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PRDM1 rs1010273 polymorphism is associated with overall survival of patients with hepatitis B virus-related hepatocellular carcinoma

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ABSTRACT

T cell exhaustion is involved in the pathogenesis of chronic hepatitis B virus (HBV) infection and hepatocellular carcinoma (HCC). B lymphocyte-induced maturation protein 1 (BLIMP-1), encoded by the *PRDM1* gene, plays a crucial role in T cell exhaustion. This study investigated *PRDM1* rs1010273 and rs2185379 polymorphisms in 403 patients with chronic HBV infection (171 chronic hepatitis, 119 liver cirrhosis and 113 HCC), 70 spontaneous HBV infection resolvers and 196 healthy controls. The results showed that the rs1010273 and rs2185379 polymorphisms had no significant differences between patients with chronic HBV infection and healthy controls or between patients with different clinical diseases. However, *PRDM1* rs1010273 polymorphism was shown to be significantly associated with the overall survival of patients with HBV-related HCC. The 1-, 3-, and 5-year survival rates of HCC patients were 70.5%, 34.6%, and 11.5%, respectively, in genotype GG carriers and 91.4%, 51.4% and 31.4%, respectively, in genotypes AA + GA carriers ($p = 0.008$). Multivariate analysis showed that *PRDM1* rs1010273 polymorphism was an independent factor associated with the overall survival of patients with HCC (odds ratio, 0.529; 95% confidence interval, 0.126-0.862; $p = 0.002$). These results provide novel evidence for a role of *PRDM1* rs1010273 in the pathogenesis of HBV-related HCC. Additional studies are needed to replicate and extend the findings of this study and to elucidate the underlying mechanisms.

1. Introduction

Chronic hepatitis B virus (HBV) infection may cause various clinical diseases [1,2] and is a leading cause of hepatocellular carcinoma (HCC), one of the most common cancers and the second most common cause of cancer associated deaths globally [3]. The different clinical outcomes of chronic HBV infection is resulted from the complex interaction between the virus and the host immune responses. Notably, T-cell dysfunction or exhaustion has an important involvement in the development of different outcomes of chronic HBV infection [4,5]. HBV-specific CD8 cells have been assumed to play a central mechanistic role in both liver damage and virus control [4] and the status of HBV specific CD8 + T cell exhaustion has been shown to be associated with the different outcomes including HCC in chronic HBV infection [6].

The B lymphocyte-induced maturation protein 1 (BLIMP-1), encoded by the *PRDM1* gene, is a transcription factor that is essential for the differentiation of plasma cells [7,8]. BLIMP-1 is also involved in the regulation and function of T lymphocytes [7,8]. During chronic viral infection, BLIMP-1 is overexpressed in virus-specific CD8 + T cells, plays a critical role in the differentiation of effector CD8+ T cells and memory CD8 + T cell and the regulation of CD8 + T cell exhaustion and memory responses [9–11]. BLIMP-1 was also shown to be a tumor suppressor in lymphomas [12–14].

In view of the fact that T cell exhaustion is associated with the development of different clinical outcomes of chronic HBV infection and the role of BLIMP-1 in T cell exhaustion of chronic viral infection and cancers, we hypothesize that BLIMP-1 may potentially play a role in chronic HBV infection and the development of different clinical diseases. However, the potential involvement of BLIMP-1 in chronic HBV infection remains largely unknown. With regards to this, this study determined polymorphisms of *PRDM1*, the encoding gene of BLIMP-1, in patients with chronic HBV infection of various liver diseases and analyzed their associations with clinical diseases in a Chinese Han population.

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2. Materials and methods

2.1. Study population

Adults with chronic HBV infection were enrolled from the First Affiliated Hospital of Xi'an Jiaotong University from March 2009 to May 2013. Fasting blood was collected in the morning. Three mL coagulation blood was used for serum separation by centrifugation and the serum samples were frozen at −20 °C until use and 2 mL of EDTA-anticoagulated whole blood was frozen at −30 °C for the extraction of human genomic DNA. The diagnosis of the patients was based on the diagnostic criteria of guidance [15]. Other liver diseases including hepatitis A, hepatitis C and hepatitis E, drug-induced liver injury, non-alcoholic fatty liver disease, alcoholic liver disease, autoimmune hepatitis, and Wilson's disease, metabolic disorders such as diabetes and hyperthyroidism, human immunodeficiency virus (HIV) infection and complications associated with other diseases other than HBV diseases were all excluded. Patients aged < 18 years and patients with pregnancy were also excluded. A history of antiviral therapy (interferon or nucleos(t)ides) were excluded in the patients with chronic HBV infection. Individuals who had no history of hepatitis with normal liver function and had no other diseases were included as healthy controls. All the individuals included are of Chinese Han ethnicity and had no close kinship. The study was approved by the Ethics Committee of the First Affiliated Hospital of Xi'an Jiaotong University and performed in accordance with the Declaration of Helsinki. All subjects completed informed consent and voluntarily participated in the study.

2.2. Genotyping of rs573775 and rs510432 polymorphisms

Human genomic DNA was extracted by commercial DNA Extraction Kit (Beijing Tiangen Biochemical Technology Co., Ltd., Beijing, China). Genotyping of *PRDM1* rs1010273 and rs2185379 was carried out using ligase detection reaction based on high temperature ligase detection reaction- polymerase chain reaction (LDR-PCR) as described previously [16].

The primers were designed using oligo6.0 and primer5.0 software and synthesized by Shanghai Biotechnology Co., Ltd (Shanghai,China). The polymorphic regions of the gene were amplified by a multiplex polymerase chain reaction (PCR) with specific primers (Supplementary Table 1). The PCR produced a 237 bp and a 262 bp products for *PRDM1* rs1010273 and rs2185379 polymorphisms, respectively. The quality of DNA was inspected by 1% agarose gel electrophoresis with 1 μL sample, and the DNA concentration was measured, and then was diluted to a final concentration of 5–10 ng/μL.

The sequences of the ligation primers for rs1010273 and rs2185379 are listed in Supplementary Table 1. The procedure of multiplex PCR, ligation and analysis for the rs1010273 and rs2185379 polymorphisms was performed as described previously [16]. In brief, for multiplex PCR, the reaction (10 μL) contained 0.5 μL 1 × GC-I buffer (TaKaRa Bio, Dalian, China), 0.5 μL Mg^{2+} (3.0 mM) (TaKaRa Bio, Dalian, China), 0.5 μL dNTP (0.3 mM) (TaKaRa Bio, Dalian, China), 0.5 μL 1U HotStar Taq Polymerase (Qiagen Inc.), 1 μL of sample DNA (5–10 ng/μL), 2 μL of multiplex PCR primers (1 μM for rs1010273 and 1μM for rs 2185379, respectively) and 5 μL ddH_2O. The PCR programs were set as follows: 1 cycle of 95 °C for 2 min; 11 cycles of 94 °C 20 s, 65 °C for 40 s and 72 °C for 1.5 min; 24 cycles of 94 °C for 20 s, 59 °C for 30 s and 72 °C for 1.5 min; and 1 cycle of 72 °C for 2 min and 4 °C forever. The 2720 Thermal Cycler (Applied Biosystems, Inc. Icarlsbad, USA) and Gel Imaging & Analysis System (Applied Biosystems, Inc. Icarlsbad, USA) were used for analysis. Multiplex PCR product was purified by addition of 5U of SAP (Promega, Madison, USA) and 2U of Exonuclease I (EXO-I, Epicentre, Madison, USA) to 10 μL of PCR product, incubated at 37 °C for 1 h, and then inactivated at 75 °C for 15 min. The ligation reaction of 10 μL contained 1 μL of 10 × ligation buffer (TaKaRa Bio, Dalian, China), 0.2 μL of high temperature ligase (New England Biolabs, Ipswich, MA, USA), 0.4 μL (1μM) of 5' ligation primer mixture (TaKaRa Bio, Dalian, China), 0.4 μL (2μM) of 3' ligation primer mixture (TaKaRa Bio, Dalian, China), 2 μL of purified multiplex PCR products and 6 μL of ddH_2O. The ligation program was as follows: 38 cycles of 94 °C for 1 min and 56 °C for 4 min; and 1 cycle of 4 °C forever. A volume of 0.5 μL of the diluted ligation product, 0.5 μL of Liz500 SIZE STANDARD (Applied Biosystems, Carlsbad, CA, USA) and 9 μL of Hi-Di (Applied Biosystems, Carlsbad, CA, USA) were mixed thoroughly, denatured at 95 °C for 5 min and then analyzed by ABI 3730 XL sequencer (ABI,USA). The data were analyzed by GeneMapper 4.1 (Applied Biosystems, CA, USA).

2.3. Statistical analysis

Statistical analysis was performed by SPSS 16.0 software (SPSS Inc. Chicago, IL, USA). The *t*-test or χ^2 test was used to compare the general data between patients with chronic HBV infection, healthy controls and HBV infection resolvers. The genotype frequencies were tested for Hardy-Weinberg equilibrium. The differences in genotype, allele, and haplotype frequencies between groups were tested by χ^2 test. Haplotype analysis was performed using SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) [17,18]. Logistist regression analysis was performed to identify independent risk factors associated with HBV-related HCC in comparison to non-HCC, and cirrhosis. Univariate and multivariate analyses by Kaplan-Meier and Cox regression analysis were performed to identify the overall survival and factors associated with the prognosis of HCC patients. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Demographics and hardy-Weinberg equilibrium of the genotypes of PRDM1 polymorphism in the study population

The gender and age between the patients, HBV infection resolvers and healthy controls had no significant differences (Supplementary Table 2). The genotypes of *PRDM1* rs1010273 and rs2185379 are all hardy-wineburg equilibrium (all $p > 0.05$, Supplementary Table 2). Of the 403 patients with chronic HBV infection, the clinical diseases were included 171 chronic hepatitis, 119 liver cirrhosis and 113 HCC. The 113 HCC patients (female/male, 98 / 15; mean age, 48.93 ± 10.85 years) had a mean follow-up of 28.0 ± 13.7 months.

3.2. Genotype and allele frequencies of PRDM1 polymorphisms in the study population

Comparisons showed that patients with chronic HBV infection had lower frequency of rs1010273 genotype GA than HBV infection resolvers (28.8% *vs.* 41.4%, $p = 0.035$, OR 0.569, 95%CI 0.336-0.965, Table 1). The frequency of rs1010273 alleles showed no significant difference between patients, resolvers and healthy controls (Table 1). The distribution of rs2185379 genotypes and alleles showed no significant differences between patients, resolvers and healthy controls (Table 1). The distribution of haplotype (rs1010273-rs2185379) between patients, resolvers and healthy controls also showed no significant differences (Table 1).

3.3. Genotype and allele frequencies of PRDM1 polymorphisms in chronic HBV-infected patients with different clinical diseases

The genotype, allele and haplotype frequencies of *PRDM1* rs1010273 and rs2185379 showed no significant differences in HBV chronically infected patients with chronic hepatitis, liver cirrhosis and HCC (Table 2).

Table 1
Genotype, allele and haplotype frequencies of *PRDM1* polymorphisms in patients with chronic hepatitis B virus (HBV) infection, HBV infection resolvers and healthy controls.

	Patients (n = 403)	Resolvers (n = 70)	Controls (n = 196)	*p*	Patients *vs.* Resolvers		Patients *vs.* Controls		Resolvers *vs.* Controls	
					p	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)
rs1010273										
Genotype										
GG	274 (67.9)	39 (55.7)	128 (65.3)	Reference						
GA	116 (28.8)	29 (41.4)	64 (36.7)	0.101	0.035	0.569(0.336-0.965)	0.378	0.847(0.585-1.226)	0.169	1.487(0.844-2.621)
AA	13 (3.3)	2 (2.9)	4 (2.0)	0.749	0.920	1.081(0.235-4.972)	0.470	1.518(0.486-4.748)	0.572	1.641(0.290-9.301)
Allele										
G	664 (82.4)	107 (76.4)	320 (81.6)	Reference						
A	142 (17.6)	33 (23.6)	72 (18.4)	0.245	0.094	0.693(0.451-1.066)	0.751	0.950(0.695-1.300)	0.184	1.371(0.860-2.186)
rs2185379										
Genotype										
GG	369 (91.6)	61 (87.1)	178 (90.8)	Reference						
GA	32 (7.9)	9 (12.9)	18 (9.2)	0.407	0.182	0.588(0.267-1.292)	0.618	0.858(0.469-1.570)	0.382	1.459(0.623-3.418)
AA	2 (0.5)	0 (0)	0 (0)	0.524	0.565	1.165(0.121-1.211)	0.326	1.482(1.399-1.571)	–	–
Allele										
G	770(95.5)	131 (93.6)	374 (95.4)	Reference						
A	36 (4.5)	9 (6.4)	18 (4.6)	0.594	0.314	0.681(0.320-1.446)	0.922	0.971(0.544-1.733)	0.395	1.427(0.626-3.256)
Haplotype (rs1010273-rs2185379)										
G-G	628(77.9)	98(70.0)	302(77.0)	Reference						
G-A	36(4.5)	9(6.4)	18(4.6)	0.465	0.221	0.624(0.292-1.336)	0.896	0.962(0.537-1.722)	0.305	1.541(0.671-3.541)
A-G	142(17.6)	33(23.6)	72(18.4)	0.195	0.071	0.671(0.435-1.037)	0.742	0.948(0.692-1.300)	0.149	1.412(0.882-2.262)

Data are presented as n (%).OR, Odds Ratio; 95%CI, 95% Confidence Intervals.

3.4. Associations of PRDM1 polymorphisms with HCC in chronic HBV infection

Univariate and multivariate logistic regression analysis of HCC risk factors in relation to LC showed that male gender ($p < 0.001$), older age ($p < 0.001$), higher ALT ($p = 0.045$), AST ($p = 0.023$) and albumin ($p = 0.010$) were main risk factors of HCC and *PRDM1* rs1010273 and rs2185379 were not shown to be risk factors of HCC (Table 3).

Univariate and multivariate logistic regression analysis of HCC risk factors in relation to HBV infection without HCC showed that male gender ($p < 0.001$), older age ($p < 0.001$), lower HBV DNA ($p = 0.048$) and higher ALT ($p < 0.001$) and AST ($p = 0.001$) were risk factors of HCC and *PRDM1* rs1010273 and rs2185379 were not shown to be risk factors of HCC (Table 4).

3.5. Associations of PRDM1 polymorphisms with overall survival of HBV-related HCC patients

The 1-, 3-, and 5-year overall survival rates of patients with HBV-related HCC were 70.5%, 34.6%, and 11.5%, respectively, in GG genotype carriers and 91.4%, 51.4% and 31.4%, respectively, in genotypes AA + GA carriers. The survival rates of HBV-related HCC patients with GG genotype were significantly lower than those with genotypes AA + GA ($p = 0.008$, Table 5, Fig. 1). Multivariate analysis showed that genotypes of *PRDM1* rs1010273 polymorphism (OR, 0.529; 95%CI, 0.126-0.862; $p = 0.002$), together with ALT ($p = 0.021$), TBIL ($p = 0.019$), and albumin ($p = 0.014$), AFP ($p = 0.033$), Child-pugh grade

Table 2
Genotype, allele and Haplotype frequencies of *PRDM1* polymorphisms in chronic hepatitis, liver cirrhosis and hepatocellular carcinoma.

PRDM1 polymorphism	CH (n = 171)	LC (n = 119)	HCC (n = 113)	*p*	CH *vs.* LC		CH *vs.* HCC		LC *vs.* HCC	
					p	OR (95%CI)	*p*	OR (95%CI)	*p*	OR (95%CI)
rs1010273										
Genotype										
GG	116(67.8)	80(67.2)	78(69.0)	Reference						
GA	51(29.8)	35(29.4)	30(26.5)	0.871	0.985	1.005(0.600-1.683)	0.624	1.143(0.670-1.951)	0.663	1.138(0.638-2.029)
AA	4(2.4)	4(3.4)	5(4.5)	0.658	0.605	0.690(0.168-2.839)	0.360	0.538(0.140-2.066)	0.718	0.780(0.202-3.013)
Allele										
G	283(82.7)	195(81.9)	186(82.3)	Reference						
A	59(17.3)	43(18.1)	40(17.7)	0.968	0.800	0.945(0.613-1.458)	0.891	0.969(0.623-1.508)	0.918	1.025(0.638-1.649)
rs2185379										
Genotype										
GG	153(89.5)	110(92.4)	106(93.8)	Reference						
GA	18(10.5)	8(6.7)	6(5.3)	0.248	0.274	1.618(0.679-3.854)	0.127	2.078(0.799-5.410)	0.652	1.285(0.431-3.828)
AA	0(0)	1(0.9)	1(0.9)	0.494	0.239	0.418(0.363-0.482)	0.231	0.409(0.354-0.474)	0.979	1.038(0.064-16.805)
GA + AA	18(10.5)	9(7.6)	7(6.2)	0.403	0.393	1.438(0.623-3.320)	0.207	1.782(0.719-4.415)	0.681	1.239(0.445-3.447)
Allele										
G	324(94.7)	228(95.8)	218(96.5)	Reference						
A	18(5.3)	10(4.2)	8(3.5)	0.619	0.557	1.267(0.574-2.795)	0.336	1.514(0.647-3.543)	0.712	1.195(0.463-3.084)
Haplotype (rs1010273-rs2185379)										
G-G	266(77.7)	185(77.7)	178(78.8)	Reference						
G-A	17(5.0)	10(4.2)	8(3.5)	0.717	0.683	1.182(0.529-2.640)	0.421	1.422(0.601-3.365)	0.704	1.203(0.464-3.117)
A-G	59(17.3)	43(18.1)	40(17.7)	0.978	0.833	0.954(0.617-1.475)	0.954	0.987(0.633-1.539)	0.890	1.034(0.642-1.667)

Data are presented as n (%). CH, chronic hepatitis; LC, liver cirrhosis; HCC, hepatocellular carcinoma; OR, odds ratio; 95%CI, 95% confidence interval.

Table 3
Univariate and multivariate analyses of risk factors associated with hepatocellular carcinoma (HCC) in comparison with cirrhosis without HCC.

Variable	LC (n = 119)	HCC (n = 113)	Univariate analysis	Multivariate analysis	
			p	OR(95%CI)	*p*
Gender (male/female)	80/39	98/15	< 0.001	0.236(0.108-0.514)	< 0.001
Mean age (years)	44.35 ± 10.86	48.93 ± 10.85	0.002	1.060(1.028-1.092)	< 0.001
HBV DNA (logIU/ml)	5.31 ± 1.63	4.91 ± 1.48	0.052	1.000(1.000-1.000)	0.110
ALT (IU/L)	50 (9-590)	54 (7-765)	0.762	0.995(0.989-1.000)	0.045
AST (IU/L)	58 (14-1022)	71 (15-1348)	0.330	1.004(1.001-1.008)	0.023
TBIL (μmol/L)	27.4 (6.5-470)	26.23(1.48-727.24)	0.056	1.005(0.999-1.011)	0.074
Albumin (g/L)	31.2 (18.8-50.7)	33.3 (21.1-51.29)	0.030	1.072(1.017-1.131)	0.010
rs1010273	80/39	78/35	0.769	0.923(0.537-1.587)	0.772
GG *vs.* GA + AA					
rs2185379	110/9	106/7	0.681	1.043(0.400-2.715)	0.932
GG *vs.* GA + AA					

HBV, hepatitis B virus; LC, liver cirrhosis; OR, odds ratio; 95% CI, 95% confidence interval; ALT, alanine aminotranferase; AST, aspartate aminotransferase; TBIL, total bilirubin.

Table 4
Univariate and multivariate analyses of risk factors associated with HBV-related hepatocellular carcinoma (HCC) in comparison with chronic HBV infection without HCC.

Variable	Patients without HCC (n = 290)	Patients with HCC (n = 113)	Univariate analysis	Multivariate analysis	
			p	OR(95%CI)	*p*
Gender (male/female)	185/105	98/15	< 0.001	0.180(0.090-0.357)	< 0.001
Mean age (y)	37.03 ± 13.23	48.93 ± 10.85	< 0.001	1.080(1.053-1.106)	< 0.001
HBV DNA (logIU/ml)	5.63 ± 1.79	4.91 ± 1.48	< 0.001	1.000(1.000-1.000)	0.048
ALT (IU/L)	55.95 (9-3629)	54 (7-765)	0.004	0.993(0.989-0.997)	< 0.001
AST (IU/L)	60 (13-4082)	71 (15-1348)	0.336	1.005(1.002-1.008)	0.001
TBIL (μmol/L)	21.6 (2-656.8)	26.23(1.48-727.24)	0.867	1.001(0.998-1.003)	0.611
Albumin (g/L)	36.7(18.7-50.8)	33.3 (21.1-51.29)	0.045	1.010(0.973-1.064)	0.436
rs1010273	196/94	78/35	0.781	0.879(0.537-1.439)	0.609
G/G *vs.* G/A and A/A					
rs2185379	263/27	106/7	0.312	1.067(0.443-2.572)	0.885
G/G *vs.* G/A and A/A					

HBV, hepatitis B virus; OR, odds ratio; 95% CI, 95% confidence interval; ALT, alanine aminotranferase; AST, aspartate aminotransferase; TBIL, total bilirubin.

($p = 0.009$), tumor size ($p = 0.010$), and TNM stage ($p = 0.005$), were factors independently associated with the overall survival of patients with HCC (Table 5).

4. Discussion

PRDM1 encodes a zinc finger transcriptional repressor, BLIMP-1, that is involved in a variety of cellular processes [19] and the differentiation of plasma cells [7,8]. Moreover, BLIMP-1 is involved in the regulation and function of T lymphocytes [7,8], the differentiation of CD8 + T cells and the regulation of CD8 + T cell exhaustion in chronic viral infection [9–11]. In chronic HBV infection, T-cell dysfunction or exhaustion is implicated in the development of different outcomes of the infection including HCC [4–6]. The present study examined the *PRDM1* rs1010273 and rs2185379 polymorphisms in a sample of Chinese Han patients with chronic HBV infection of various clinical diseases including HBV-related HCC. The results showed that the genotypic frequencies of the studied polymorphisms, rs1010273 and rs2185379, did not significantly differ in relation to chronic HBV infection and clinical diseases although patients with chronic HBV infection had lower frequency of *PRDM1* rs1010273 genotype GA than HBV infection resolvers. However, HCC patients who carry rs1010273 GG genotype were found to have a significantly shorter duration of overall survival than patients who carry rs1010273 AA + GA genotypes. The rs1010273 GG genotype was also shown to be an independent risk factor for the overall survival of patients with HBV-related HCC.

So far, studies of the *PRDM1* polymorphisms have been primarily involved in malignant lymphoma. For instance, *PRDM1* gene mutations within the exon 2–intron 2 splice sites that result in large deletions of the PRDM1 protein have been reported in diffuse large B-cell lymphomas [20,21]. A study in Hodgkin's lymphoma children treated with radiation therapy indicated that the variants related to decreased basal expression of PRDM1 and impaired induction of the PRDM1 protein after radiation exposure were associated with radiation therapy-induced second malignant neoplasms [22]. We examined for the first time the relationship between *PRDM1* polymorphisms and different diseases of chronic HBV infection including HBV-related HCC in the current study. Although the frequencies of the rs1010273 and rs2185379 polymorphic genotypes in patients with chronic HBV infection, infection resolvers and healthy controls had no significant difference, rs1010273 GG genotype, in comparison with genotypes AA + GA, was found to be significantly associated with shorter overall survival of HCC patients. The rs1010273 GG genotype was also shown to be an independent risk factor for the overall survival of patients with HBV-related HCC. These results indicate that rs1010273 genotype GG is predisposing while genotypes AA + GA are protective for the disease progression of HBV-related HCC. *PRDM1* rs1010273 is an A/G single-nucleotide variation on chromosome 6 associated with the formation of a synonymous codon. It is suggested that rs1010273 genotype GG might functionally or quantitatively impair PRDM1 and thus predispose to the progression of HCC. However, these results should be interpreted with caution, given the small number of HCC patients genotyped and followed-up as well as the lack of potential functional clarification of the rs1010273 polymorphism.

Functionally, BLIMP-1 was shown to be a tumor suppressor in activated B cell-like diffuse large B cell lymphoma [12,13] and natural killer cell lymphoma (NKCL) [14]. In addition, BLIMP-1 was

Table 5
Univariate and multivariate analysis of the overall survival of patients with hepatitis B virus-related hepatocellular carcinoma.

	No. of patients	Overall Survival (%)			Univariate analysis	Multivariate analysis	
		1 year	3 year	5 year	*p*	OR (95%CI)	*p*
Gender					0.252	1.396 (0.568-3.129)	0.622
Male	98	78.5	41.8	17.3			
Female	15	66.7	26.7	20.0			
Age (year)					0.477	1.193 (0.286-1.661)	0.289
≤50	64	79.7	46.9	18.8			
> 50	49	73.5	30.6	16.3			
HBV DNA (IU/mL)					0.430	1.665 (0.647-2.017)	0.658
$\leq 10^4$	35	82.9	45.7	14.3			
$> 10^4$	78	74.3	37.2	19.2			
ALT (IU/L)					0.709	2.735 (0.196-3.921)	0.021
≤40	36	83.3	44.4	13.9			
> 40	77	74.0	37.7	19.5			
AST (IU/L)					0.072	2.015 (0.877-4.151)	0.074
≤40	29	93.1	51.7	20.7			
> 40	84	71.4	35.7	16.7			
TBIL (μmol/L)					< 0.001	2.421 (1.027-3.965)	0.019
≤40	78	89.7	50.0	20.5			
> 40	35	48.6	17.1	11.4			
Albumin (g/L)					< 0.001	0.536 (0.127-0.926)	0.014
≤32	50	68.0	44.0	6.0			
> 32	63	84.1	52.4	26.9			
AFP (ng/mL)					0.043	1.927 (1.018-2.962)	0.033
≤200	62	85.5	50.0	20.9			
> 200	51	66.7	27.5	13.7			
Child-pugh grade					0.004	3.651 (1.029-4.029)	0.009
A	73	87.7	42.5	17.8			
B + C	40	57.5	35.0	17.5			
Tumor size (cm)					0.016	1.286 (1.004.-2.316)	0.010
≤5	61	78.7	47.5	24.6			
> 5	52	75.0	30.8	9.6			
TNM stage					0.008	3.428 (1.086-4.581)	0.005
I + II	83	80.7	43.4	20.5			
III	30	66.7	30.0	10.0			
rs1010273					0.008	0.529 (0.126-0.862)	0.002
GG	78	70.5	34.6	11.5			
AA + GA	35	91.4	51.4	31.4			
rs2185379					0.830	0.784 (0.236-1.678)	0.105
GG	106	75.5	39.6	17.9			
AA + GA	7	100	42.9	14.3			

HBV, hepatitis B virus; AFP, alpha-fetoprotein; OR, odds ratio; 95% CI, 95% confidence intervals; ALT, alanine aminotranferase; AST, aspartate aminotransferase; TBIL, total bilirubin.

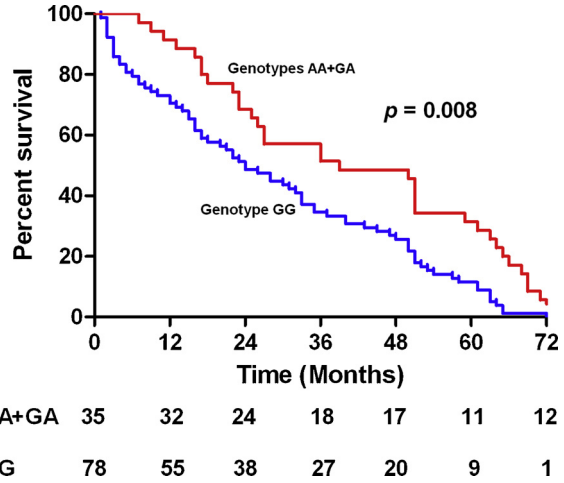

Fig. 1. Overall survival of patients with hepatitis B virus-related hepatocellular carcinoma according to *PRDM1* rs1010273 genotypes.

demonstrated to have an important inhibitory effect on T cell response in acute myeloid leukemia (AML) [23]. Therefore, it is unclear whether the association of *PRDM1* rs1010273 polymorphism with the disease prognosis of HBV-related HCC may be potentially implicated in the effect of *PRDM1* on HCC directly or through host immune response related to HCC or both.

Studies showed that the phenotypes, functional states and underlying mechanisms of CD8 + T cell exhaustion between chronic hepatitis B and HCC are somewhat different [24], patients with or without HCC have a quantitative and functional hierarchy of tumor-specific T cells [25], and T cells from non-HCC patients were polyfunctional (IFN-gamma+, TNF-alpha+, CD107a+) and those from HCC patients displayed an exhausted phenotype (IFN-gamma+, CD107a+) [25]. Whether the *PRDM1* rs1010273 polymorphism is potentially implicated in the differential manifestations of T cell dysfunction/exhaustion between chronic hepatitis B (non-HCC) and HCC is also an interesting issue to be investigated.

PRDM1 rs2185379 is an A/G single-nucleotide variation on human chromosome 6 which may cause alteration of protein product. Although *PRDM1* rs2185379 is a functionally relevant polymorphism, we did not show any association of this polymorphism in relation to the disease phenotypes of chronic HBV infection and disease progression of HBV-related HCC. Therefore, the role of rs2185379 polymorphism in chronic HBV infection and HBV-related clinical diseases including HCC needs to be further investigated in large sample size of patient and control populations considering the relatively small sample size of patients and control individuals in the present study.

Serum PRDM1 levels are differentially expressed between patients

with chronic HBV infection and healthy controls and between patients with chronic hepatitis, cirrhosis and HCC [26]. It is indicated that PRDM1/BLIMP1 is involved in the pathogenesis and disease progression of chronic HBV infection. Whether the *PRDM1* polymorphisms are potentially associated with the levels of *PRDM1/BLIMP1* expression also needs to be investigated in future studies.

This study has limitations on the small size of patient and control samples, limited numbers of polymorphisms genotyped and lack of functional investigations. These limitations may compromise the power of the study. Therefore, further clinical studies in large sample sizes of patients and controls and functional studies are required to elucidate the role of *PRDM1* rs1010273 and rs2185379 and other *PRDM1* polymorphisms in chronic HBV infection and HCC.

5. Conclusion

In conclusion, this study investigated the *PRDM1* rs1010273 and rs2185379 polymorphisms in a sample of Chinese patients with chronic HBV infection of various clinical diseases including HBV-related HCC, showing that *PRDM1* rs2185379 polymorphism appears to have no significant association with chronic HBV infection and HBV-related diseases including HCC while the *PRDM1* rs1010273 polymorphism is associated with the overall survival of HBV-related HCC patients and is an independent risk factor for the survival of patients with HBV-related HCC. In general, HBV infection resolvers had higher frequency of *PRDM1* rs1010273 genotype GA than patients with chronic HBV infection and HCC patients with rs1010273 AA + GA genotypes had a significantly longer duration of overall survival than those with rs1010273 GG genotype. Speculatively, the rs1010273 GA genotype may be related to a better immune response, possibly resulting in spontaneous resolution of the viral infection on the one side, and in improved anti-tumor immunity on the other side although many additional experiments are needed to demonstrate this hypothesis. Anyway, additional studies are needed to replicate and extend the findings of this study and to elucidate the role of *PRDM1* rs1010273 and rs2185379 and other *PRDM1* polymorphisms and the underlying mechanisms between the associated polymorphisms and chronic HBV infection including HBV-related HCC.

Contributorship

All the authors contributed to this work by recruiting the cohorts or performing the genetic and statistical analysis and writing the manuscript.

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Declaration of Competing Interest

None of the authors have competing interests related to this work.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imlet.2019.07.007.

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