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ORIGINAL RESEARCH

Genetic Variants in p53 Pathway Genes Affect Survival of Patients with HBV-Related Hepatocellular Carcinoma

Liming Qin[1,2,*], Moqin Qiu[3,*], Jingmei Tang[2], Shuyan Liu[4], Qiuling Lin[5], Qiongguang Huang[2], Xiaoxia Wei[5], Qiuping Wen[1], Peiqin Chen[6], Zihan Zhou[7], Ji Cao[7], Xiumei Liang[8], Qian Guo[9], Cunli Nong[9], Yizhen Gong[5], Yuying Wei[1], Yanji Jiang[10], Hongping Yu[1,2,11–13], Yingchun Liu[1]

[1]Department of Experimental Research, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China; [2]School of Public Health, Guangxi Medical University, Nanning, People's Republic of China; [3]Department of Respiratory Oncology, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China; [4]Key Laboratory of Biological Molecular Medicine Research, Education Department of Guangxi Zhuang Autonomous Region, Guangxi Medical University, Nanning, People's Republic of China; [5]Department of Clinical Research, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China; [6]Editorial Department of Chinese Journal of Oncology Prevention and Treatment, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China; [7]Department of Cancer Prevention and Control, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China; [8]Department of Disease Process Management, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China; [9]Liuzhou Worker's Hospital, Liuzhou, People's Republic of China; [10]Department of Scientific Research, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China; [11]Key Laboratory of Early Prevention and Treatment for Regional High Frequency Tumor, Ministry of Education, Guangxi Medical University, Nanning, People's Republic of China; [12]Guangxi Key Laboratory of Early Prevention and Treatment for Regional High Frequency Tumor, Nanning, People's Republic of China; [13]Key Cultivated Laboratory of Cancer Molecular Medicine of Guangxi Health Commission, Guangxi Medical University Cancer Hospital, Nanning, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yingchun Liu; Hongping Yu, Department of Experimental Research, Guangxi Medical University Cancer Hospital, 71# Hedi Road, Qingxiu District, Nanning, People's Republic of China, Tel +86-15877191237; +86-771-5330850, Fax +86-771-5312000, Email liuyingchun@stu.gxmu.edu.cn; yuhongping@stu.gxmu.edu.cn

Purpose: *P53* is a suppressor gene closely related to carcinogenesis. However, the associations between genetic variants in the p53 signaling pathway and prognosis in hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) remain unknown. The current study aims to analyze associations between the single nucleotide polymorphisms (SNPs) in p53 pathway-related genes and survival of patients with HBV-HCC.

Methods: We evaluated the associations between 4698 SNPs in 70 genes of the p53 pathway and overall survival (OS) of 866 patients in additive genetic models by using Cox proportional hazards regression analysis. Stepwise multivariable Cox regression analysis was conducted to determine the independent effects of identified SNPs in single-locus analyses. The expression of quantitative trait loci (eQTL) was also analyzed using data from GTEx and 1000 Genomes Project, and functional prediction of SNPs was performed by using RegulomeDB v2.2, 3DSNP v2.0, HaploReg v4.2 and VannoPortal.

Results: We found that two novel SNPs of *CD82* rs7925603 A > G and *PMAIP1* rs4396625 A > T, were significantly and independently associated with OS [adjusted hazards ratios (HRs) and 95% confidence intervals (CI) were 1.27 (1.10–1.48) and 0.77 (0.66–0.91), respectively; $P = 0.001$ and = 0.002, respectively] and that the combined risk genotypes of these SNPs showed a significant association with OS in patients with HBV-HCC ($P_{trend} < 0.001$). Further eQTL analysis in the GTEx dataset showed that the rs7925603 G allele was associated with lower *CD82* mRNA expression levels, while the rs4396625 T allele was associated with higher *PMAIP1* mRNA expression levels in whole blood cells.

Conclusion: We identified two observed survival-associated SNPs in *CD82* and *PMAIP1* in the p53 pathway, which influenced HBV-HCC survival possibly through a mechanism of altering mRNA expression. Large studies are warranted to validate our findings.

Keywords: hepatocellular carcinoma, hepatitis B virus, p53 signaling pathway, genetic variants, survival

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Introduction

Liver cancer is a significant public health issue and a leading cause of cancer-related deaths. According to global cancer statistics, there were approximately 905,677 reported cases of liver cancer and 830,180 related deaths in 2020, making it the third most common cause of cancer deaths.[1] Hepatocellular carcinoma (HCC), as the main type of liver cancers, accounted for about 80% of all the cases.[2] Chronic active hepatitis caused by hepatitis B virus (HBV) infection is a major risk factor for HCC,[3] and 87.7% of HCC patients have contracted with HBV in Southern China.[4] Despite advances in treatment strategies such as surgical resection, liver transplantation, percutaneous ablation and radiation as well as transarterial and systemic therapies, the 5-year survival rate for HCC patients remains discouraging at around 12.1%.[5] Additionally, individual patients with the similar clinical characteristics and histopathological types may experience different survival outcomes, when received the same treatment methods. Even patients with early-stage HCC who received hepatectomy still have a variable survival,[6] suggesting genetic susceptibility to risk of death (or survival) after surgery, indicating that genetic factors, such as single nucleotide polymorphisms (SNPs), may play a role.[7] As a result, there is an urgent need to identify prognostic biomarkers for survival of HBV-HCC in the key genes and pathways to guide personalized management and treatment for HBV-HCC.

Previous studies have shown that Reticulons 3 (*RTN3*) is downregulated in HCC compared with normal hepatocytes, restraining HCC growth and inducing apoptosis by activating p53.[8] Further research suggests that hepatitis B antigen (HBxAg) can bind to p53, leading to the inactivation of p53 function and "gain of function" mutations may occur during tumor progression. This combination is important for the occurrence and development of HCC.[9–11] These findings indicate that abnormal activation of the p53 pathway plays a crucial role in the carcinogenesis and progression of HCC. The tumor suppressor protein, p53, is closely related to carcinogenesis and tumorigenesis. It consists of five main structural regions, including a proline-enriched region, a DNA binding region, tetramer structural region, a C-terminal regulatory structural region and a transcriptional activation structural region.[12] The p53 could be activated upon different cell stresses, initiating the transcriptional program that plays an essential role in cell cycle arrest, cell apoptosis, cellular senescence, DNA recombination and repair, inhibition of angiogenesis, regulation of metabolism or other cellular responses.[13,14] As a transcription factor, p53 is strictly regulated by a rich network of post-translational modifications, such as phosphorylation, acetylation, and ubiquitination. These modifications affect the stability, DNA binding ability, and transcriptional activity of p53, thereby exerting its role in tumor suppression. Mutations in the p53 gene are one of the most common events in cancer, and mutant p53 may completely lose its tumor suppressive function, and may even acquire new oncogenic functions. The roles of mutant p53 in tumor cells include promoting cell proliferation, invasion, metastasis, and treatment resistance in cancer.[15–17] The MDM2 protein is a key negative regulator of the p53 pathway. One study found that SNP309 in the *MDM2* promoter could increase the affinity of transcription activator Sp1 and promote the expression of MDM2 RNA and protein, leading to subsequent inactivation of the p53 pathway, which was associated with accelerating cancers formation.[13] Other key genes in the p53 signaling pathway, such as tumor protein p63 (*TP63*) and tumor protein p73 (*TP73*), have also been shown to play important roles in the development of cancers.[18]

Genome-wide association studies (GWAS) were considered a powerful tool for genetic analysis of complex traits and diseases, including cancers.[19] In the post-GWAS era, the method based on the biological pathway was a promising hypothesis-driven approach, which has been used to identify functionally reasonable SNPs related to patient survival.[20] A previous study found that the TP73 rs747828 C allele in the p53 signaling pathway was significantly associated with reduced progression-free survival in colorectal cancer and the functional genetic variants of TP73 mediated the response to chemotherapy in colorectal cancer.[21] However, the specific genetic variants in candidate genes that affect the function of the p53 pathway in the development and progression of HCC are still unknown. Considering that the *P53* gene plays an important role in many cancers, it is reasonable to assume that naturally occurring genetic variants in the p53 pathway may also influence the development and progression of HCC. However, there are currently no reports on the associations between genetic variants in the p53 pathway genes and survival of HBV-HCC patients based on the method of biological pathway. It is crucial to identify potentially functional SNPs in the p53 pathway genes as predictive biomarkers for survival of HBV-HCC. Therefore, the aim of the current study is to analyze the associations between SNPs in p53 pathway-related genes and overall survival (OS) of patients with HBV-HCC by utilizing a two-stage study design.

Materials and Methods

Study Populations

In the current study, patients were eligible if they were pathologically diagnosed with HBV-HCC and had undergone hepatectomy at the Guangxi Medical University Cancer Hospital from July 2007 to December 2017. The specific inclusion and exclusion criteria and basic characteristics of patients have been described in the previous study.[22,23] Briefly, peripheral whole blood samples were collected for genotyping, and clinical data such as age, sex, smoking and drinking status, alpha-fetoprotein (AFP) level, cirrhosis, cancer embolus, and Barcelona clinic liver cancer (BCLC) stage were also collected. According to the Chinese Guideline of surgical treatment for HCC patients,[24] the operable patients included some patients with BCLC B or C stage. According to the inclusion and exclusion criteria, 866 HBV-HCC patients were ultimately included in the current study and were randomly divided into a discovery dataset and a replication dataset in a 1:1 ratio. Their age varied from 20 to 81 years (median age of 47 years old). All these patients were followed up every three months in the first two years after hepatectomy and followed up every six months in the following years via phone until the death of the patient or March 2020. The endpoint of the current study was overall survival (OS), defined as the time from hepatectomy to death or the last effective follow-up. All the above-mentioned basic characteristics of patients are defined as co-variables to be used for subsequent multivariable analyses. The current study has been approved by the Institutional Review Board of Guangxi Medical University Cancer Hospital (Approval number: LW2023189), and written informed consent has been obtained from all participants.

Genotyping, Imputation, Gene and SNP Selection

The whole-genome DNA from the peripheral blood of each HCC patient was extracted by using blood DNA extraction kit (Concert, Xiamen, China). Genotyping was performed by using the Illumina Infinium® Global Screening Assay genotyping chip (GSA, GSAMD-24v1-0, Illumina, San Diego) at Genenergy Biotechnology (Shanghai, China) using the Illumina iScan System. SNP imputation was performed by a web-based platform Michigan Imputation Server (Minimac3) according to the International 1000 Genomes projects (1000G, Phase 3 v5) by using the Minimac3 software.

We selected the genes involved in the p53 pathway using the keyword "p53" by searching the Molecular Signatures Database (http://software.broadinstitute.org/gsea/msigdb/index.jsp). This database uses gene set enrichment analysis methods to construct a gene set of multiple genes with similar positions or functions from multiple perspectives such as gene location, function, metabolic pathways, and target binding, in order to compensate for the deficiency that a single gene cannot explain disease phenomena. After excluding duplicated genes, genes on the sex chromosome, and genes absent in the human genome reference (hg19), we identified a total of 70 candidate genes for further analysis (Table S1). We extracted relevant SNPs within ± 2kb flanking regions of candidate genes from the Chinese Han population in Beijing (CHB) in the 1000 Genomes Project using Plink (version 1.09) (http://pngu.mgh.harvard.edu/purcell/plink/) for analysis. We screened SNPs that met the following criteria: (1) call rate ≥ 95%, (2) MAF ≥ 5%, and (3) HWE test $P \geq 1.0\times10^{-6}$. Ultimately, a total of 4698 SNPs from the discovery dataset were available for further analysis.

Functional Prediction and Expression Quantitative Trait Loci (eQTL) Analysis

Furthermore, the linkage disequilibrium (LD) analysis between the significant SNPs was analyzed by using Haploview software with r^2 threshold of 0.8, and functional prediction of the significant SNPs was carried out by using various web-based tools, including Regulome DB v2.2 (https://www.regulomedb.org/regulome-search/), 3DSNP v2.0 (https://www.omic.tech/3dsnpv2/), HaploReg v4.2 (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) and VannoPortal (http://www.mulinlab.org/vportal). Among those significant SNPs in LD, the predicted functional SNPs will be chosen for further analysis. The correlation between the independently functional SNPs and their respective mRNA expression levels were evaluated by using eQTL analysis. The mRNA expression data from the genotype-tissue expression (GTEx) project, 1000 Genomes Project and TCGA dataset were used. GTEx v8 has 208 normal liver tissues and 607 whole blood cells from donors, and 1000 Genomes has 358 Europeans and 76 Han Chinese in Beijing, China (CHB), were used in this analysis. Additionally, we also compared the mRNA expression levels between tumor and adjacent normal tissues by using the TCGA data from the UALCAN database (https://ualcan.path.uab.edu/analysis.html) as well as the mutation

rates in the identified genes in the tumor tissues by using the cBioPortal database (https://www.cbioportal.org/). The Haploview software was used to draw a Manhattan plot.

Statistical Analysis

In the single-locus analysis, we used multivariate Cox proportional hazards regression analysis with adjusted for age, sex, smoking and drinking status, AFP level, cirrhosis, cancer embolus, and BCLC stage to evaluate the associations between the SNPs in 70 genes of the p53 pathway and OS of the HBV-HCC patients in additive genetic models. Univariable and multivariable Cox proportional hazards regression analyses were also used to calculate the hazard ratio (HRs) and their 95% confidence interval (CI) for each of SNPs. Multiple testing corrections were performed by using false-positive report probability (FPRP)[25] and the Bayesian false discovery probability (BFDP)[26] as recommended. We chose BFDF with a cut-off value of 0.80 and a prior probability of 0.10 for the multiple testing corrections, because many SNPs tested in the single-locus analysis were in LD or in the same block as a result of imputation.[26] Replication of the SNPs found in the discovery dataset was conducted by using the replication dataset, and the independent SNPs were identified by using multivariable stepwise Cox regression analysis with the adjustments for age, sex, smoking and drinking status, AFP level, cirrhosis, cancer embolus, and BCLC stage. We used bootstrap method for 1000 times random sampling to verify that the results of SNPs were not accidental.[27] Kaplan–Meier curves analysis was also performed to assess survival for the combined risk genotypes. Subgroup analysis was conducted to explore the potential interactions between the combined risk genotypes and each of covariables on the HBV-HCC survival. All these statistical analyses were conducted by using R software (versions 3.1.3 and 4.1.2) and PLINK (version 1.9), and two-sided $P < 0.05$ was considered statistically significant.

Results

Association Between SNPs in p53 Pathway Genes and OS of the HBV-HCC Patients

The current study design is illustrated in Figure 1, a total of HBV-HCC 866 patients were included in the current study and were randomly divided into a discovery dataset and a replication dataset. The specific characteristics of the patients

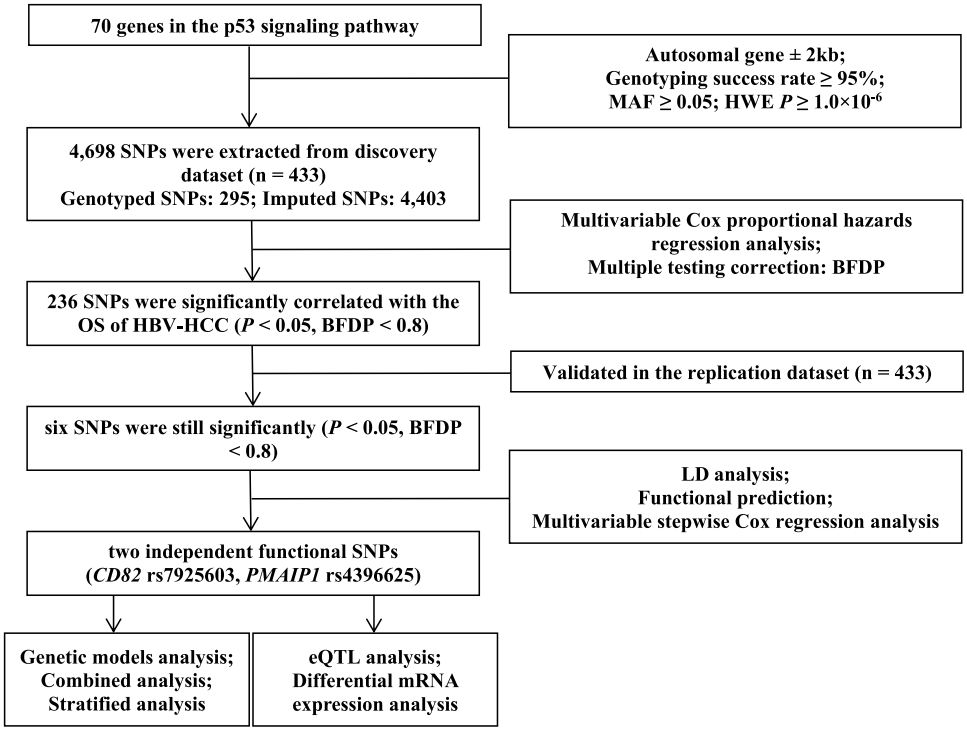

Figure 1 Flow chart of the study design.

are shown in Table S2. In the discovery dataset, a total of 4698 SNPs (295 genotyped and 4403 imputed SNPs) in 70 genes of the p53 pathway were identified. In the single-locus analysis, the associations between these SNPs and the survival of patients with HBV-HCC were evaluated through multivariable Cox regression analysis with adjustment for the co-variables. After multiple testing correction, 236 SNPs were found to be significantly associated with the OS of patients with HBV-HCC ($P < 0.05$, BFDP < 0.8). Further replication identified six SNPs that remained statistically noteworthy ($P < 0.05$, BFDP < 0.8, Table 1 and Figure 2). Among these six SNPs, four were located in *CD82*, and two were in *PMAIP1*. The results are summarized in the Manhattan plots (Figure S1).

Functional Prediction and LD Analysis

In order to identify potentially functional SNPs among these six identified SNPs, we used bioinformatics tools, including RegulomeDB v2.2, 3DSNP v2.0, HaploReg v4.2 and VannoPortal to explore their potential functions. As shown in Table 2, the four SNPs in the *CD82* gene were all located in intron regions. Among them, the RegulomeDB v2.2 rank and 3DSNP v2.0 score of rs7925603 are 2b and 28.45, respectively; and the rs7925603 was located in the enhancer histone marks and DNase hypersensitive sites (IPSC, KID, BLD), and it may affect nine motifs and multiple-histone modifications. The two SNPs in the *PMAIP1* both were downstream gene variants, among which the RegulomeDB v2.2 rank and 3DSNP v2.0 scores of rs4396625 were 4 and 85.26, respectively, and the rs4396625 located in the promoter histone marks, enhancer histone marks and DNase hypersensitive sites, and it may affect various histone modifications. In addition, we conducted LD analysis on SNPs in *CD82* and *PMAIP1*. It was found that there was a high LD between *CD82* rs744631 and rs7925603, *CD82* rs7129118 and rs7925603, and *PMAIP1* rs4396625 and rs4396625 ($r^2 > 0.80$, Figure S2). Based on functional prediction and LD analysis, we selected functional SNPs rs7925603 and rs4396625 for further analysis.

Independent SNPs and OS of HBV-HCC in the Whole Dataset

To evaluate the independent effects of two functional SNPs (ie, rs7925603 and rs4396625) on the survival of HBV-HCC patients, a stepwise multivariable Cox regression analysis was conducted using the whole dataset of 866 HBV-HCC. As a result, the *CD82* rs7925603 A > G (HR = 1.24, 95% CI = 1.07–1.44 and $P = 0.004$) and *PMAIP1* rs4396625 A > T (HR = 0.78, 95% CI = 0.66–0.92 and $P = 0.004$) were found to be independently associated with the OS of HBV-related HCC patients in the presence of covariables (ie, age, sex, smoking, drinking, AFP, Cirrhosis, Cancer embolus, and BCLC stage) (Table 3).

Associations Between CD82 rs7925603 and PMAIP1 rs4396625 Genotypes with HBV-HCC OS

As shown in Table 4, the *CD82* rs7925603 G allele was significantly associated with a poor survival for HCC patients, while the *PMAIP1* rs4396625 T allele was significantly associated with a better for HCC patients, in a dose–response manner in the additive models (both $P_{trend} = 0.003$). In the dominant genetic model, *CD82* rs7925603 AG/GG was associated with a poor survival compared with AA genotypes (HR = 1.27, 95% CI = 1.04–1.54, $P = 0.019$), while *PMAIP1* rs4396625 AT/TT was associated with a better survival in HBV-HCC patients compared with AA genotypes (HR = 0.75, 95% CI = 0.62–0.92, $P = 0.006$).

Subsequently, to verify that the results of these two SNPs were not accidental, we used bootstrap method for 1000 times random sampling and calculated HR values by using multivariable Cox regression analysis. The results showed that the HR values followed a normal distribution, and their 95% CI included the HR values of these two SNPs, which indicated that the results were reliable (Figure S3).

To analyze the accumulative effect of the two independent SNPs on survival of HBV-HCC patients, their risk genotypes (rs7925603 AG/GG and rs4396625 AA) were classified into a genetic score, the number of risk genotypes (NRGs), to categorize all HBV-HCC patients into three groups with 0, 1, and 2 NRGs. Multivariate analysis showed that a high NRG was significantly associated with a poor survival in a dose–response manner ($P_{trend} < 0.001$) with adjustments for the covariables (Table 4). Additionally, all the patients were divided into low-risk (0–1 NRG) and high-

Table 1 Association Between Six Significant SNPs and OS of HBV-HCC in Multivariable Cox Regression Models for the Single-Locus Analysis

SNPs	Gene	Allele[a]	Discovery Dataset (n = 433)					Replication Dataset (n = 433)					Whole Dataset (n = 866)				
			MAF	HR (95% CI)[b]	*P*[b]	BFDP	FPRP	MAF	HR (95% CI)[b]	*P*[b]	BFDP	FPRP	MAF	HR (95% CI)[b]	*P*[b]	BFDP	FPRP
rs744631	*CD82*	C > G	0.274	1.29 (1.03–1.62)	0.029	0.768	0.221	0.289	1.33 (1.07–1.66)	0.010	0.623	0.221	0.281	1.30 (1.12–1.52)	0.001	0.192	0.009
rs35969493	*CD82*	A > G	0.269	1.35 (1.07–1.69)	0.010	0.567	0.088	0.286	1.38 (1.11–1.72)	0.004	0.416	0.088	0.278	1.35 (1.16–1.58)	< 0.001	0.050	0.002
rs7129118	*CD82*	C > T	0.301	1.26 (1.01–1.56)	0.041	0.797	0.244	0.331	1.30 (1.06–1.59)	0.013	0.615	0.244	0.316	1.27 (1.10–1.48)	0.001	0.324	0.020
rs7925603	*CD82*	A > G	0.301	1.26 (1.01–1.56)	0.041	0.797	0.244	0.331	1.30 (1.06–1.59)	0.013	0.615	0.244	0.316	1.27 (1.10–1.48)	0.001	0.324	0.020
rs4396625	*PMAIP1*	A > T	0.241	0.76 (0.60–0.96)	0.022	0.723	0.182	0.225	0.78 (0.61–0.98)	0.034	0.787	0.182	0.233	0.77 (0.66–0.91)	0.002	0.311	0.020
rs1942919	*PMAIP1*	G > T	0.241	0.76 (0.60–0.96)	0.022	0.723	0.182	0.226	0.78 (0.62–0.98)	0.035	0.787	0.182	0.234	0.77 (0.66–0.91)	0.002	0.311	0.020

Notes: [a]reference allele > effect allele; [b]The adjusted variables for multivariable Cox proportional hazards regression analysis were age, sex, smoking, drinking, AFP, Cirrhosis, Cancer embolus, BCLC stage.

Abbreviations: SNPs, single nucleotide polymorphisms; OS, overall survival; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; MAF, minor allele frequency; HR, hazards ratio; CI, confidence interval; BFDP, Bayesian false-discovery probability; FPRP, false-positive report probability.

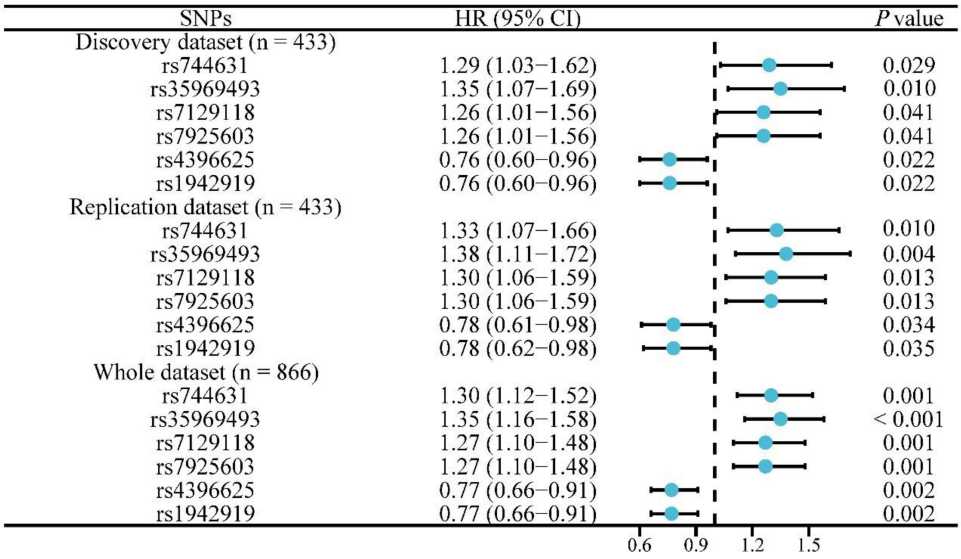

Figure 2 Forest plot of the association between six significant SNPs and OS of HBV-HCC in multivariable Cox regression models.

risk (two NRGs) groups. Compared with the low-risk group, the high-risk group had a significantly poor survival for OS (HR = 1.38, 95% CI = 1.13–1.69, P = 0.002). Kaplan–Meier curves were constructed to represent the associations between risk genotypes and the OS of patients with HBV-HCC (Figure 3).

Stratified Analysis Between Risk Genotypes with HBV-HCC OS

In further stratified analysis, we investigated whether the combined risk genotypes interplayed with all the covariables on the survival of HBV-HCC patients in the whole dataset. It was shown that among subgroups of patients aged less than or equal to 47 years, male, never drinking, never smoking, AFP levels less than or equal to 400 ng/mL, liver cirrhosis, no cancer embolus, and BCLC stage, the high-risk group had a poor survival for OS. In addition, we did not find any interaction between the combined risk genotypes and each of these covariables on HBV-HCC survival, except for sex (P = 0.011, Table 5).

The eQTL Analysis and Differential mRNA Expression Analysis

To explore the association between the two independently functional SNPs and the respective mRNA expression, we performed the eQTL analysis. In the GTEx project dataset, for *CD82* rs7925603, it was found that the G allele was significantly associated with lower *CD82* mRNA expression levels in whole blood cells (P = 0.034, Figure 4A), while there was no significant association was observed in normal liver tissues (P = 0.246, Figure S4A). For another SNP *PMAIP1* rs4396625, the T allele was significantly associated with higher mRNA expression levels in whole blood cells (P < 0.001, Figure 4C) instead of normal liver tissues (P = 0.378, Figure S4D). We also performed the eQTL analysis by using data from the 1000 Genomes Project. However, in both European descendants and CHB population, no significant correlations were found between both *CD82* rs7925603 G and *PMAIP1* rs4396625 T allele with the mRNA expression levels of corresponding genes (both P > 0.05, Figure S4B, C, E and F). Besides, the two SNPs information were not available in TCGA dataset.

Subsequently, we further compared the mRNA expression levels of these two genes between HCC tissues and adjacent normal tissues by using the TCGA database. It was found that the expression levels of *PMAIP1* mRNA in HCC tissue were significantly lower than in adjacent normal tissues (P = 0.001, Figure 4D). Similar trend was also observed in *CD82*, but there was no statistically significant (P = 0.444, Figure 4B). In addition, we used the TCGA data to investigate the somatic mutations of *CD82* and *PMAIP1* in HCC tissues. It was found that the mutation rates of these two genes in HCC tissues were both less than 1.5% (Figure S5), indicating that somatic mutations of these two genes may play a minor role in their expression levels.

Table 2 Functional Annotation of the Six SNPs

SNPs	Gene	Location	RegDB v2.2[a]	3DSNP v2.0[b]		HaploReg v4.2[c]				VannoPortal[d]
			Rank	Score	TFBS	Promoter Histone Marks	Enhancer Histone Marks	DNAse	Motifs Changed	Mark
rs744631	*CD82*	intron	5	2.85	0	–	7 tissues	–	–	H3K79me2
rs35969493	*CD82*	intron	5	6.25	0	–	7 tissues	–	6 altered motifs	H3K36me3
rs7129118	*CD82*	intron	4	17.17	1	–	6 tissues	LNG	SREBP	H3K27ac, H3K4me1
rs7925603	*CD82*	intron	2b	28.45	0	–	13 tissues	IPSC, KID, BLD	9 altered motifs	DNase, H3K27ac, H3K36me3, H3K4me1
rs4396625	*PMAIP1*	downstream	4	85.26	30	ESC, IPSC, BLD	19 tissues	15 tissues	–	DNase, H3K27ac, H3K4me2, H3K4me3
rs1942919	*PMAIP1*	downstream	5	46.20	18	ESC, BLD	10 tissues	ESDR, SKIN	GR	H3K27ac, H3K4me1, H3K4me2, H3K4me3, H3K79me2

Notes: [a]http://www.regulomedb.org/index; [b]https://www.omic.tech/3dsnpv2/; [c]https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php; [d]http://www.mulinlab.org/vportal.

Table 3 Stepwise Cox Regression Analysis of Clinical Factors and SNPs in the Whole Dataset

Variables	Cases	Multivariable Analysis	
		HR (95% CI)[a]	*P*[a]
Age[b]			0.016
≤ 47	434	1.00	
> 47	432	0.79 (0.65–0.96)	
Sex			0.095
Female	106	1.00	
Male	760	1.32 (0.95–1.83)	
AFP (ng/mL)			0.019
≤ 400	522	1.00	
> 400	344	1.27 (1.04–1.56)	
Cancer Embolus			< 0.001
No	636	1.00	
Yes	230	1.77 (1.40–2.23)	
BCLC Stage			< 0.001
0/A	427	1.00	
B/C	439	1.95 (1.54–2.48)	
rs7925603			0.004
AA/AG/GG	399/386/81	1.24 (1.07–1.44)	
rs4396625			0.004
AA/AT/TT	516/296/54	0.78 (0.66–0.92)	

Notes: [a]The adjusted variables for Cox proportional hazards regression analysis were age, sex, smoking, drinking, AFP, Cirrhosis, Cancer embolus, BCLC stage, rs7925603 and rs4396625; [b]The median age in the whole dataset was 47 years old.
Abbreviations: SNPs, single nucleotide polymorphisms; HR, hazards ratio; CI, confidence interval; AFP, alpha-fetoprotein; BCLC, Barcelona clinic liver cancer.

Table 4 Associations Between Genotypes of Two Independent SNPs and OS of HBV-HCC

Genotype	Cases	Deaths (%)	Multivariable Analysis	
			HR (95% CI)[b]	*P*[b]
***CD82* rs7925603**				
AA	399	179 (44.86)	1.00	
AG	386	192 (49.74)	1.20 (0.97–1.47)	0.088
GG	81	48 (59.26)	1.65 (1.19–2.27)	0.002
P_{trend}				0.003
AG/GG	467	240 (51.39)	1.27 (1.04–1.54)	0.019
***PMAIP1* rs4396625**				
AA	516	265 (51.36)	1.00	
AT	296	134 (45.27)	0.79 (0.64–0.97)	0.026
TT	54	20 (37.04)	0.59 (0.38–0.94)	0.026
P_{trend}				0.003
AT/TT	350	154 (44.00)	0.75 (0.62–0.92)	0.006

(*Continued*)

Table 4 (Continued).

Genotype	Cases	Deaths (%)	Multivariable Analysis	
			HR (95% CI)[b]	*P*[b]
NRGs[a]				
0	169	71 (42.01)	1.00	
1	411	191 (46.47)	1.27 (0.96–1.68)	0.089
2	286	157 (54.90)	1.64 (1.23–2.18)	0.001
Trend				<0.001
0–1	580	262 (45.17)	1.00	
2	286	157 (54.90)	1.38 (1.13–1.69)	0.002

Notes: [a]NRG: number of protective genotypes (ie, rs7925603 AG/GG; rs4396625 AA); [b]The adjusted variables for Cox proportional hazards regression analysis were age, sex, smoking, drinking, AFP, Cirrhosis, Cancer embolus, BCLC stage.

Abbreviations: SNPs, single nucleotide polymorphisms; OS, overall survival; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazards ratio; CI, confidence interval; NRGs, number of risk genotypes.

Discussion

In the current study, we identified two novel SNPs (ie, *CD82* rs7925603 A > G and *PMAIP1* rs4396625 A > T) in the p53 pathway genes that were significantly associated with the survival of HBV-HCC patients. Specifically, the *CD82* rs7925603 G allele was associated with a poor survival, while the *PMAIP1* rs4396625 T allele was associated with a better survival of HBV-HCC patients. Additionally, their combined risk genotypes were associated with poor HBV-HCC survival. Further eQTL analysis revealed that the rs7925603 G allele was significantly correlated with reduced mRNA expression levels of *CD82*, while the rs4396625 T allele was significantly correlated with elevated mRNA expression levels of *PMAIP1*. The *CD82* rs7925603 A > G and *PMAIP1* rs4396625 G > T change may have an effect on its binding to enhancer histone marker, promoter histone marker, motif modification, and histone modifications. Collectively, our study provided some evidence for the associations between genetic variants in the p53 pathway genes and the prognosis of HBV-HCC patients. These potentially functional SNPs may serve as prognostic markers for HBV-HCC, which may be beneficial for guiding personalized management and treatment of HBV-HCC patients.

CD82, also known as *KAI1*, is situated on the human chromosome 11p11.2. It belongs to the transmembrane 4 superfamily and has been implicated in various biological processes including signal transduction, adhesion, proliferation, migration, movement, protein trafficking, and aggregation.[28,29] For example, *CD82* functions as a tumor suppressor by inhibiting tumor invasion and metastasis, generally expressed in normal tissues and down-regulated in various cancers including HCC.[30–33] Noticeably, the *CD82* promoter contains a binding motif of transcription factor p53, which activates the *CD82* gene at the transcriptional level through binding by p53 to the specific site in its 5'upstream region; thus, the expression of *CD82* is positively regulated by p53, but the loss of p53 function may down-regulate the expression of *CD82*, promoting tumor metastasis and leading to a poor survival of cancers.[30,34] Another study found that the HBx protein, a component of HBV, induced *CD82* promoter methylation and inhibited *CD82* expression at the transcriptional level, accelerating the progression of HCC.[35] These findings suggested that *CD82* may have inhibit the progression of HBV-HCC. Consistent with previous studies, we found that the mRNA expression levels of *CD82* in HCC tissues were higher than that in normal adjacent tissues, and the rs7925603 G allele was associated with lower *CD82* mRNA expression levels.

Another p53 pathway-related gene, *PMAIP1* identified by the rs4396625 SNP, is located on chromosome 18q21.32 and belongs to the pro-apoptotic subfamily of the B-cell lymphoma-2 (Bcl-2) protein family. It only contains the BCL-2 homology domain 3 (BH3-only) and is mainly located in mitochondria, regulating apoptosis,[36] and it is a type of programmed cell death, which is essential for organogenesis during embryonic development and tissue homeostasis in the adults.[37] *PMAIP1* mainly antagonizes the activity of the anti-apoptotic protein Mcl-1 in the Bcl-2 family, promoting the activation of BAX/BAK protein on the surface of mitochondrial membrane, thereby

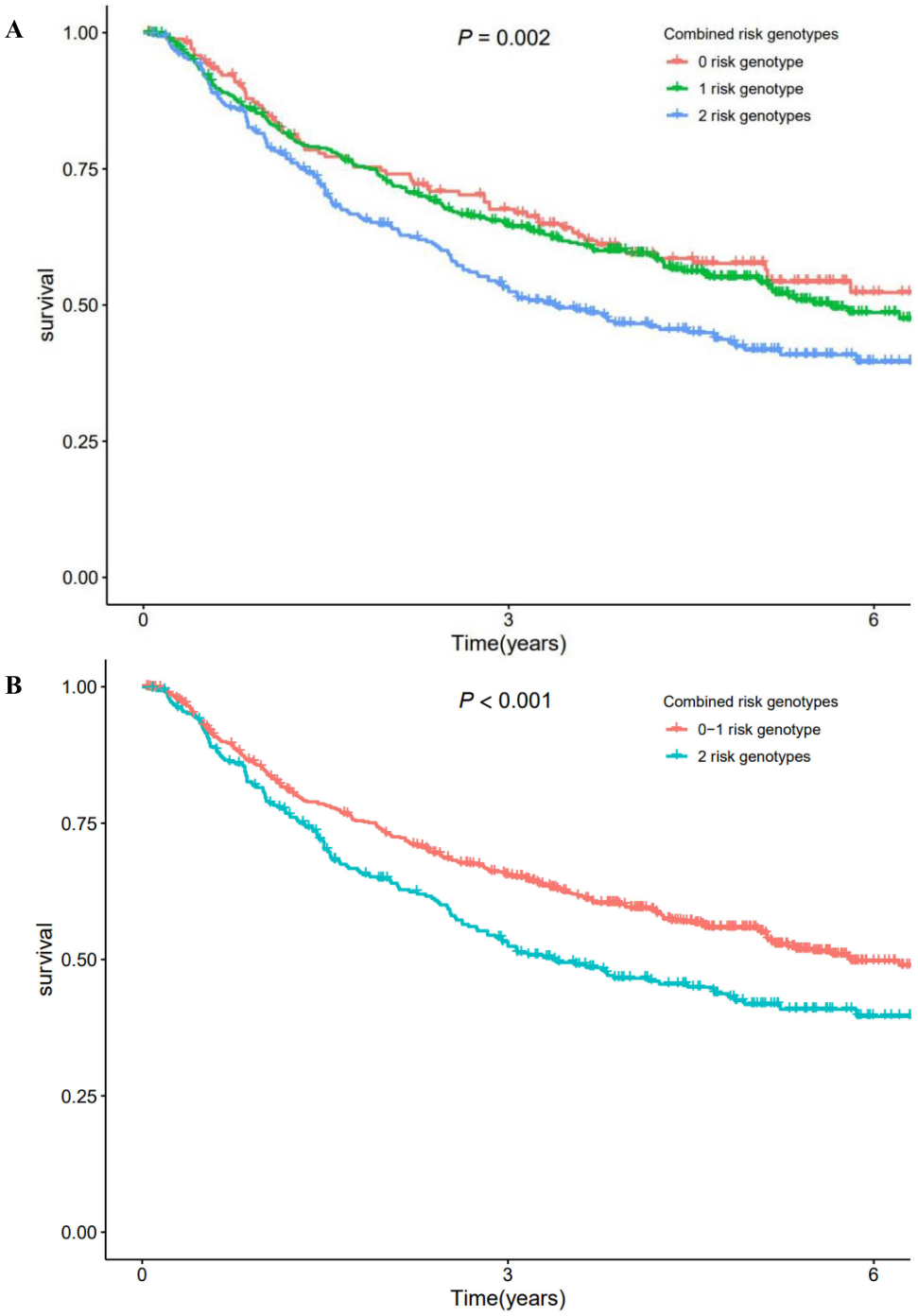

Figure 3 Kaplan–Meier curves of combined protective genotypes and OS of HBV-HCC in the whole dataset. (**A**) Kaplan–Meier curves of NRGs; (**B**) Kaplan–Meier curves of low-risk group (0–1 risk genotype) and high-risk group (2 risk genotypes).

exerting a pro-apoptotic effect.[38,39] Multiple studies have shown that *PMAIP1* can be activated and regulated by p53-dependent pathways, playing an important role in cell apoptosis, the occurrence and development of various malignant tumors, and their treatment.[39–41] For instance, low *PMAIP1* expression in tumor samples was correlated with a worse survival in a clinical trial for relapsed/refractory B-cell lymphoma using tandem CD19/20 CAR T-cells.[42] In liver cancer cells,[43] *PMAIP1* specifically induced cell apoptosis and inhibited the migration and invasion of HepG2 cells, while having minimal effect on the survival of normal liver HL-7702 cells. Here, our

Table 5 Stratified Analysis of Combined Risk Genotypes and OS of HBV-HCC

Variables	0–1 NRG[a]		2 NRGs[a]		Multivariable Analysis		
	Cases	Death (%)	Cases	Death (%)	HR (95% CI)[b]	P^b	$P_{inter}{}^c$
Age[d]							0.080
≤ 47	296	142 (47.97)	138	91 (65.94)	1.68 (1.28–2.01)	< 0.001	
> 47	284	120 (42.25)	148	66 (44.59)	1.13 (0.83–1.53)	0.446	
Sex							0.011
Female	66	28 (42.42)	40	14 (35.00)	0.69 (0.35–1.37)	0.290	
Male	514	234 (45.53)	246	143 (58.13)	1.52 (1.23–1.88)	< 0.001	
Smoking							0.125
Never	360	161 (44.72)	185	107 (57.84)	1.54 (1.20–1.97)	0.001	
Ever	220	101 (45.91)	101	50 (49.51)	1.17 (0.83–1.64)	0.382	
Drinking							0.475
Never	409	178 (43.52)	205	114 (55.61)	1.43 (1.13–1.82)	0.003	
Ever	171	84 (49.12)	81	43 (53.09)	1.29 (0.89–1.88)	0.179	
AFP (ng/mL)							0.938
≤ 400	353	149 (42.21)	169	83 (49.11)	1.44 (1.14–1.84)	0.003	
> 400	227	113 (49.78)	117	74 (63.25)	1.27 (0.87–1.84)	0.212	
Cirrhosis							0.541
No	261	119 (45.59)	119	65 (54.62)	1.28 (0.94–1.75)	0.112	
Yes	319	143 (44.83)	157	92 (58.60)	1.46 (1.12–1.90)	0.006	
Cancer Embolus							0.620
No	432	204 (47.22)	162	98 (60.49)	1.46 (1.13–1.88)	0.004	
Yes	148	100 (67.57)	82	59 (71.95)	1.28 (0.92–1.77)	0.140	
BCLC Stage							0.889
0/A	303	97 (32.01)	124	49 (39.52)	1.45 (1.03–2.06)	0.036	
B/C	277	165 (59.57)	162	108 (66.67)	1.34 (1.04–1.71)	0.021	

Notes: [a]NRG: number of protective genotypes (ie, rs7925603 AG/GG; rs4396625 AA); [b]The adjusted variables for Cox proportional hazards regression analysis were age, sex, smoking, drinking, AFP, cirrhosis, cancer embolus, BCLC stage; [c]P_{inter}: *P* for interaction analysis between NRG and covariables; [d]The median age in the whole dataset was 47 years old.

Abbreviations: OS, overall survival; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; NRGs, number of risk genotypes; HR, hazards ratio; CI, confidence interval; AFP, alpha-fetoprotein; BCLC, Barcelona clinic liver cancer.

results indicated that the *PMAIP1* rs4396625 T allele was associated with higher *PMAIP1* mRNA expression levels, and the *PMAIP1* mRNA expression levels were significantly lower in HCC tissues compared with adjacent normal tissues in TCGA dataset. Furthermore, we also found that the *PMAIP1* rs4396625 T allele is located in promoter and enhancer histone marks, and it may affect the modifications of various histones, such as H3K27ac, H3K4me1, H3K79me2, etc. All these provide strong biological evidence to support our findings of the association between the *PMAIP1* rs4396625 SNP and survival of patients with HBV-HCC.

However, there were several limitations in the current study. Firstly, the above findings may not be generalizable to other populations, because we have enrolled participants from southern China only. Secondly, the sample size of the study population was limited, which did not provide us opportunity to examine subgroup effects. Thirdly, we cannot exclude selection bias of the patients as they all originated from a single institution, where some clinical information was not made available, such as treatment and detailed testing results of hepatitis B infection. Fourthly, this study was unable to collect more comprehensive clinical data, such as detailed treatment information of patients. This study failed to adjust for these confounders, which may influence the associations observed. Finally, the exact molecular mechanisms of the identified independent SNPs that may influence survival of HBV-HCC patients remain unclear. Therefore, additional comprehensive mechanistic studies are needed to validate our findings.

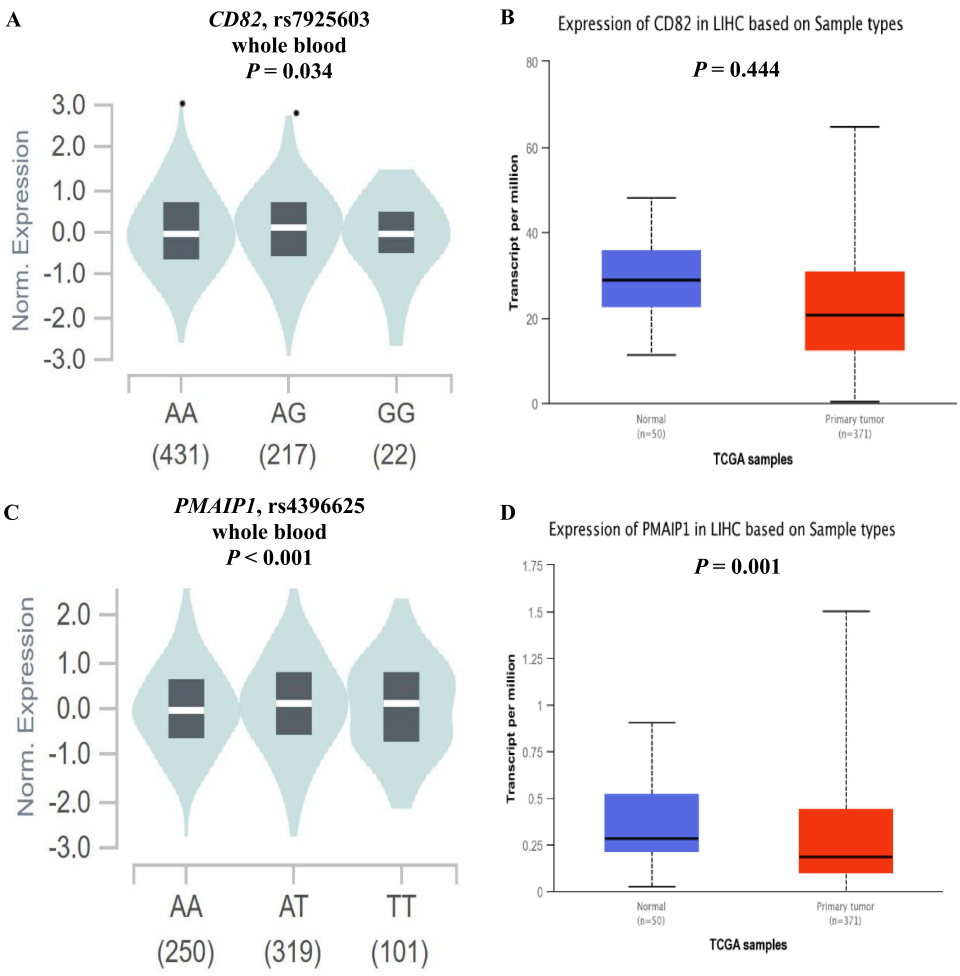

Figure 4 The e-QTL analysis of significant SNPs *CD82* rs7925603 A > G and *PMAIP1* rs4396625 A > T, and differential mRNA expression analysis of HCC from the UALCAN using the TCGA data. (**A**) The association between *CD82* rs7925603 A > G and mRNA expression levels of *CD82* in whole blood samples from the GTEx database; (**B**) *CD82* mRNA expression levels were down-regulated in tumor tissues but not statistically significant from the UALCAN using the TCGA data; (**C**) The association between *PMAIP1* rs4396625 A > T and mRNA expression levels of *PMAIP1* in whole blood samples from the GTEx database; (**D**) *PMAIP1* mRNA expression levels were down-regulated in tumor tissues from the UALCAN using the TCGA data.

Conclusion

The current study has identified two independent SNPs that were significantly associated with the OS of HBV-HCC. These novel SNPs were predicted to have potentially biological functions in modifying targeted gene expression. Our results provide a rationale for further exploring the roles of survival-related SNPs in p53 pathway genes in the progression of HBV-HCC.

Data Sharing Statement

The data that support the findings of this study are available on request from the corresponding authors. The data are not publicly available due to privacy or ethical restrictions.

Ethics Statement

The current study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board of the Guangxi Medical University Cancer Hospital (Approval number: LW2023189).

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

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Disclosure

The authors report no conflicts of interest in this work.

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