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Original Article

Genome-wide association study identified PLCE1- rs2797992 and EGFR- rs6950826 were associated with TP53 expression in the HBV-related hepatocellular carcinoma of Chinese patients in Guangxi

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Abstract: Objective: The genome-wide association approach was employed to explore the association between single nucleotide polymorphisms (SNPs) and TP53 expression in the HBV-related hepatocellular carcinoma (HCC) of Chinese patients in Guangxi. Methods: 403 HBV-related HCC patients were recruited into this study and classified according to the TP53 expression in the cancer by immunohistochemistry. DNA was extracted from the cancer and genotyped with the Human ExomeBeadChip 12v1-1 system; quality control and principal-component analysis (PCA) were applied for data analysis. Results: The Genome-wide association analysis indicated that rs2797992 with a *P* value of 4.35×10^{-5} locus in PLCE1 gene and rs6950826 with a *P* value of 2.2×10^{-3} locus in EGFR gene were associated with TP53 expression in the HCC. A allele of rs2797992 predicted a decreased risk for TP53 expression in HCC. In contrast, A allele of rs6950826 increased the risk for TP53 expression. There was no strong LD locus in the tested regions. PLCE1 and EGFR were associated with TP53 in pathway and at HCC mRNA level. Conclusion: rs2797992 of PLCE1 gene and rs6950826 of EGFR gene are associated with TP53 expression, but not with the prognosis of HBV-related HCC in HBV-related HCC of Chinese patients in Guangxi.

Keywords: TP53, hepatocellular carcinoma, hepatitis B virus, PLCE1, EGFR

Introduction

Liver cancer, a type of cancer with high mortality, is much more common in developing countries. Global Cancer Statistics reported that half of the new liver cancer cases and deaths worldwide during 2012 were estimated to occur in China [1]. Liver cancer has been the third leading cause of cancer related death in China and the fourth most common cancer in China although its incidence is reducing [2]. The vast majority (85% to 90%) of primary liver cancer is hepatocellular carcinoma (HCC) [3]. HCC is a genetically heterogeneous tumor and a complex disease. It has multiple genetic and epigenetic alterations with involvement of several signal transduction pathways, including TP53 [4], Ras and MAPK [5]. Multiple predisposing factors of HCC have been defined, including hepatitis virus B (HBV) infection, HCV infection, excessive alcohol consumption, obesity, aflatoxin [6] and smoking [7]. HBV infection plays an important role in the pathogenesis of HCC [8]. The high prevalence of HCC in parts of Asia is largely associated with the elevated prevalence of HBV infection (over 5% of the populations in this region chronically infected with HBV) [1]. In addition, epidemiological studies have shown that chronic exposure to aflatoxin is not only an independent risk factor for liver cancer, but drastically increases the carcinogenicity of HBV infection [9].

TP53 protein encoded by the human gene TP53 is a key tumor suppressor and can prevent the tumorigenesis and cancer progression. TP53

Table 1. Clinicopathological characteristics in HCC P53 negative and P53 positive patients

Characteristics	TP53 negative (n=154)	TP53 positive (n=233)	OR (95% CI)	P
Age (years)				
≤ 60	133	211	1	
> 60	21	22	0.66 (0.35-1.247)	0.201
Sex				
Male	141	207	1	
Female	13	26	0.734 (0.365-1.477)	0.386
Race				
Han	103	142	1	
Minority	51	91	1.294 (0.845-1.983)	0.236
BMI				
≤ 25	121	181	1	
> 25	33	52	1.053 (0.643-1.725)	0.836
Smoking status				
None	97	147	1	
Ever	57	86	0.996 (0.653-1.518)	0.984
Drinking status				
None	91	134	1	
Ever	63	99	1.067 (0.706-1.613)	0.758
Child-Pugh†				
A	116	189	1	
B	28	30	0.658 (0.374-1.156)	0.146
Cirrhosis				
No	12	27	1	
Yes	142	206	0.645 (0.316-1.315)	0.228
Radical resection‡				
Yes	82	133	1	
None	67	94	0.865 (0.57-1.313)	0.496
Portal hypertension&				
No	69	124	1	
Yes	75	87	0.645 (0.421-0.989)	0.044
Pathological grade$				
Well differentiated	14	10	1	
Moderately differentiated	126	190	2.111 (0.909-4.901)	0.082
Poorly differentiated	1	10	14 (1.536-127.621)	0.019
Serum AFP¥				
≤ 400 (ng/mL)	87	110	1	
> 400 (ng/mL)	58	105	1.432 (0.935-2.193)	0.099
Antiviral therapy				
NO	95	157	1	
Yes	59	76	0.779 (0.51-1.192)	0.25
Tumor behaviors				
Tumor size				
≤5 cm	72	85	1	
>5 cm	82	148	1.529 (1.011-2.313)	0.044
Tumor number				

can not only suppress the abnormal cell proliferation and prevent abnormal DNA replication, but eliminate cells with abnormal growth [10]. In addition, TP53 may also induce cell apoptosis, cell-cycle arrest and senescence in response to stress. It is suggested that TP53 is important in the tumor suppression [11]. Mutant TP53 is frequently detectable in cancers [12]. As compared to other tumor suppressor genes, TP53 has a higher mutation rate. A Chinese study conducted in GuangXi population shows that HBV infection and aflatoxin B1 (AFB1) exposure are closely associated with the increased incidence of TP53 mutation. The high AFB1 exposure level and high HBV infection rate in Guangxi result in a high rate (34%) TP53 gene mutation at codon 249 in exon 7 of Guangxi population in China [13]. Thus, the population in this region is representative to investigate the relationship of HBV infection, aflatoxin exposure and TP53 gene mutation with HCC. It has been shown that TP53 expression in the cancer is closely associated with HCC prognosis. A meta-analysis on the basis of 24 studies evaluates the correlation between TP53 expression and survival in HCC patients, and results show that TP53 expression is associated with the poor prognosis of HCC patients [14]. Other related studies in HCC patients also got the simi-

Single	111	169	1	
Multiple	43	64	0.978 (0.62-1.54)	0.922
Status of tumor capsule				
Complete	126	201	1	
Incomplete	28	32	0.716 (0.412-1.247)	0.238
Regional invasion				
Absence	131	199	1	
Presence	23	34	0.973 (0.549-1.726)	0.926
BCLC stage				
A	87	129	1	
B	28	38	0.915 (0.523-1.6)	0.756
C	39	66	1.141 (0.706-1.845)	0.59
PVTT				
No	130	189	1	
Yes	24	44	1.261 (0.731-2.175)	0.404

Notes: BMI, body mass index; AFP, α-fetoprotein, BCLC, Barcelona Clinic Liver Cancer; OR, odds ratio; CI, confidence interval; PVTT, portal vein tumor thrombus; †Child-Pugh-class was unavailable in 24 patients. ‡Information of radical resection was unavailable in 11 patients. &Informationof portal hypertension was unavailable in 32 patients. ΦInformation of pathological diagnosiswasunavailable in 36 patients. ℐInformation of serum AFP level was unavailable in 27 patients.

lar conclusion [15-17]. In this study, the exome single nucleotide polymorphism (SNP) was detected in the HBV related HCC tissues and its relationship with TP53 expression determined by immunohistochemistry and post-operative prognosis was evaluated in Chinese patients from Guangxi Province, a south region of China.

Materials and methods

Study population

Our study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University with an Ethics approval number of 2015 (KY-E-032). A total of 403 patients were consecutively recruited into present study. These patients were newly diagnosed with HCC by histological examination in the First Affiliated Hospital of Guangxi Medical University between 2001 and 2013. All the patients were positive for HBV surface antigen in the serum. The TP53 expression in the cancer tissues was detected by immunohistochemistry. The cancer tissues were collected in the surgery and immediately stored at -80°C for further use.

Genotyping

Total DNA was extracted from cancer tissues for genotyping with the Human ExomeBeadChip 12v1-1 system (Illumina, Inc.; San Diego, CA), which includes 242,898 markers of protein-altering variants. The nonsynonymous SNPs, SNPs in splice sites, stop variants, SNPs in promoter regions, SNPs in extended MHC region, GWAS tag markers and HLA tags were included in these markers. All the samples were processed according to the instructions of Illumina® HD Assay Ultra manual and imaging BeadChip on the iScan system. Genotype calling was performed using the Genotyping Module v1.0 in Genome Studio version 2011.1, and average call rate was 99.98%.

Genome-wide association analysis

Before data analysis, a standard quality control (QC) procedure [18] was employed: Step1 for the simples: if the simple has the following scenarios, it would fail to pass the quality control: (i) an overall genotyping rate of < 95%; (ii) ambiguous gender; (iii) genome-wide identity-by-descent (IBD) > 0.1875; (iiii) outliers in principal component analysis (PCA) for ancestry and population stratification. Step 2 for the SNPs: if the SNPs had one of the following scenarios, it would fail to continue the next analysis. The scenarios included a call rate of < 95%; a Hardy-Weinberg equilibrium (HWE) $P < 1\times10^{-6}$; a minor allele frequency (MAF) < 0.01. Those steps were completed with Plink version 1.07, R 3.0.1 and EIGENSOFT package.

Guangxi province is a multiracial region in China. All the patients in this study were local residents or lived for a long time in Guangxi region. Ananalysis of population stratification was performed to eliminate the influence of races using PCA with the EIGENSOFT package [19]. Genomic inflation factor (GIF), which was calculated by MATLAB 7.0 [20], was used to investigate residual population stratification. Single Variant Test [21] (the Logistic Score Test) was used to analyze the association of variants with TP53 expression in HCC with

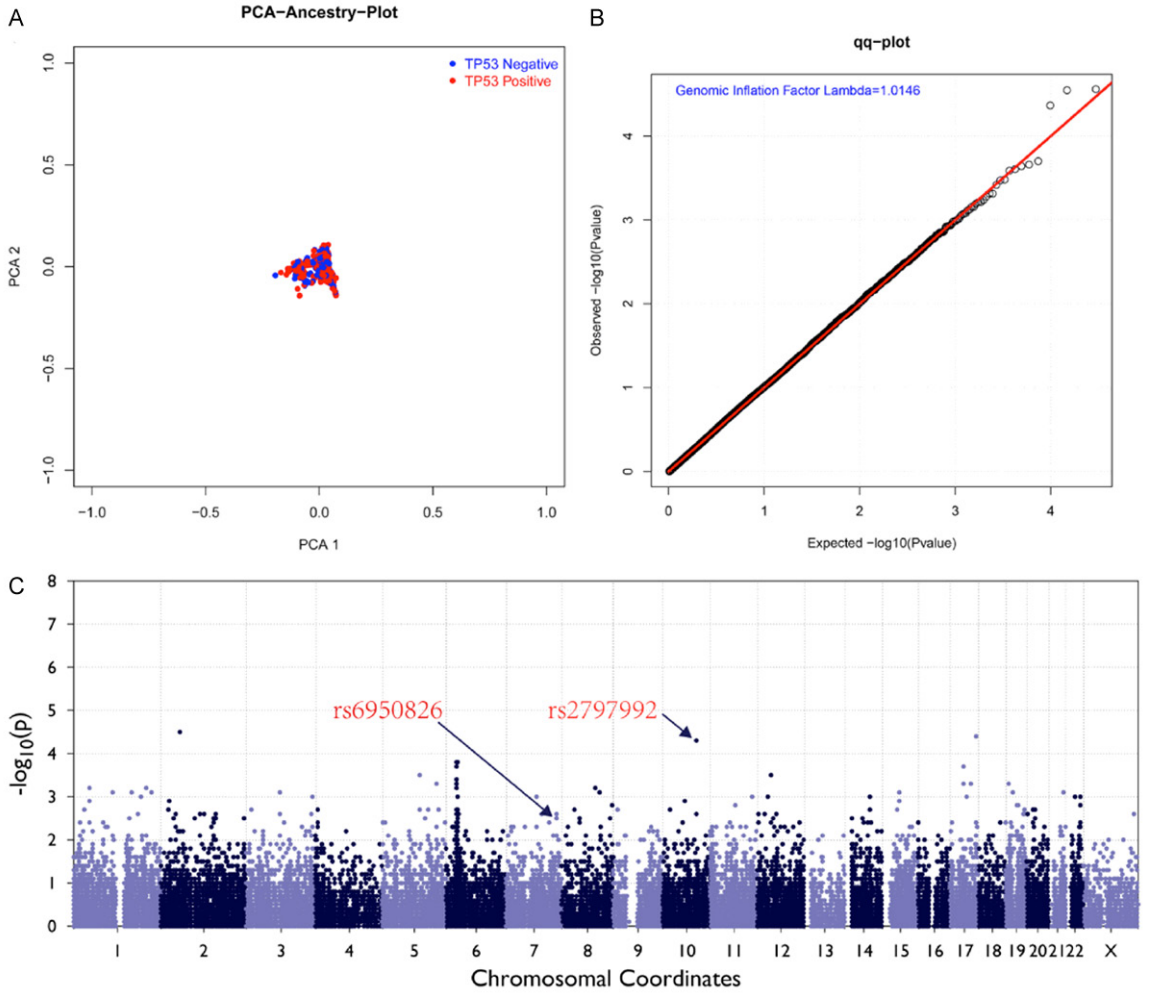

Figure 1. A: Principal-componentanalysis (PCA) analysis of study population. Results show no abnormal outlier samples in this study population. B: Quantile-quantile (Q-Q) plots are from Single Variant Test. Genomic inflation factor is from MATLAB7.0, which calculates based on the *P* value. λ (Lambda)=1.0146. C: Manhattan plot of the Single Variant Test association analysis (-log10 *P* value) plots against genomic position (GRCH37/hg19).

EPACTS package version 3.2.6 after adjustment forage, gender, race, Barcelona-Clinic-Liver-Cancer (BCLC) stage [22], cirrhosis, smoking status, drinking status [23, 24].

Association analysis

Logistic regression model was used to analyze the characteristics of patients between two groups and the genetic model of the selected SNPs. The association between clinical characteristics and selected SNPs was evaluated with the logistic regression model. The data were analyzed with the bionary logistic regression, and odds ratio (OR) and corresponding 95% confidence interval (95% CI) were calculated for the estimation of relative risk of TP53 expression in HBV-related HCC and clinical characteristics associated with the selected SNPs. Ananalysis for local linkage disequilibrium (LD) and recombination patterns near by the selected SNPs was performed using Locus zoom [25]. Genotype frequencies were examined for Hardy-Weinberg equilibrium using the Chi-square test and all were found to be consistent ($P > 0.05$). To identify the association of expression of selected genes with TP53 expression at mRNA level, the data from Gene Expression Omnibus (GEO, GSE14520) were analyzed. The Spearman correlation coefficient was used to assess the correlation. Gene function prediction website (GENE MANIA: http://www.genemania.org/) was alsoused for correlation analysis between genes.

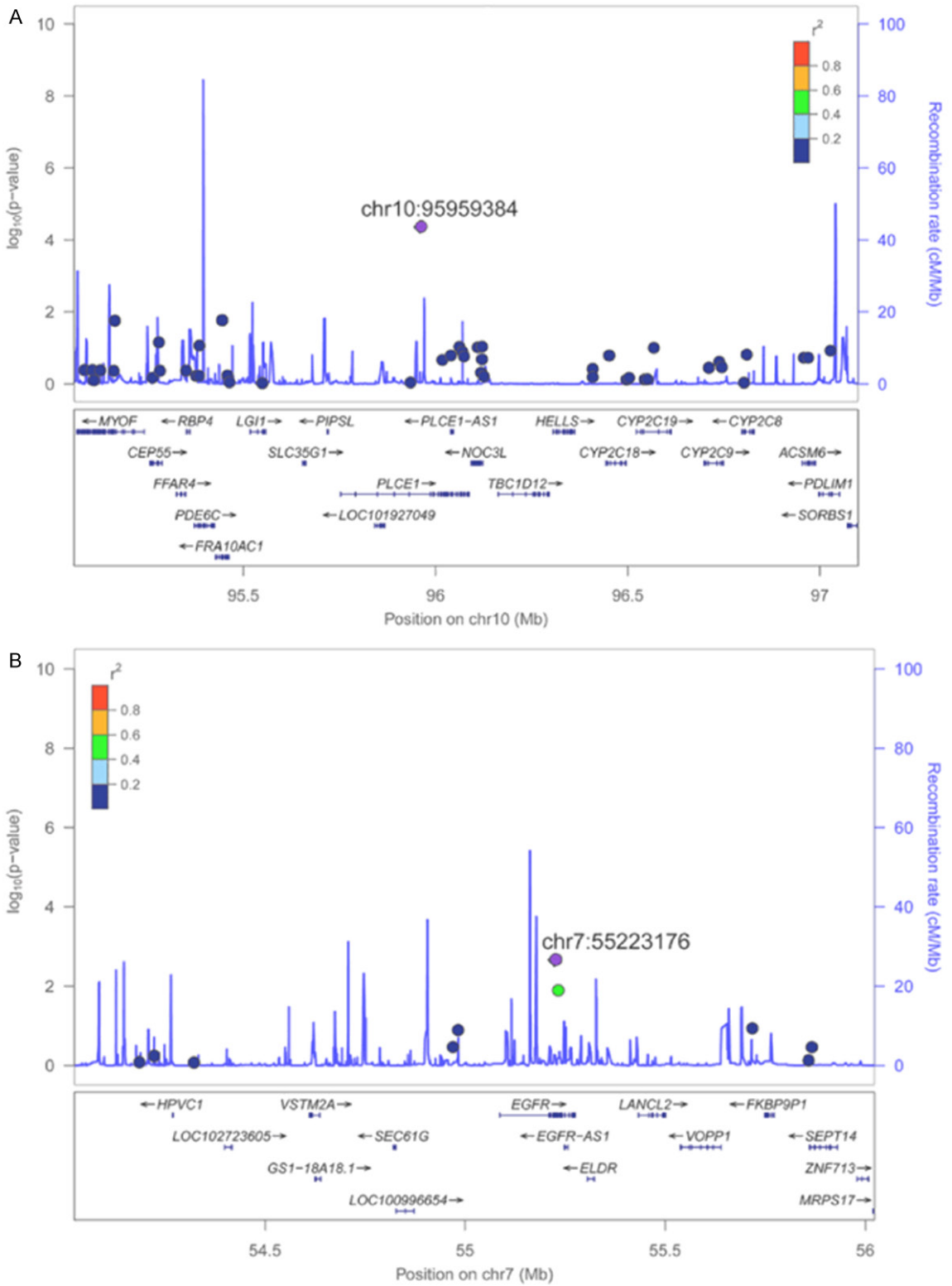

Figure 2. Plots show the 1.5 Mb window centers on the association peak. The top panel shows all SNPs in the region plotted according to the significance of their association withstudy population. The bottom panel shows the LD structure among SNPs.

Table 2. Genotype distribution of PLCE1 rs2797992 and EGFR rs6950826 in TP53 negative and P53 positive patients (genetic model)

Variable	TP53 negative	TP53 positive	Crude OR (95% CI)	Adjusted OR (95% CI)	Adjusted P§
PLCE1 rs2797992					
GG	57	125	1	1	
AG	70	94	0.612 (0.394-0.951)	0.592 (0.372-0.94)	0.026
AA	27	14	0.236 (0.115-0.485)	0.224 (0.106-0.472)	0.000084
AA+AG	97	108	0.508 (0.335-0.770)	0.489 (0.316-0.758)	0.001
EGFR rs6950826					
GG	86	99	1	1	
AG	57	101	1.539 (0.997-2.377)	1.611 (1.019-2.548)	0.041
AA	11	33	2.606 (1.242-5.468)	2.671 (1.24-5.749)	0.012
AA+AG	68	134	1.712 (1.135-2.581)	1.787 (1.159-2.755)	0.009

Notes: §Adjustment for age, gender, race, body mass index, smoking status, drinking status, BCLC stage, cirrhosis, radical resection, antiviral therapy, status of tumor capsule, regional invasion, portal vein tumor thrombus in logistic regression model. OR: odds ratio; 95% CI: 95% confidence intervals.

Follow-up and survival analysis

In this study, patients were followed up after surgery until the final follow-up or death. The final follow-up was conducted in September 2014. A total of 387 patients received complete follow up successfully, with the lost to follow-up rate of 7%. The duration of follow up ranged from 12 months to 125 months, and the median survival time was 48 months. Survival analysis was performed using the Kaplan-Meier method with log-rank test in different groups and genotypes. Cox proportional hazards regression analysis was performed to calculate the crude or adjusted hazard ratio (HR) and 95% CI in univariate analysis and multivariate analysis after adjustment for age, gender, race, body mass index (BMI), smoking status, drinking status, BCLC stage, cirrhosis, radical resection, antiviral therapy, status of tumor capsule, regional invasion and portal vein tumor thrombus (PVTT). A value of $P < 0.05$ was considered statistically significant. All the statistical analyses were conducted with SPSS version 20.0.

Results

Baseline characteristics

In this study, a total of 387 HBV-related HCC patients were included for final analysis. Of them, 154 were negative for TP53 expression and 233 positive for TP53 expression. The characteristics of these patients are presented in **Table 1**. Most of the patients were male (n=348, 89.9%) with the median age of 46 years. More than half of the patients were Han Chinese (n=245, 63.3%), and 85 (21.9%) patients were diagnosed with overweight (BMI > 25). 143 patients and 163 patients were smoker and drinker, respectively, and 163 had elevated serum AFP (> 400 ng/mL) before surgery. The majority of patients had liver cirrhosis (n=348, 89.9%), but most was classified as Child-Pugh class A (n=305, 78.8%) and the others were class B. HBV infection was found in all the patients before hepatectomy, but only 135 received antiviral therapy for HBV infection after surgery. As shown in **Table 1**, there were significant differences in the tumor size, portal hypertension and poorly differentiated HCC between TP53 positive patients and TP53 negative patients ($P < 0.05$). The tumor number, status of tumor capsule, regional invasion, BCLC stage and PVTT were comparable between two groups. Radical resection was conducted in 215 patients (55.5%), 179 (46.25%) patients died before the final follow-up and the median survival time (MST) was 48 months.

Genome-wide association analysis

After the QC, 387 patients with 28952 SNPs were included for further analysis. Successful genotyping was performed in 99.33% of patients. PCA (**Figure 1A**) showed there were no abnormal outlier samples. We focus on the MAF > 0.01, Q-Q plot and Manhattan plot as shown in **Figure 1B** and **1C**. The SNPs rs2797992 (MAF=0.3189, $P=4.35 \times 10^{-5}$) in phospholipase C epsilon 1 (PLCE1) gene and rs6950826 (MAF=0.32345, $P=2.2\times 10^{-3}$) in

Table 3. Stratified analysis of associations of PLCE1 rs2797992 and EGFR rs6950826 genotypes with clinicopathological characteristics

Characteristics	PLCE1 rs2797992		OR (95% CI)	P	EGFR rs6950826		OR (95% CI)	P
	GG	AA+AG			GG	AA+AG		
Tumor size								
≤ 5 cm	76	81	1		84	73	1	
> 5 cm	106	124	1.098 (0.731-1.648)	0.653	101	129	1.47 (0.978-2.209)	0.064
Tumor number								
Single	134	146	1		136	144	1	
Multiple	48	59	1.128 (0.721-1.765)	0.597	49	58	1.118 (0.715-1.747)	0.625
Child-Pugh class†								
A	142	163	1		149	156	1	
B	28	30	0.933 (0.532-1.637)	0.81	25	33	1.261 (0.716-2.221)	0.422
BCLC stage								
A	103	113	1		106	110	1	
B	27	39	1.317 (0.753-2.302)	0.335	30	36	1.156 (0.665-2.01)	0.607
C	52	53	0.929 (0.583-1.481)	0.757	49	56	1.101 (0.69-1.757)	0.686
Serum AFP⸸								
≤ 400 (ng/mL)	93	104	1		99	98	1	
> 400 (ng/mL)	75	88	1.049 (0.692-1.591)	0.821	73	90	1.245 (0.821-1.899)	0.301
Radical resection‡								
Yes	99	116	1		102	113	1	
None	76	85	0.955 (0.634-1.438)	0.824	74	87	1.061 (0.705-1.598)	0.776
Status of tumor capsule								
Complete	158	169	1		158	169	1	
Incomplete	24	36	1.402 (0.801-2.456)	0.237	27	33	1.143 (0.657-1.986)	0.636
Regional invasion								
Absence	150	180	1		165	165	1	
Presence	32	25	0.651 (0.37-1.147)	0.137	20	37	1.85 (1.03-3.321)	0.039
PVTT								
No	145	174	1		154	165	1	
Yes	37	31	0.698 (0.413-1.181)	0.18	31	37	1.114 (0.659-1.884)	0.687
Pathological grade⁂								
Well differentiated	12	12	1		14	10	1	
Moderately differentiated	137	179	1.307 (0.569-2.998)	0.528	144	172	1.672 (0.721-3.878)	0.231
Poorly differentiated	5	6	1.2 (0.287-5.021)	0.803	5	6	1.68 (0.399-7.075)	0.479

Notes: †Information of Child-Pughclass was unavailable in 24 patients. ‡Information of radical resection was unavailable in 11 patients. ⁂Information of pathological diagnosis was unavailable in 36 patients. ⸸Information of serum AFP level was unavailable in 27 patients. AFP, α-fetoprotein; BCLC, Barcelona Clinic Liver Cancer; PVTT, portal vein tumor thrombus; OR, odds ratio; 95% CI, 95% confidence intervals.

epidermal growth factor receptor (EGFR) had a moderate association with HBV-related HCC. The allele frequencies met the Hardy-Weinberg equilibrium as shown by the goodness-of-fit χ^2-test (rs2797992: χ^2=1.324, P=0.25; rs6950826: χ^2=0.20, P=0.65). LD analysis (**Figure 2**) showed that there was no strong LD locus detectable in the tested region.

Genetic model analysis of rs2797992 and rs6950826

The genotype distributions of rs2797992 and rs6950826 in TP53 negative patients and TP53 positive patients are shown in **Table 2**. In codominant genetic model, after adjustment for the age, gender, race, BMI, smoking status, drinking status, BCLC stage and cirrhosis, radical resection, antiviral therapy, status of tumor capsule, regional invasion and PVTT, single locus analyses revealed that genotype A of PLCE1 rs2797992 was associated with a significantly decreased risk for TP53 expression in HBV-related HCC (P=0.026; adjusted OR=0.592; 95% CI=0.372-0.94 for AG vs GG and P=0.000084; adjusted OR=0.224; 95% CI =0.106-0.472 for AA vs GG) compared with GG genotype. Patients with A allele (AA/AG) also had significantly decreased risk for TP53 expression, compared with patients without A allele (GG) (P=0.001; adjusted OR=0.489; 95% CI=0.316-0.758). For rs2797992, A allele significantly decreased the risk for HBV-related

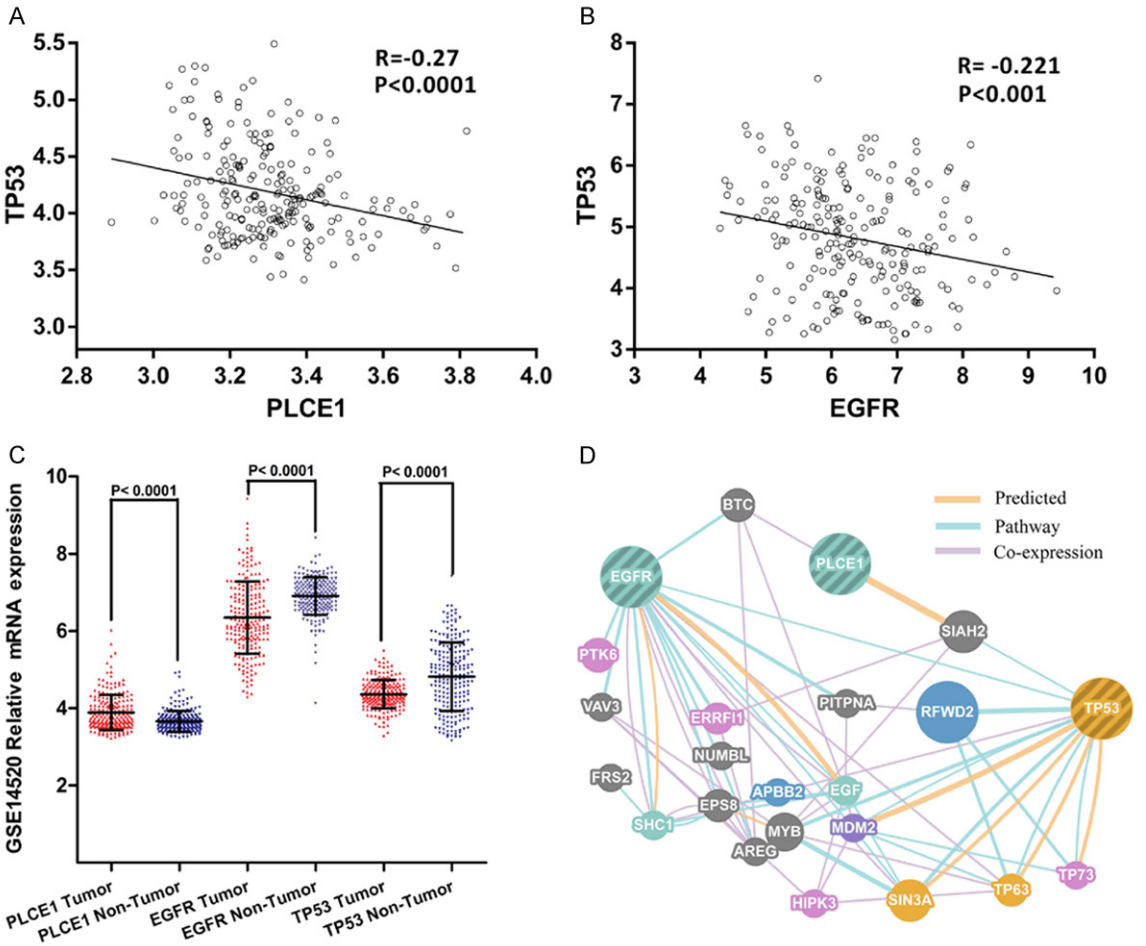

Figure 3. A: Correlation between PLCE1 gene and TP53 gene. B: Correlation between EGFR gene and TP53 gene. C: The mRNA expression of PLCE1 and EGFR,TP53 in HCC and adjacent normal tissues. D: Interaction of PLCE1 gene and EGFR gene with TP53 gene predicts network graph by GeneMANIA.

HCC TP53 expression compared with G allele, and this decreased risk was an additive effect. On the contrary, A genotype of rs6950826 was associated with a significantly increased risk for TP53 expression in HBV-related HCC (P=0.041; adjusted OR=1.611; 95% CI=1.019-2.548 for AG vs GG and P=0.012; adjusted OR=2.671; 95% CI =1.24-5.749 for AA vs GG) compared with GG genotype. The patients with A allele (AA/AG) also had significantly increased risk for TP53 expression compared with patients without A allele (GG) (P=0.009; adjusted OR=1.787; 95% CI=1.159-2.755) and this increased risk function was also an additive effect.

Stratified analysis

The association between genotypes of selected SNPs and clinicopathological characteristics is shown in **Table 3**. Results showed that the genotypes of rs2797992 had no relationship with the clinicopathological characteristics in this study population. Regional invasion was significantly associated with genotypes AA, AG and GG, suggesting that EGFR plays an important role in the regional invasion of HCC. The other clinicopathological characteristics had no statistically significant association with the genotypes of rs6950826.

Bioinformatics analysis

In order to confirm above results, the correlation between PLCE1 mRNA expression and TP53 mRNA expression in HCC by using the GSE14520 database. Results showed a weak negative correlation between them (r=-0.27, P < 0.0001, **Figure 3A**). Similar result was also found between EGFR mRNA expression and

Table 4. Survival analysis of HCC patients according to the rs2797992 genotypes, rs6950826 genotypes and TP53 expression status

Genotypes	Patients (n)	MST (months)	Crude HR (95% CI)	Adjusted HR (95% CI)	Adjusted P#
rs2797992					
GG	182	52	1	1	
AG	164	51	1.022 (0.744-1.404)	0.917 (0.656-1.281)	0.611
AA	41	31	1.492 (0.943-2.36)	1.241 (0.768-2.005)	0.379
AG+AA	205	41	1.107 (0.823-1.49)	0.982 (0.72-1.341)	0.91
rs6950826					
GG	185	58	1	1	
AG	158	44	1.188 (0.863-1.636)	1.158 (0.823-1.629)	0.4
AA	44	40	1.48 (0.951-2.301)	1.243 (0.782-1.977)	0.248
AG+AA	202	42	1.256 (0.935-1.687)	1.181 (0.862-1.617)	0.301
TP53 status					
TP53 negative	154	52	1	1	
TP53 positive	233	43	1.161 (0.855-1.575)	1.191 (0.867-1.636)	0.281

Notes: #Adjustment for age, gender, race, body mass index, smoking status, drinking status, BCLC stage, cirrhosis, radical resection, antiviral therapy, status of tumor capsule, regional invasion, PVTT (portal vein tumor thrombus) in Cox proportional hazards regression model; BCLC, Barcelona Clinic Liver Cancer; PVTT, portal vein tumor thrombus; CI, confidence interval; HR, hazard ratio; MST, median survival time.

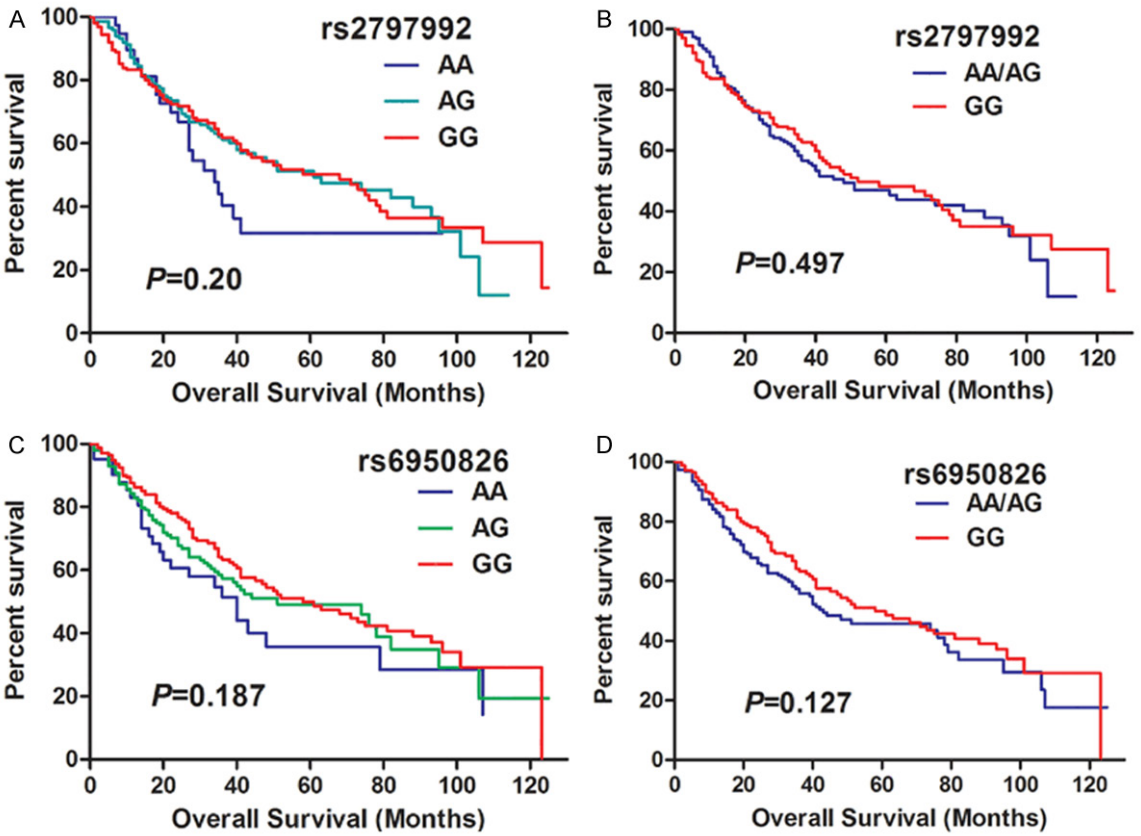

Figure 4. Survival curves of patients with different rs2797992 and rs6950826 genotypes. A: Kaplan-Meier survival curve among patients with genotypes AA, AG and GG of rs2797992. B: Kaplan-Meier survival curve of patients with genotypesAA/AG and GG of rs2797992. C: Kaplan-Meier survival curve of patients with genotypes AA, AG and GG of rs6950826. D: Kaplan-Meier survival curve of patients with genotypes AA/AG and GG of rs6950826.

Table 5. Joint effects analysis of rs2797992 genotypes, rs6950826 genotypes and TP53 expression status

Group	Genotype	TP53 status	Patients	MST (months)	Crude HR (95% CI)	Adjusted HR (95% CI)	Adjusted P#
	rs2797992						
1	GG	Negative	57	71	1	1	
2	GG	Positive	125	44	1.345 (0.825-2.194)	1.298 (0.787-2.138)	0.307
3	AG+AA	Negative	97	45	1.309 (0.781-2.194)	1.107 (0.647-1.893)	0.712
4	AG+AA	Positive	108	40	1.41 (0.857-2.32)	1.238 (0.744-2.058)	0.411
	rs6950826						
1	GG	Negative	86	71	1		
2	GG	Positive	99	41	1.471 (0.942-2.296)	1.257 (0.79-1.999)	0.334
3	AG+AA	Negative	68	39	1.721 (1.055-2.806)	1.265 (0.753-2.124)	0.375
4	AG+AA	Positive	134	43	1.498 (0.973-2.308)	1.385 (0.891-2.152)	0.148

Notes: #Adjustment for age, gender, race, BMI body mass index, smoking status, drinking status, BCLC, cirrhosis, radical resection, antiviral therapy, status of tumor capsule, regional invasion, PVTT in Cox proportional hazards regression model; BCLC, Barcelona Clinic Liver Cancer; PVTT, portal vein tumor thrombus; CI, confidence interval; HR, hazard ratio; MST, median survival time.

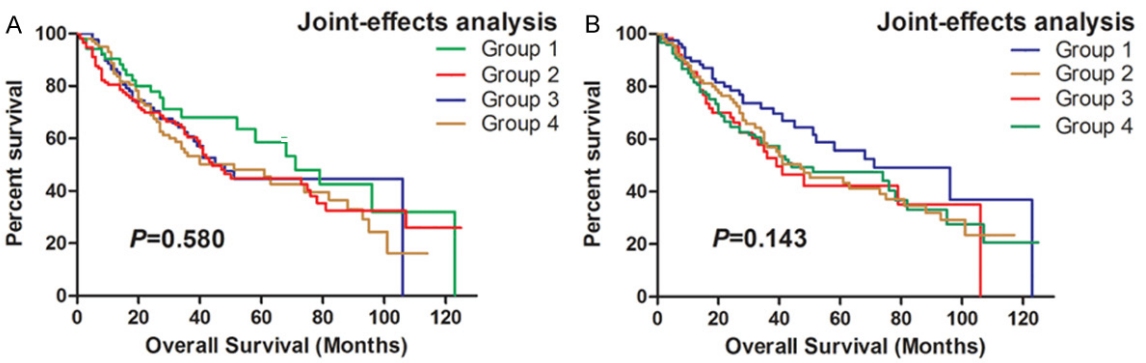

Figure 5. Kaplan-Meier survival curve for joint effects analysis of rs2797992 and rs6950826 in patients with different TP53 expression statuses. A: Group 1, genotype GG of rs2797992 and TP53 negative patients; Group 2, genotype GG of rs2797992 and TP53 positive patients; Group 3, genotype AA/AG of rs2797992 and TP53 negative patients; Group 4, genotype AA/AG of rs2797992 and TP53 positive patients. B: Group 1, genotype GG of rs6950826 and TP53 negative patients; Group 2, genotype GG of rs6950826 and TP53 positive patients; Group 3, genotype AA/AG of rs6950826 and TP53 negative patients; Group 4, genotype AA/AG of rs695082 and TP53 positive patients.

TP53 mRNA expression (r=-0.221, P=0.0009, **Figure 3B**). The mRNA expression of EGFR, PLCE1 and TP53 was significantly different between HCC tissues and adjacent normal tissues (P < 0.0001, **Figure 3C**). Gene interaction network determined with GENE MANIA is shown in **Figure 3D**. Results showed PLCE1 was predicted in SIAH2 to TP53 pathway, and the EGFR was on the TP53 pathway.

Relationship of SNPs and TP53 status with survival time

In univariate analysis of rs2797992, patients with A allele (AA/AG) had a poorer MST than those without A allele (GG) (41 months vs 52 months, log-rank P=0.497, **Table 4**, **Figure 4B**), but the MST of patients with genotypes AA and AG was slightly shorter than that of patients with GG genotype, respectively (31, 51 vs 52 months, log-rank P=0.20, **Table 4**, **Figure 4A**). Similar results were found in genotypes of rs6950826 (MST AA/AG vs GG, 42 vs 58 months, log-rank P=0.127; AA, AG vs GG, 40, 44 vs 58 months, log-rank P=0.187; **Table 4**, **Figure 4C** and **4D**). After adjustment for risk factors in Cox proportional hazards regression analysis, the MST was comparable in patients with different genotypes of both rs2797992

and rs6950826. In addition, TP53 negative patients seemed to have a longer MST than TP53 positive patients (52 vs 43 months) although there was no significant difference.

Joint-effects analysis

We further analyze the TP53 and SNPs mutual association with HBV-related HCC survival outcomes. For rs2797992 and rs6950826, TP53 negative patients with GG genotype had a longer MST (**Table 5**, **Figure 5**) as compared to other patients. After adjustment for age, gender, race, BMI, smoking status, drinking status, BCLC stage, cirrhosis, radical resection, antiviral therapy, status of tumor capsule, regional invasion and PVTT in Cox proportional hazards regression model, the MST was similar in patients with different genotypes.

Discussion

The PLCE1 gene is mapped to 10 q23 and encodes PLCE1 protein which is a member of phosphoinositide-specific phospholipase C (PLC) family. PLCE1 protein, like other PLC family members, is composed of PLC catalytic domain, PH domain, EF domain and C2 domain. However, PLCE1 protein has unique regions, two RA domains at its C terminus and a CDC25-like domain at its N terminus. Especially, the two RA domains directly interact with several GTP ases of Ras family. Thus, PLCE1, as a multifunctional signaling protein, plays an important role in the cell growth, differentiation, gene expression and oncogenesis [26]. Several studies have shown that PLCE1 functions as an effector of Ras and is a major participant in the progression of various cancers, include lung cancer [27], gallbladder cancer [28], colorectal cancer [29] and head and neck cancer [30]. A genome-wide association study (GWAs) also reports that gastric adenocarcinoma and esophageal squamous cell carcinoma shared a susceptibility in PLCE1 [31]. These findings suggest that PLCE1 may affect the risk for some cancers in human. Some SNPs (such as rs2274223) in PLCE1 also affect the gene expression or protein functions as well as the risk for esophageal cancer [32]. Recently, study indicates that PLCE1 level is associated with the suppression of TP53 expression in esophageal cancer cells, and PLCE1 can interfere with STAT3 phosphorylation, thereby inhibiting the p53 expression [33]. Another study shows that PLCE1 is able to inhibit P53 expression in lung cancer; inhibition of PLCE1 increases the p53 expression in non-small-cell lung cancer (NSCLC) cells and further induces NSCLC cell apoptosis [27]. Both studies indicate a negative correlation between PLCE1 expression and p53 expression in NSCLC cells and esophageal cancer cells. These findings suggest that PLCE1 plays a role as an oncogene in the lung cancer and esophageal cancer. Similar findings were found in the present study via the analysis of GEO database for the relationship between TP53 and PLCE1 at mRNA level in HCC. A gene set enrichment analysis study on 242 HCC patients shows that patients who had a high PLCE1 expression had a high risk for metastasis, and thusa poorer prognosis [34]. However, the role of PLCE1 gene in HCC has not been reported. Our study for the first time employed Genome-wide association analysis and found PLCE1- rs2797992 was associated with TP53 expression in HBV-related HCC, but rs2797992 genotypes was not associated with the prognosis of HBV-related HCC in Chinese patients of Guangxi province. These findings provide a theoretical basis for further investigations on the relationship between TP53 expression and PLCE1 SNPs in HCC. GEO database and GeneMANIA analysis also support this finding. Many previous studies have demonstrated that TP53 expression in HCC is aprognostic factor of clinicopathological characteristics [17]. Even so, different from previous studies, the survival time was not different between TP53 negative patients and TP53 positive patients in our study, due to the limitation of study population. But, our findings can still be used to assess the relationship of PLCE1 SNPs and HCC prognosis.

Another SNP, EGFR- rs6950826, was also identified in HBV related HCC. EGFR, encoded by the c-erbB1 proto-oncogene, is a receptor of epidermal growth factor family members and a ligand of extracellular proteins [35]. Lots of studies reveal the role of EGFR signaling pathway in cancers, including HCC. There is evidence showing that HCC patients with early recurrence have a significant increase in EGFR mRNA expression in HCC tissues [36]. EGFR is not only associated with the histological grade of HCC, but with other clinicopathological characteristics, such as serum AFP. Thus, EGFR may serve as a candidate biomarker to predict

the malignant behaviors of HCC and for the diagnosis of HCC.EGFR also correlates with the prognosis of HCC and can be used as a new target in the treatment of HCC [37, 38]. The activation of EGF-EGFR signaling pathway is associated with the progression of CK19-positive HCC and may lead to a poor prognosis of HCC patients [39]. Similar results in EGFR-positive liver macrophages can also promote the tumorigenesis of HCC and is associated with a poor survival [40]. Available findings suggest that soluble-EGFR is a potential biomarker of HCC metastasis [41] and the EGFR inhibitor, erlotinib, can be used to attenuate liver fibrosis and block the progression of HCC [42]. In HBV-related HCC, the EGFR system may promote the immune tolerance and viral amplification after HBV infection, then affecting prognosis. As mentioned above, EGFR is a prognostic marker and a risk factor of HCC recurrence [43]. Different signaling pathways activated in hepatocarcinogenesis and EGFR system are recognized as a "signaling hub". Precisely, EGFR will be a promising target in the therapy of HCC due to the complex role of EGFR in HCC [44]. TP53 expression was significantly correlated with EGFR in HCC, but this relationship did not elaborate [45]. Previous study has reported that HSC1 λ cells over-expressing EGFR have significant decreased TP53 expression [46]. Consistent with findings from many EGFR related studies on HCC, our study also indicated that rs6950826 was related to HBV-related HCC and TP53 expression in HCC. However, survival analysis showedrs6950826 genotypes were not associated with the prognosis of HCC in this population. Analysis of GEO database revealed that EGFR expression and TP53 expression at mRNA level were negatively correlated in HCC and GeneMANIA indicated that EGFR was involved in the TP53 pathway.

In conclusion, rs2797992 in PLCE1 and rs6950826 in EGFR have a moderate association with TP53 expression in immunohistochemistry in Chinese HBV-related HCC patients of Guangxi. Our study for the first evaluates the association between TP53 expression and SNPs of PLCE1 and EGFR in HBV-related HCC. With the help of GSE14520 database, a negative correlation of both PLCE1 mRNA expression and EGFR mRNA expression with TP53 is found in HCC, but the specific mechanism underlying this relationship needs to be further validated in future studies. Due to limitation of relatively small sample size, rs2797992 and rs6950826 were not related to the prognosis of HCC in this population. Further investigations are required to confirm our results.

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Disclosure of conflict of interest

None.

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