73(2.28~3.25)	2.04(1.81~2.33)	1.88(1.41~2.30)	2.97(2.24~3.46)	<0.05
hs-CRP (mg/dL)	3.23(1.20~4.58)	2.50(0.66~8.36)	1.18(0.33~5.18)	6.04(1.25~21.07)	<0.001
HBVDNA(IU/mL)	N	14200.00(347.00~376000.00)	169.00(100.00~118000.00)	100.00(100.00~11900.00)	<0.001
FGL1(ng/ml)	0.74(0.48~1.14)	25.36(16.79~27.23)	21.24(10.00~25.88)	37.89(20.75~114.35)	<0.001
AFP (ng/mL)	3.00(2.20~4.35)	7.90(1.78~128.75)	4.85(2.20~11.98)	109.60(8.70~8497.35)	<0.001

3.3 Concentrations of AFP and FGL1 In Different Groups

The Kruskal-Wallis test was used to analyze the different expression of AFP and FGL1 concentrations in serum in the normal control population, CHB group, LC group and HCC group. FGL1 in CHB group [25.36(16.79~27.23) ng/ml], LC group [21.24(10.00~25.88) ng/ml] and HCC group [37.89(20.75~114.35) ng/ml] was significantly higher than that of the healthy control group [0.74(0.48~1.14) ng/ml], which was statistically significant ($P<0.001$, $P<0.001$, $P<0.001$). The expression of FGL1 in HCC group [37.89(20.75~114.35) ng/ml] was obviously higher than CHB group [25.36(16.79~27.23) ng/ml] and LC group [21.24(10.00~25.88) ng/ml], with statistically significant ($P<0.001$, $P<0.001$), but there was no statistically significant difference in the concentrations of FGL1 between the CHB group and the LC group ($P>0.05$). The expression of FGL1 in AFP-negative CHB group [22.13(9.37~28.57) ng/ml], AFP-negative LC group [19.43(7.95~27.64) ng/ml] and AFP-negative HCC group [39.05(27.24~115.56) ng/ml] was significantly higher than that of the healthy control group [0.74(0.48~1.14) ng/ml], which was statistically significant ($P<0.001$, $P<0.001$, $P<0.001$). The expression of FGL1 in the AFP-negative HCC group [39.05(27.24~115.56) ng/ml] was significantly higher than that in the AFP-negative CHB group [22.13(9.37~28.57) ng/ml] and the AFP-negative LC group [19.43(7.95~27.64) ng/ml], with statistically significant, ($P<0.001$, $P<0.001$), but there was no obvious difference in the expression of FGL1 between the AFP-negative CHB group and the AFP-negative LC group. The dissimilar expression was not statistically significant ($P>0.05$). The expression of AFP in the HCC group [109.60(8.70~8497.35) ng/mL] was higher than that in the HC group [3.00(2.20~4.35) ng/mL], the CHB group [7.90(1.78~128.75) ng/mL] and the LC group [4.85(2.20~11.98) ng/mL], the different concentrations were statistically significant, ($P<0.001$, $P<0.001$, $P<0.001$). In the LC group [21240.12(9998.42~25882.08) pg/ml] and the CHB group [7.90(1.78~128.75) pg/ml], the expression of AFP was also significantly higher than that of the HC group [3.00(2.20~4.35) ng/mL], Is statistically significant, ($P<0.001$, $P<0.001$). As shown in Table 2, Figure 2.

3.4 The Diagnostic Value Of FGL1 And AFP Alone And In Combination In The HC Group, CHB Group, LC Group And HCC Group

When the cutoff value of AFP was 12.1 ng/mL and the cutoff value of FGL1 was 30.07 ng/ml; in the HCC group and healthy control group, FGL1 showed good diagnostic performance, and its area under the curve (AUC) was 0.999 [95% confidence interval (CI), 0.959 to 1.000] is higher than AFP (0.917; 95%CI, 0.840 to 0.965), and the combination of the two makes the diagnosis efficiency better (1.000; 95%CI, 0.960 to 1.000). When the CHB group, LC group, and HC group were used as controls for the HCC group, the area under the curve of FGL1 was 0.807 (95% CI, 0.735 to 0.866) than the area under the curve of AFP (0.833; 95% CI, 0.764 to 0.888), but the area under the curve of the indicator after the combination of the two is increased to 0.912 (95% CI, 0.856 to 0.952). CHB and LC are important factors in the onset of HCC. Differential diagnosis of HCC in CHB and LC patients has extremely high diagnostic value, so further analysis. When the CHB group and the LC group were used as the control group of the HCC group, the area under the FGL1 curve was 0.695 (95% CI, 0.603 to 0.777) than the area under the AFP curve (0.784; 95% CI, 0.699 to 0.855), but two the AUC increased to 0.862 (95% CI, 0.786 to 0.918) after the combination of patients. The specificity of FGL1 and AFP in the diagnosis of HBV-related HCC were 98.39% and 70.97%, respectively, and the specificity of the combination of the two was 93.55%. When distinguishing between the LC group and the HCC group, although the area under the FGL1 curve (0.706; 95% CI, 0.602 to 0.797) is lower than the area under the AFP curve (0.754; 95% CI, 0.653 to 0.839), FGL1 is specific the degree of 100% is distinctly higher than that of AFP (80.65%), and the combination of the two increases the area under the curve to 0.883 (95% CI, 0.799 to 0.941). When distinguishing the HCC group from the CHB group, the area under the curve of AFP (0.828; 95% CI, 0.733 to 0.899) was significantly higher than that of FGL1 (0.653; 95% CI, 0.546 to 0.751), but under the curve after the two were combined the area increased distinctly to 0.869 (95% CI, 0.782 to 0.931). When distinguishing between the LC group and the HC group and the CHB group and the HC group, FGL1 showed good diagnostic efficiency, and the area under the curve was 0.999 (95%CI, 0.945 to 1.000) and 0.972 (95%CI, 0.899 to 0.997), respectively, the diagnostic efficiency in the CHB group was significantly higher than that of AFP (0.537; 95% CI, 0.411 to 0.659). In Table 3 and Figure 3.

Table 3. Use separate or combined diagnosis ROC curve to distinguish HC group, CHB group, LC group and HCC group

3.5 The Diagnostic Efficiency Of FGL1 And AFP Alone And In Combination In The Three Stages Of A, B, And C In BCLC

Because the treatment and prognosis of different BCLC stages are quite diverse, the prognosis of both B and C stages is poor, so it is of great significance to diagnose and distinguish BCLC stages in patients with HCC. In patients with stage A and stage B, the AUC of FGL1 (0.584; 95% CI, 0.390 to 0.760) is the same as AFP (0.584; 95% CI, 0.390 to 0.760), and the two are combined the AUC is 0.647 (95% CI, 0.452 to 0.812). In patients with stage B and C, the combination of FGL1 (0.685; 95% CI, 0.523 to 0.819) and AFP (0.767; 95% CI, 0.611 to 0.883) failed to increase the area under the curve (0.767; 95% CI, 0.611 to 0.883), but the sensitivity is improved (80.00%) while maintaining the AUC, even though the specificity (70.59%) is slightly decreased. FGL1 did not show good diagnostic efficiency in the comparison between group A and group C as well as group A and group B+C. As shown in Table 4 and figure 4.

Table 4. The use of separate or combined diagnostic ROC curves to distinguish BCLC staging

Group	Biomarkers	AUC	95%CI	SEN%	SPE%
HCC vs HC	AFP	0.917	0.840 to 0.965	78.18	100.00
	FGL-1	0.999	0.959 to 1.000	98.18	100.00
	Combination	1.000	0.960 to 1.000	100.00	100.00
HCC vs LC	AFP	0.754	0.653 to 0.839	58.33	80.65
	FGL-1	0.706	0.602 to 0.797	53.33	100.00
	Combination	0.883	0.799 to 0.941	81.67	87.10
HCC vs CHB	AFP	0.828	0.733 to 0.899	88.14	64.52
	FGL-1	0.653	0.546 to 0.751	54.24	100.00
	Combination	0.869	0.782 to 0.931	83.05	87.10
HCC vs	AFP	0.833	0.764 to 0.888	70.91	81.63
Controls +LC+CHB	FGL-1	0.807	0.735 to 0.866	56.36	98.98
	Combination	0.912	0.856 to 0.952	80.00	91.84

Group	Biomarkers	AUC	95%CI	SEN%	SPE%	Group	Biomarkers	AUC	95%CI	SEN%	SPE%
HCC vs LC+CHB	AFP	0.784	0.699 to 0.855	70.91	70.97	A-B	AFP	0.584	0.390 to 0.760	70.59	61.54
	FGL-1	0.695	0.603 to 0.777	56.36	98.39		FGL-1	0.584	0.390 to 0.760	52.94	69.23
	Combination	0.862	0.786 to 0.918	74.55	93.55		Combination	0.647	0.452 to 0.812	41.18	84.62
LC vs HC	AFP	0.711	0.587 to 0.815	51.61	100.00	B-C	AFP	0.767	0.611 to 0.883	60.00	88.24
	FGL-1	0.999	0.945 to 1.000	100.00	97.22		FGL-1	0.685	0.523 to 0.819	64.00	70.59
	Combination	1.000	0.946 to 1.000	100.00	100.00		Combination	0.767	0.611 to 0.883	80.00	70.59
CHB vs HC	AFP	0.537	0.411 to 0.659	32.26	100.00	A-C	AFP	0.843	0.689 to 0.940	60.00	100.00
	FGL-1	0.972	0.899 to 0.997	96.77	100.00		FGL-1	0.603	0.432 to 0.758	64.00	61.54
	Combination	0.972	0.899 to 0.997	96.77	100.00		Combination	0.818	0.660 to 0.924	60.00	100.00
LC+CHB vs HC	AFP	0.624	0.520 to 0.720	41.94	100.00	A-B+C	AFP	0.615	0.474 to 0.743	35.71	100.00
	FGL-1	0.986	0.938 to 0.999	96.77	100.00		FGL-1	0.527	0.388 to 0.664	52.38	61.54
	Combination	0.986	0.938 to 0.999	98.39	100.00		Combination	0.607	0.466 to 0.736	35.71	100.00

3.6 The Diagnostic Efficiency Of FGL1 In HBV-Related HCC When AFP Is Negative

Although AFP is widely used in the laboratory diagnosis of HBV-related HCC, not all HBV-related HCC secrete high level AFP, and AFP also increases in cases of liver cirrhosis or hepatitis. The diagnostic efficiency of FGL1 was further analyzed in AFP-negative patients, and good results were obtained. In the HCC group and the normal control group, FGL1 can achieve an excellent diagnostic effect (1.000; 95% CI, 1.000 to 1.000). In comparison with

all control groups, the area under the curve of FGL1 is 0.931 (95 % CI, 0.858 to 0.974). When distinguishing the CHB group from the HCC group, the AUC of FGL1 was 0.839 (95% CI, 0.682 to 0.939), and the AUC of FGL1 in the LC group and the HCC group was 0.866 (95% CI, 0.719 to 0.954). When CHB patients and LC patients were used as a control group for diagnostic analysis, the area under the FGL1 curve was 0.849 (95% CI, 0.724 to 0.932). In the comparison between the LC group and the HC group, FGL1 showed a good diagnostic effect, and the AUC was 0.998 (95% CI, 0.930 to 1.000). In the comparison between the CHB group and the HC group, the AUC was 1.000 (95% CI, 0.930 to 1.000), in the comparison between the HC group and the LC group + CHB group, the AUC is 0.999 (95% CI, 0.945 to 1.000). As shown in Table 5 and figure 5.

Table 5. Use ROC curve to diagnose AFP-negative patients

Group	AUC	95%CI	SEN%	SPE%
HCC vs Controls	1.000	1.000 to 1.000	100.00	100.00
HCC vs LC	0.866	0.719 to 0.954	72.73	100.00
HCC vs CHB	0.839	0.682 to 0.939	72.73	100.00
HCC vs Controls +LC+CHB	0.931	0.858 to 0.974	72.73	100.00
HCC vs LC+CHB	0.849	0.724 to 0.932	72.73	100.00
LC vs HC	0.998	0.930 to 1.000	100.00	97.22
CHB vs HC	1.000	0.930 to 1.000	100.00	100.00
HC vs LC+CHB	0.999	0.945 to 1.000	100.00	97.22

3.7 Immunofluorescence Quantitative Analysis

We calculate the mean fluorescence intensity of FGL1 in the field of view, it was found that the mean fluorescence intensity of the HCC group [77.67(43.29~88.88) AU] was lower than that of the normal control group [87.47(78.92~92.97) AU], and the difference was statistically significant. ($P<0.05$, $U=120.5$) In figure 6.

4. Discussion

Hepatocellular carcinoma is a primary tumor with extremely high morbidity and mortality worldwide. In order to reduce that of hepatitis-related cancers, it is necessary to focus on prevention and conduct large-scale screening and treatment [9]. Liver biopsy is usually used to confirm the diagnosis of HBV-related HCC, but this method is traumatic to patients and is not easy to be accepted by patients [10]. Therefore, it is urgent to find a non-invasive method for HBV-related HCC detection, but the mechanism of HBV-related HCC is complicated and related to multiple factors, and it is difficult to be diagnosed with a single biomarker. Therefore, it is very important to find an effective biomarker to improve the diagnostic efficiency of HCC.

In this study, the key molecule FGL1 was screened by analyzing the expression of hepatocellular carcinoma-related genes in the OncoLnc database [11], and the interactive analysis tool of GEPIA was used to obtain that the expression of FGL1 in liver cancer tissue was lower than that in normal liver tissue [12], Consistent with the reported literature [13]. Reported studies have shown that FGL1 is located on the short arm of human chromosome 8, which is rich in tumor suppressor genes that are often deleted in HCC [14]. FGL1 belongs to the fibrinogen family and has a high degree of homology with the carboxyl terminus of fibrinogen β and γ subunits. In normal physiological conditions, FGL1 protein is mainly secreted by liver cells and contributes to the mitogenic and metabolic functions of normal liver cells.

This study found that the expression of FGL1 in benign liver disease and HBV-related HCC is significantly different, indicating that FGL1 may be an indicator of the progression of HCC from precancerous lesions to cancer. AFP concentrations were higher in the HCC group than in other groups, which is in line with previous studies [15]. The results showed that the diagnostic efficiency of FGL1 alone was higher than that of AFP when compared with normal people, and was lower than that of AFP when compared with patients with benign liver disease. However, after combining with AFP, the diagnostic efficiency was significantly improved in each group. When AFP is negative, FGL1 shows good diagnostic efficiency and specificity, which can supplement the diagnostic indicators of AFP-negative HBV-related HCC patients and reduce the missed diagnosis rate. Further studies of the ROC curve show that FGL1 has extremely high diagnostic efficiency when distinguishing HCC from HC. It is speculated that FGL1 is specifically expressed by the liver, so the specificity of FGL1 is also higher than that of AFP, which supplements the lack of specificity of AFP. High defects. In HBV-related HCC patients with different BCLC stages, FGL1 combined with AFP increased the area under the diagnostic curve for patients with stage A and stage B [16]. Although the diagnostic efficiency of FGL1 alone is not as high as that of AFP, its high specificity makes up for the diagnostic defects of AFP, and it also has a good area under the diagnostic curve and diagnostic specificity in AFP-negative patients.

The immunofluorescence experiment proved that FGL1 is lower expressed in liver cancer tissues than in normal liver tissues, which is consistent with the analysis results obtained by the TCGA database. However, the level of FGL1 concentration in the serum of patients with liver cancer is

significantly higher than that of the normal control population. We speculate that this part of secreted FGL1 is secreted by normal liver cells and binds to the corresponding receptor in the blood circulation to play a tumor suppressor effect. Recent studies have shown that FGL1 is a high-affinity ligand of lymphocyte activating 3 (LAG-3) and is independent of MHC-II molecules. It is speculated that FGL1 can bind to LAG-3 as immune checkpoint inhibitor (ICIs). Studies have shown that the density of LAG-3⁺ cells in tumor tissues is consistent with the expression level of FGL1. FGL1 can mediate T lymphocytes to produce tumor immune tolerance. In FGL1 knockout mice, colon tumors grew significantly slower than tumors in wild-type mice of the same type, and the anti-LAG-3 monoclonal antibody could control the growth rate of mouse tumors. Prove that FGL1/LAG-3 is an important tumor immune escape pathway^[17]. It is speculated that FGL1 is closely related to the occurrence and development of HCC.

This study has certain limitations. The first is that the small sample size may have some influence on the results. Second, there are individual differences among patients in the included population. This difference makes the measured FGL1 and AFP levels have high variability. Therefore, in order to further explore the role of FGL1 in HBV-related HCC, it is necessary to further include more sample sizes and conduct longitudinal studies.

5. Conclusion

In summary, this study first found that FGL1 is closely related to HCC through the TCGA database, and speculated that it can be used as a non-invasive diagnostic index for HCC, and then confirmed the differential expression of FGL1 in HCC and normal controls by immunofluorescence semi-quantitative experiments. The FGL1 content in human serum was further detected and combined with AFP, a commonly used indicator for HCC diagnosis, to obtain a good diagnostic efficiency. This shows that the expression of FGL1 in liver cancer has certain clinical significance, and the relationship between it and liver cancer can be further explored.

List Of Abbreviations

HCC
hepatocellular carcinoma
LC
liver cirrhosis
CHB
chronic hepatitis B
HC
healthy people
FGL1
Fibrinogen like protein 1
AFP
alpha fetoprotein
LAG-3
lymphocyte activating 3
ICIs
immune checkpoint inhibitor
AUC
area under the receiver operating curve
HBV
hepatitis B virus
ALT
Alanine aminotransferase
AST
aspartate aminotransferase
ALP
alkaline phosphatase
γ-GT
γ-glutamyl transpeptidase
ALB
albumin
TBIL
Total bilirubin
DBIL
direct bilirubin
Hs-CRP

Hypersensitive C-reactive protein
BCLC
Barcelona clinic liver cancer
CI
Confidence interval

Declarations

Ethics approval and consent to participate:

The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study has been reviewed and approved by the Medical Ethics Review Committee of Renmin Hospital, Wuhan University and informed consent was taken from all individual participants.

Consent for publication:

Not applicable.

Competing interests:

The authors declare that they have no competing interests, financial competing interests and non-financial competing interests.

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Figures

Figure 1

Pan-cancer analysis and FGL1 expression analysis based on the GEPIA database. Note: (A) The expression of FGL1 was significantly increased in LIHC. ($P<0.05$)(B) The expression of FGL1 in LIHC was lower than that in normal tissues,* $P<0.05$ .GEPIA, Gene Expression Profiling Interactive Analysis; LIHC, Liver hepatocellular carcinoma; LGG, Brain Lower Grade Glioma; LUAD, Lung adenocarcinoma; PAAD, Pancreatic adenocarcinoma; ESCA, Esophageal carcinoma; T, tumor; N, normal.

Figure 2

Expression levels of FGL1 and AFP in HC, CHB, LC and HCC groups. Note: (A) Expression level of FGL1 in HC, CHB, LC and HCC groups; (B) The expression level of FGL1 in HC, CHB, LC and HCC groups when AFP was negative; (C) AFP expression levels in HC, CHB, LC and HCC groups; All data is converted to Log10. #, P<0.001 compared with HC group. &, P<0.001 compared with CHB group. *, P<0.001 compared with LC group.

Figure 3

ROC curves of FGL-1 and AFP alone and in combination in the HC group, CHB group, LC group and HCC group. Note: (A) ROC curve analysis of HCC group and HC group; (B) ROC curve analysis of HCC group and LC group; (C) ROC curve analysis of HCC group and CHB group; (D) ROC curve analysis of HCC group and HC + LC + CHB group; (E) ROC curve analysis of HCC group and LC + CHB group; (F) ROC curve analysis of LC group and HC group; (G) ROC curve analysis of CHB group and HC group; (H) ROC curve analysis of LC + CHB group

Figure 4

ROC curves of FGL-1 and AFP alone and in combination in the three stages of A, B, and C in BCLC. Note: (A) ROC curve analysis of A and B stages in BCLC staging; (B) ROC curve analysis of B and C stages in BCLC staging; (C) ROC curve analysis of A and C stages in BCLC staging; (D) ROC curve analysis of stage A and stage B+C in BCLC staging

Figure 5

ROC curve of FGL-1 in HBV-related HCC when AFP is negative. Note: (A) ROC curve analysis of HCC group and HC group; (B) ROC curve analysis of HCC group and LC group; (C) ROC curve analysis of HCC group and CHB group; (D) HCC group and HC group+ ROC curve analysis of LC group + CHB group; (E) ROC curve analysis of HCC group and LC group + CHB group; (F) ROC curve analysis of LC group and HC group; (G) ROC curve of CHB group and HC group Analysis; (H) ROC curve analysis of LC group + CHB group

Figure 6

Fluorescence expression of FGL1 in liver cancer and normal tissues. Note: (A) and (B) Fluorescence photography with 200× visual field of HCC group; (C) and (D) Fluorescence photography with 400× visual field of HCC group; (E) and (F) Fluorescence photography with 200× visual field of HC group; (G) and (H) Fluorescence photography with 400× visual field of HC group; (I) and (K) Fluorescence photography with 200× and 400× visual field of tumor in autologous control; (J) and (L) Fluorescence photography with 200× and 400× visual field of normal tissue in autologous control.