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Original Article

Genetic polymorphisms in apoptosis-related genes and the prognosis of hepatocellular carcinoma

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Abstract: The apoptotic pathway is important in the control of vital processes of hepatocellular carcinoma (HCC). In the current study, we aimed to determine whether apoptotic gene-related polymorphisms modified HCC prognosis. We genotyped 16 single nucleotide polymorphisms (SNPs) in 10 core genes (*TP53*, *TP53INP1*, *TP53BP1*, *CDKN2A*, *CDKN1A*, *CDKN1B*, *MDM2*, *BAX*, *CCDN1* and *BCL2*) in the apoptotic pathway by using DNA from blood samples of 362 HCC patients receiving surgical resection of HCC tumor. The associations between genotypes/haplotypes of the 10 genes and overall survival (OS) of HCC patients were assessed using the Cox proportional hazards model. We found one *CDKN1B* haplotype CCT/ACT (constructed by rs36228499 C>A, rs34330 C>T and rs2066827 T>G) significantly associated with decreased OS of HCC patients, compared to the common haplotype ACT/CTT both in univariate analysis (*P*=0.013, HR=1.198, 95% CI: 1.039-1.381) and multivariate analysis (*P*=0.006, HR=1.224, 95% CI: 1.059-1.413). We also find two SNPs (rs560191 G>C and rs2602141 T>G) in *TP53BP1* shown to be marginally significantly associated with decreased OS of HCC patients. However, none of the other SNPs or haplotypes were significantly associated with HCC OS. Our results illustrated the potential use of *CDKN1B* haplotype as a prognostic marker for HCC patients with surgical resection of tumor.

Keywords: Hepatocellular carcinoma, survival, apoptosis, CDKN1B, genetic polymorphisms

Introduction

Hepatocellular carcinoma (HCC) is diagnosed in more than half a million people worldwide every year, and it is one of the leading causes of cancer-related deaths worldwide [1]. China alone accounts for about 50% of the total number of HCC cases and deaths [2]. In 2012, estimated 782,500 new HCC cases and 745,500 cancer-related deaths occurred worldwide [1], making the incidence and mortality rates almost equal. Although multiple clinical factors of HCC, such as large tumor size, vascular invasion, positive portal vein thrombosis, increased serum α-fetoprotein (AFP) and advanced tumor nodes metastasis (TNM) stage have been indicated to be useful to evaluate HCC patients' prognosis [3], they cannot meet clinical requirements for precise prediction of HCC course. Therefore, it is of great significance to identify potential biomarkers for improving the efficiency of prognosis prediction, thus establishing more appropriate cancer management strategies and improving better clinical outcomes of HCC.

Apoptosis is a genetically controlled cell suicide mechanism, which enables multicellular organ-

Table 1. The selected functional SNPs in 10 apoptosis-related genes and their allele frequencies

Gene	Chromosome	Location	Position	SNP	Allele*	MAF (CHB)†	MAF (observed)‡
TP53	17p13.1	exon 4	7579472	rs1042522	G/C	0.489	0.413
TP53INP1	8q22	3'UTR (miR-330-5p target site)	95938422	rs7760	G/T	0.109	0.113
TP53BP1	15q15-q21	promoter	43803621	rs1869258	T/G	0.489	0.452
		exon 9	43767774	rs560191	C/G	0.444	0.424
		exon 17	43724646	rs2602141	G/T	0.478	0.425
CDKN2A	9p21	exon 3	21968199	rs11515	C/G	0.011	0.022
		exon 3	21968159	rs3088440	T/C	0.107	0.123
CDKN1A	6p21.2	exon 2	36651971	rs1801270	A/C	0.449	0.488
		3'UTR	36653597	rs1059234	T/C	0.442	0.496
CDKN1B	12p13.1-p12	promoter	12869936	rs36228499	A/C	0.408	0.414
		promoter	12870695	rs34330	T/C	0.470	0.461
		exon 1	12871099	rs2066827	G/T	0.044	0.022
MDM2	12q14.3-q15	promoter	69202580	rs2279744	T/G	0.358	0.465
BAX	19q13.3-q13.4	promoter	49457938	rs4645878	A/G	0.051	0.068
CCND1	11q13	exon 4	69462910	rs603965 (rs9344)	G/A	0.438	0.422
BCL2	18q21.3	P2 promoter (5'flanking)	60986837	rs2279115	A/C	0.433	0.411

Abbreviations: MAF, minor allele frequency; CHB, Chinese Han in Beijing. *Minor allele/major allele. †Obtained from the International Hap Map Project database (http://hapmap.ncbi.nlm.nih.gov/). ‡Estimated from 362 HCC patients receiving surgical resection of the tumor.

isms to regulate cell number in tissues and to eliminate unnecessary or damaged cells [4]. Defects in apoptosis are implicated in tumor progression and metastasis through maintaining survival of tumor cells, leading to clonal expansion within tumor and further invading surrounding tissues [5]. It is assumed that a decreased ability to eliminate cells with DNA damage may facilitate the accumulation of somatic mutations, and thereby contribute to tumor initiation, progression, and metastasis [6-9]. There are considerable inter-individual variations in apoptotic capacity, which are largely attributed to an individual's genetic constitution [10, 11]. Many studies have demonstrated that several polymorphisms in apoptosis-related genes affect either the expression or the activities of enzymes, and thus associated with the risk of various human cancers, including HCC [12-15]. Accordingly, it is reasonable to suggest that alterations in apoptotic capacity related polymorphisms of apoptosis-related genes could affect prognosis of patients with HCC. However, evidence is still limited to the demonstration of the effects of apoptotic gene-related polymorphisms on the prognosis of HCC.

In this study, we systematically selected 16 potentially functional single nucleotide polymorphisms (SNPs) from 10 genes in the apoptotic pathway, including *TP53*, *TP53INP1*, *TP53BP1*, *CDKN2A*, *CDKN1A*, *CDKN1B*, *MDM2*, *BAX*, *CCDN1* and *BCL2* to assess their prognostic significance for HCC in a Chinese cohort of 362 HCC patients.

Materials and methods

Patients and samples collection

A total of 362 newly diagnosed HCC patients receiving surgical resection of HCC tumor were recruited by the Qidong Liver Cancer Institute in Qidong, Jiangsu province, China from April 1996 to September 2009. All of the HCC patients were Han Chinese. The clinical outcomes of HCC were recorded until October 2014, with a median follow-up time of 53.0 months (range 2-110 months).

The diagnosis of HCC was based on histopathological examination and the National Comprehensive Cancer Network (NCCN) clinical practice guidelines in oncology. All tumors were proven to be HCC by two pathologists. All patients had no other cancers as determined by initial screening examination and were followed up prospectively every 3 months from the time of enrollment by personal or family contacts until death or last time of follow-up.

There were no recruitment restrictions on age, gender and tumor stage. 5 ml whole blood for each subject was extracted. Clinical information was collected at the time the blood specimens were collected from medical records with patients' consent. The histologic grade of tumor

differentiation was assigned by the Edmondson grading system. The clinical typing of tumors was determined according to the TNM classification system of International Union against Cancer (edition 6). The study endpoint was OS, which was calculated from the date of pathologic diagnosis/recruitment to death or the end of available follow-up.

The methods were carried out in accordance with the approved guidelines and in accordance with the Helsinki Declaration as revised in 2000. This study was approved by the Department of Scientific Research of Fudan University and the Qidong Liver Cancer Institute, and a written informed consent with a signature was obtained from each patient before enrollment.

SNP selection

To select the potential functional SNPs of apoptosis-related genes, we utilized the International HapMap Project database (http://hapmap.ncbi.nlm.nih.gov/), and dbSNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) to search for candidate variants in the promoter region, all exons including intron-exon boundaries and the 3'-untranslated region (3'-UTR). We also selected SNPs previously reported to be associated with the outcome of cancers. Finally, a total of 16 potentially functional SNPs were selected for genotyping (**Table 1**).

DNA extraction, genotyping, and haplotypes reconstruction

Genomic DNA was extracted from blood samples using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany). Genotyping was performed with Sequenom MassARRAY iPLEX platform by use of allele-specific MALDI-TOF mass spectrometry assay. Polymerase chain reaction (PCR) and extension primers for these 16 SNPs were designed using the MassARRAY Assay Design 3.0 software (Sequenom). PCR and extension reactions were performed according to the manufacturer's instructions, and extension product sizes were determined by mass spectrometry using the Sequenom iPLEX system. Duplicate test samples and two water samples (PCR negative controls) that were blinded to the technician were included in each 96-well plate. Genotyping quality was examined by a detailed QC procedure consisting of >95% successful call rate, duplicate calling of genotypes, internal positive control samples.

The linkage disequilibrium (LD) status among SNPs was measured with Lewontin D and r^2 by using the Haploview software package (http://www.broad.mit.edu/mpg/haploview). LD blocks were inferred from the definition proposed by Gabriel and colleagues [16]. Probable haplotypes were calculated on the basis of a Bayesian algorithm [17] using PHASE software (ver 2.1.1, Seattle, WA, USA).

Statistical analysis

The effects of the study variables including clinical variables, single SNP and haplotype on HCC OS were assessed using the Cox proportional hazards model. For single SNP analysis, the major homozygote genotype was regarded as reference, the heterozygote and minor homozygote genotypes as well as the combination of heterozygote and minor homozygote genotype were compared to the major homozygote genotype. While for haplotype analysis, the most popular haplotype was considered as reference and other haplotypes were compared to it. HRs and 95% CIs were estimated for each analysis. In multivariate analysis of single SNP and haplotype, only clinical variables found to be significant in univariate analysis were considered in the Cox model. Survival curves were estimated according to the Kaplan-Meier method, and the statistical differences in the survival curves of different subgroups of subjects were analyzed using the log-rank test. Data analysis, with the exception of haplotype construction, was performed with SPSS software version 22 (SPSS, Chicago, IL). All tests were two-sided and a $P<0.05$ was considered statistically significant.

Results

Patient characteristics and clinical outcomes

This study included 362 HCC patients with an overall median survival time (MST) of 34.0 months and median follow-up time of 53.0 months. At the time of analysis, 225 (62.2%) of the patients had died. The clinical pathologic characteristics and their association with OS are summarized in **Table 2**. By univariate analysis, tumor size and venous invasion were significantly associated with overall survival (OS) ($P<0.05$). Therefore, we calculated hazard ratio (HR) and its corresponding *P*-value for each single SNP and haplotype in multivariate analy-

Table 2. Clinical characteristics and their prediction of overall survival in 362 HCC patients receiving surgical resection for the tumor

Characteristics	No of patients	No of events	5-y-survival (%)	Overall survival		
				MST (95% CI)	Hazard ratio (95% CI)	*P*
Number	362	225	30	34.0 (27.4-40.6)		
Age (year)						
≤50	186	113	30	35.0 (23.2-46.7)	1.000	
>50	176	112	29	33.0 (24.2-41.8)	1.098 (0.845-1.426)	0.483
Sex						
Female	63	41	27	31.0 (24.2-37.8)	1.000	
Male	299	184	30	37.0 (27.5-46.5)	0.885 (0.631-1.242)	0.481
Smoking						
Never	224	144	26	31.0 (23.2-38.8)	1.000	
Ever	138	81	37	39.0 (27.3-50.7)	0.858 (0.653-1.127)	0.270
Drinking						
Never	142	86	28	35.0 (19.6-50.4)	1.000	
Ever	220	139	31	33.0 (25.0-41.0)	1.071 (0.818-1.401)	0.619
Family history						
Absent	263	158	31	37.0 (27.3-46.7)	1.000	
Present	81	55	25	29.0 (17.4-40.6)	1.192 (0.877-1.620)	0.262
Unknow	18	12				
HbsAg						
Negative	59	40	35	22.0 (6.7-37.3)	1.000	
Positive	303	185	28	37.0 (30.2-43.8)	0.913 (0.648-1.287)	0.603
AFP						
Negative	142	95	26	33.0 (25.7-40.3)	1.000	
Positive	214	127	32	35.0 (26.1-43.9)	0.892 (0.684-1.164)	0.400
Unknow	6	3				
Tumor size (cm)						
≤5	183	107	35	39.0 (28.1-49.9)	1.000	
>5	179	118	24	30.0 (21.0-39.0)	1.343 (1.033-1.747)	0.028†
Differentiation						
I+II	196	122	28	37.0 (27.5-46.5)	1.000	
III+IV	155	96	32	34.0 (26.0-42.0)	0.926 (0.708-1.120)	0.572
Unknow	11	7				
Tumor capsule						
Absent	177	113	28	31.0 (22.7-39.3)	1.000	
Present	181	110	31	37.0 (26.1-47.9)	0.913 (0.702-1.188)	0.499
Unknow	4	2				
Venous invasion						
Absent	257	150	33	39.0 (29.3-48.7)	1.000	
Present	102	73	22	26.0 (20.1-31.9)	1.368 (1.033-1.811)	0.029†
Unknow	3	2				
Cirrhosis						
Absent	121	79	30	27.0 (13.6-40.4)	1.000	
Present	239	145	30	36.0 (29.6-42.4)	0.949 (0.721-1.249)	0.708
Unknow	2	1				
Tumor number						
Solitary	279	172	30	34.0 (26.0-42.0)	1.000	

Multiple	83	53	27	35.0 (24.5-45.5)	1.061 (0.780-1.445)	0.704
pTNM stage						
I+II	309	188	31	37.0 (30.7-43.3)	1.000	
III+IV	39	27	24	22.0 (13.0-31.0)	1.280 (0.855-1.916)	0.231
Unknow	14	10				

Abbreviations: MST, median survival time; CI, confidence interval; AFP, α-fetoprotein. †P<0.05.

Table 3. Univariate and multivariate Cox regression analysis of genotype of all selected SNPs in 362 HCC patients with surgical resection for the tumor

Genotype	No of patients	No of events	5-y-survival (%)	MST (95% CI)	Overall survival			
					Univariate analysis		Multivariate analysis	
					Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
TP53_rs1042522								
GG	114	73	30	36.0 (25.8-46.2)	1.000		1.000	
GC	191	114	32	30.0 (22.1-37.9)	0.960 (0.715-1.288)	0.785	0.967 (0.720-1.300)	0.825
CC	52	33	26	51.0 (38.5-63.5)	0.910 (0.603-1.374)	0.654	0.899 (0.590-1.371)	0.622
CC+GC	243	147	30	35.0 (23.2-46.8)	0.950 (0.717-1.258)	0.719	0.946 (0.713-1.255)	0.699
TP53INP1_rs7760								
TT	279	178	28	31.0 (23.5-38.5)	1.000		1.000	
GT	75	44	32	41.0 (25.0-57.0)	0.838 (0.602-1.166)	0.294	0.875 (0.628-1.218)	0.428
GG	3	2	33	16.0 (0.0-33.6)	1.244 (0.309-5.018)	0.759	1.467 (0.361-5.956)	0.592
GG+GT	78	46	32	38.0 (21.7-54.3)	0.850 (0.614-1.176)	0.326	0.891 (0.643-1.233)	0.485
TP53BP1_rs1869258								
TT	112	62	38	46.0 (23.0-69.0)	1.000		1.000	
GT	172	112	26	35.0 (25.4-44.6)	1.223 (0.897-1.669)	0.204	1.214 (0.887-1.659)	0.225
GG	77	50	27	30.0 (20.9-39.1)	1.263 (0.870-1.834)	0.220	1.307 (0.899-1.902)	0.161
GG+GT	249	162	26	33.0 (26.7-39.3)	1.239 (0.924-1.660)	0.152	1.249 (0.930-1.677)	0.140
TP53BP1_rs560191								
GG	127	70	38	46.0 (25.5-66.5)	1.000		1.000	
CG	163	107	24	36.0 (26.1-45.9)	1.260 (0.932-1.704)	0.133	1.264 (0.933-1.713)	0.130
CC	72	48	26	30.0 (19.2-40.8)	1.161 (0.966-1.396)	0.112	1.176 (0.978-1.415)	0.085
CC+CG	235	155	25	33.0 (26.7-39.3)	1.288 (0.971-1.708)	0.080	1.303 (0.980-1.732)	0.068
TP53BP1_rs2602141								
TT	126	69	39	46.0 (25.7-66.3)	1.000		1.000	
GT	162	107	24	35.0 (25.3-44.7)	1.282 (0.947-1.736)	0.108	1.284 (0.946-1.742)	0.108
GG	72	48	26	30.0 (19.2-40.8)	1.357 (0.938-1.963)	0.105	1.393 (0.961-2.019)	0.080
GG+GT	234	155	25	33.0 (26.7-39.3)	1.306 (0.983-1.736)	0.065	1.320 (0.992-1.757)	0.057
CDKN2A_rs11515								
CC	344	213	30	35.0 (29.0-41.0)	1.000		1.000	
GC	16	11	31	12.0 (0.0-27.7)	1.312 (0.715-2.406)	0.381	1.382 (0.752-2.540)	0.298
GG	0	0						
CDKN2A_rs3088440								
CC	277	173	29	33.0 (26.1-39.9)	1.000		1.000	
CT	79	48	32	36.0 (21.8-50.2)	0.922 (0.660-1.269)	0.617	0.964 (0.699-1.330)	0.825
TT	5	4	12	61.0 (35.2-86.8)	0.844 (0.313-2.277)	0.738	0.860 (0.318-2.325)	0.767
TT+CT	84	52	30	39.0 (25.3-52.7)	0.915 (0.671-1.247)	0.574	0.955 (0.699-1.304)	0.771
CDKN1A_rs1801270								
AA	97	55	36	36.0 (12.0-60.0)	1.000		1.000	
CA	177	115	27	35.0 (29.1-40.9)	1.215 (0.881-1.676)	0.236	1.170 (0.846-1.617)	0.342
CC	88	55	28	28.0 (11.1-44.9)	1.270 (0.873-1.848)	0.211	1.193 (0.817-1.742)	0.361
CC+CA	265	170	27	33.0 (26.0-40.0)	1.230 (0.907-1.667)	0.183	1.186 (0.873-1.610)	0.275
CDKN1A_rs1059234								
CC	93	59	28	27.0 (11.4-42.6)	1.000		1.000	
TC	179	115	27	37.0 (31.1-42.9)	0.937 (0.684-1.283)	0.685	0.924 (0.673-1.267)	0.623

TT	90	51	37	35.0 (11.4-58.6)	0.783 (0.538-1.140)	0.202	0.842 (0.575-1.233)	0.377
TT+TC	269	166	30	36.0 (29.5-42.5)	0.883 (0.656-1.188)	0.411	0.893 (0.662-1.204)	0.457
CDKN1B_rs36228499								
CC	127	74	34	33.0 (21.1-44.9)	1.000		1.000	
CA	170	109	28	37.0 (28.1-45.9)	1.080 (0.804-1.451)	0.609	1.045 (0.776-1.406)	0.773
AA	65	42	26	30.0 (16.5-43.5)	1.147 (0.785-1.675)	0.479	1.082 (0.739-1.585)	0.685
AA+CA	235	151	27	35.0 (28.1-41.9)	1.101 (0.834-1.455)	0.497	1.062 (0.803-1.405)	0.672
CDKN1B_rs34330								
TT	110	66	33	31.0 (20.7-41.2)	1.000		1.000	
CT	169	105	29	38.0 (28.8-47.2)	0.946 (0.695-1.287)	0.723	0.907 (0.666-1.237)	0.539
CC	82	54	27	24.0 (15.5-32.5)	1.176 (0.820-1.685)	0.378	1.108 (0.771-1.593)	0.578
CC+CT	251	159	28	37.0 (29.3-44.7)	1.019 (0.764-1.357)	0.900	0.975 (0.731-1.300)	0.862
CDKN1B_rs2066827								
TT	346	215	30	35.0 (28.2-41.8)	1.000		1.000	
GT	16	10	26	33.0 (12.9-53.1)	1.093 (0.579-2.060)	0.784	0.992 (0.524-1.877)	0.981
GG	0	0						
MDM2_rs2279744								
GG	103	60	32	39.0 (23.6-54.4)	1.000		1.000	
GT	179	112	28	35.0 (26.2-43.8)	1.082 (0.791-1.481)	0.622	1.070 (0.779-1.468)	0.677
TT	78	51	32	28.0 (15.2-40.8)	1.118 (0.769-1.626)	0.558	1.157 (0.790-1.694)	0.454
TT+GT	257	163	29	31.0 (24.6-37.4)	1.092 (0.812-1.468)	0.561	1.094 (0.811-1.474)	0.577
BAX_rs4645878								
GG	313	190	32	34.0 (26.2-41.8)	1.000		1.000	
GA	49	35	14	37.0 (28.3-45.7)	1.197 (0.834-1.717)	0.329	1.200 (0.834-1.725)	0.326
AA	0	0						
CCND1_ rs9344								
AA	121	80	23	35.0 (26.2-43.8)	1.000		1.000	
GA	175	101	36	39.0 (25.5-52.5)	0.852 (0.635-1.144)	0.287	0.893 (0.663-1.202)	0.455
GG	65	44	23	24.0 (16.3-31.7)	1.110 (0.923-1.335)	0.266	1.085 (0.900-1.308)	0.392
GG+GA	240	145	32	34.0 (25.7-42.3)	0.941 (0.716-1.237)	0.664	0.965 (0.733-1.271)	0.802
BCL2_rs2279115								
CC	126	75	31	37.0 (27.7-46.3)	1.000		1.000	
CA	173	110	30	31.0 (17.3-44.7)	1.067 (0.795-1.431)	0.666	1.043 (0.777-1.401)	0.778
AA	62	39	28	31.0 (23.6-38.4)	1.109 (0.752-1.634)	0.602	1.124 (0.759-1.665)	0.559
AA+CA	235	149	30	31.0 (23.4-38.6)	1.076 (0.815-1.420)	0.606	1.058 (0.801-1.398)	0.691

Abbreviations: MST, median survival time; CI, confidence interval.

sis using Cox proportional hazard models, adjusted for tumor size and venous invasion.

Association analysis of individual SNPs with OS of HCC patients

Table 3 shows the data for all the 16 SNPs among 10 genes (*TP53*, *TP53INP1*, *TP53BP1*, *CDKN2A*, *CDKN1A*, *CDKN1B*, *MDM2*, *BAX*, *CCDN1* and *BCL2*) analyzed for OS of HCC patients. In the univariate analysis, of all the 16 SNPs, only two SNPs (rs560191 and rs2602141), which are resided in TP53B*P1* gene, showed suggestive evidence of an association with OS of HCC patients (**Table 3**). We observed rs560191 CC+CG genotype has a marginally significant association with decreased OS (*P*=0.080; HR=1.288, 95% confident interval [CI]: 0.971-1.708), compared with the GG genotypes (**Table 3**, **Figure 1A**). Similar result was found for rs2602141 GG+GT genotype, which was marginally significantly associated with decreased OS (*P*=0.065; HR=1.306, 95% CI: 0.983-1.736), compared with the TT genotypes (**Table 3**; **Figure 1B**). However, none of the other 14 SNPs examined were significantly associated with OS (**Table 3**).

A multivariate analysis of genotype effects on OS of HCC patients was conducted using Cox proportional hazards models adjusted for tumor size and venous invasion, and similar results were found as the univariate analysis. As shown in **Table 3**, only two SNPs (rs560191 and rs2602141) in *TP53BP1* were confirmed to be marginally significantly associated with clini-

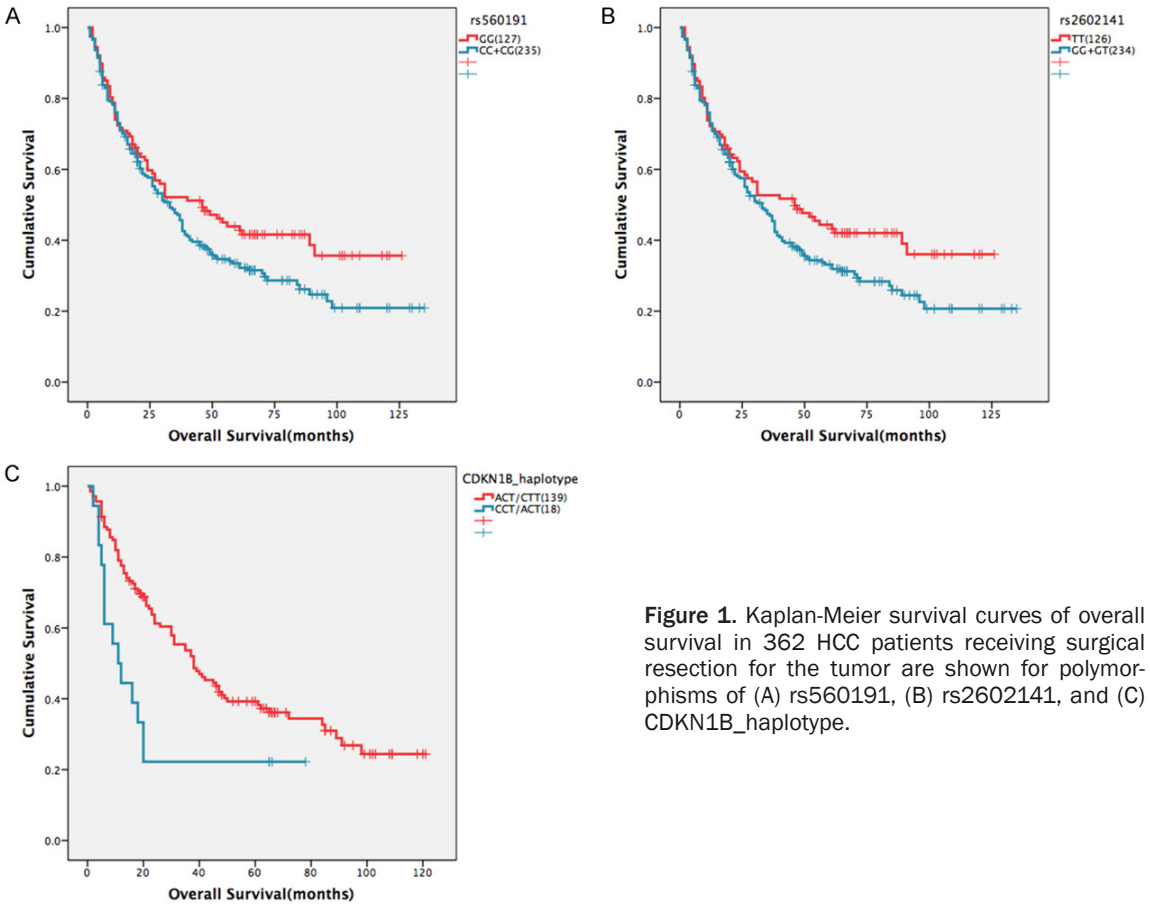

Figure 1. Kaplan-Meier survival curves of overall survival in 362 HCC patients receiving surgical resection for the tumor are shown for polymorphisms of (A) rs560191, (B) rs2602141, and (C) CDKN1B_haplotype.

cal outcomes of HCC patients, with the CC+CG genotype of rs560191 presenting a suggestively negative effect on OS (*P*=0.068, HR=1.303, 95% CI: 0.980-1.732), compared to the common GG genotype, and with the GG+GT genotype of rs2602141 presenting a suggestively negative effect on OS (*P*=0.057, HR=1.320, 95% CI: 0.992-1.757), compared to the common TT genotype (**Table 3**).

Association analysis of haplotypes with OS of HCC patients

Furthermore, we examined the associations of the haplotypes with OS of HCC patients. When examining combinations of SNPs for the *TP53BP1* (rs1869258 G>T, rs560191 G>C and rs2602141 T>G), *CDKN2A* (rs11515 G>C and rs3088440 C>T), *CDKN1A* (rs1801270 C>A and rs1059234 C>T), *CDKN1B* (rs36228499 C>A, rs34330 C>T and rs2066827 T>G), which has at least two tested SNPs in this study, we attained 4 haplotypes of *TP53BP1*, 3 haplotypes of *CDKN2A*, 4 haplotypes of *CDKN1A*, and 6 haplotypes of *CDKN1B*. The inferred haplotypes and their associations with OS are shown in **Table 4**. It shows that only one *CDKN1B* haplotype CCT/ACT was significantly related with OS (*P*=0.013, HR=1.198, 95% CI: 1.039-1.381), compared to the common haplotype ACT/CTT in univariate analysis (**Table 4**; **Figure 1C**).

Similar result was found in multivariate analysis adjusted for tumor size and venous invasion, the *CDKN1B* CCT/ACT haplotype (*P*=0.006, HR=1.224, 95% CI: 1.059-1.413) present an independent negative effect on OS, compared to the common haplotype ACT/CTT in *CDKN1B* (**Table 4**). None of the haplotypes carrying variant alleles from *TP53BP1*, *CDKN2A* and *CDKN1A* showed any significant association with OS.

Discussion

HCC has a highly variable clinical courses and includes several subgroups with distinct path-

Table 4. Univariate and multivariate Cox regression analysis of haplotype of the apoptosis-related genes in 362 HCC patients with surgical resection for the tumor

Haplotype	No of patients	No of events	5-y-survival (%)	MST (95% CI)	Overall survival			
					Univariate analysis		Multivariate analysis	
					Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
TP53BP1_haplotype								
GCG/TGT	157	104	24	35.0 (25.4-44.6)	1.000		1.000	
TGT/TGT	112	62	38	47.0 (24.3-69.7)	0.793 (0.579-1.087)	0.150	0.799 (0.582-1.098)	0.166
GCG/GCG	71	47	27	30.0 (22.0-38.0)	1.054 (0.746-1.489)	0.765	1.099 (0.777-1.553)	0.593
Rare*	22	12	38	46.0 (1.5-90.5)	0.786 (0.432-1.429)	0.430	0.812 (0.446-1.478)	0.495
CDKN2A_haplotype								
CC/CC	263	163	29	35.0 (27.5-42.5)	1.000		1.000	
CC/CT	77	47	31	36.0 (22.0-50.0)	0.945 (0.683-1.307)	0.732	1.002 (0.723-1.390)	0.989
Rare*	20	14	27	26.0 (8.6-43.4)	1.1639 (0.673-2.008)	0.588	1.203 (0.695-2.082)	0.510
CDKN1A_haplotype								
AT/CC	172	111	27	37.0 (31.2-42.8)	1.000		1.000	
AT/AT	90	51	37	35.0 (11.4-58.6)	0.840 (0.603-1.171)	0.304	0.880 (0.630-1.230)	0.455
CC/CC	88	55	28	28.0 (11.1-44.9)	1.016 (0.864-1.195)	0.845	1.021 (0.868-1.201)	0.803
Rare*	12	8	25	30.0 (0.0-69.8)	1.002 (0.788-1.272)	0.990	1.030 (0.808-1.313)	0.814
CDKN1B_haplotype								
ACT/CTT	139	87	29	38.0 (26.8-49.2)	1.000		1.000	
CTT/CTT	101	61	32	28.0 (20.3-35.7)	1.078 (0.777-1.496)	0.654	1.132 (0.814-1.575)	0.460
ACT/ACT	56	34	31	31.0 (8.5-53.5)	1.034 (0.848-1.261)	0.739	1.017 (0.832-1.242)	0.871
CTT/CCT	21	10	42	61.0 (/-/)	0.875 (0.703-1.088)	0.875	0.876 (0.702-1.092)	0.239
CCT/ACT	18	14	22	11.0 (4.8-17.2)	1.198 (1.039-1.381)	0.013†	1.224 (1.059-1.413)	0.006†
Rare*	27	19	21	34.0 (13.2054.8)	1.054 (0.954-1.165)	0.299	1.044 (0.945-1.155)	0.398

Abbreviations: MST, median survival time; CI, confidence interval. *All that genotypes with number less than 18 (5% of 362) were combined as rare genotypes. †$P<0.05$.

ways of hepatocarcinogenesis [18]. These processes share common mechanisms with embryogenesis and can be considered as an aberrant form of organogenesis [19]. However, the critical steps both with respect to molecular genetics and phenotypic characteristics in the prognosis of HCC are still not well characterized. While some germline genetic factors have been suspected of playing an important role in prognosis, none have been firmly established [20, 21]. Most investigations into SNPs in apoptosis-related genes have just focused on their effects on risk rather than prognosis of HCC [12-15, 22, 23]. The aim of our study was to evaluate the role of genetic variants of apoptosis-related genes in determining the clinical outcomes of HCC patients. To the best of our knowledge, this is the first evidence showing the relationship between genetic variants of apoptosis-related genes and the prognosis of HCC patients.

In the present study, we found that one haplotype in *CDKN1B* gene was significantly associated with OS in 362 HCC patients. The haplotype GCT/TCT (constructed by rs36228499 C>A, rs34330 C>T and rs2066827 T>G) in *CDKN1B* gene was significantly associated with decreased OS, compared with the common TCT/GTT haplotype both in univariate analysis and in multivariate analysis adjusted for tumor size and venous invasion. This haplotype presents an independent negative effect on OS and could be used to predict which HCC patients are at risk for poor clinical outcomes in the future.

CDKN1B ($p27^{Kip1}$), encoded by *CDKN1B* gene, is an enzyme inhibitor in humans and belongs to the cip/kip family of CDKI [24]. CDKN1B shares significant homology with its other family members (p21 and p57), specifically in the amino terminal domain [25]. The protein was firstly identified as an inhibitor of CDK2 containing complexes in G1 arrested lung epithelial cells under contact inhibition or when treated with transformation growth factor beta (TGF-β) [26]. Subsequently, the gene encoding CDKN1B was cloned and also identified in a yeast tri-hybrid screen as a cyclin D-CDK4 interacting protein [25, 27]. Since then CDKN1B has not only emerged as a prime regulator of cell cycle progression but has also been implicated in

numerous malignancies including HCC [28]. In cancer cells, CDKN1B can also be mislocalized to the cytoplasm in order to facilitate metastasis. The mechanisms by which it acts on motility differ between cancers. In HCC cells, CDKN1B co-localizes with actin fibers to act on GTPase Rac and induce cell migration [29], and CDKN1B promotes cell migration in metastatic HCC cells through the regulation of RhoA activity [30]. Moreover, studies in several tumor types indicate that CDKN1B expression levels have both prognostic and therapeutic implications [31]. To date, accumulating evidence has suggested that decreased CDKN1B expression can be considered as an adverse prognostic biomarker in HCC [32-38].

Besides *CDKN1B* gene, none of additional genetic polymorphisms reached significance and could be served as an independent prognostic factor for OS. One explanation is that even though we selected and investigated these SNPs in a systematical way, due to limited techniques, labor and resources, we missed some key SNPs which play a predominant role in regulating the expression of the apoptosis-related genes. For this reason, we are not capable of concluding that the SNPs and the other haplotypes of these genes are not associated with the prognosis of HCC. Instead, a more comprehensive analysis of polymorphisms in the apoptosis related-genes is imperative to illustrate the close correlation between apoptosis related-genes and HCC prognosis.

It is worth mentioning that there were some limitations in our study. Firstly, the cohort size of the present study was relatively small. Therefore, larger well-designed longitudinal follow-up studies and functional evaluation are warranted to confirm our findings. Secondly, though several clinical and pathologic characteristics showed significant associations with OS, including tumor size and venous invasion, it is regretful that we failed to collect adequate and accurate information of these factors in our study. In order to make the greatest use of the genotype polymorphisms information of the 362 HCC patients, we had to operate the multivariate analysis by adjusting all these potential prognostic factors. Future studies are essential to investigate the role of genetic polymorphisms in HCC patients with more complete and comprehensive clinical pathologic characteristics. Last but not the least, as mentioned above, all of our samples are blood from each HCC patients treated with surgery. This drawback prevented us to conduct analysis of the relationship between apoptosis related genes expression in tissues and HCC prognosis. Accordingly, analyses of tissue samples are urgent to figure out the unknown modulation of these genes in HCC prognosis.

In summary, our results demonstrated the potential use of *CDKN1B* gene haplotype as a prognostic marker for HCC patients. However, neither the SNPs nor the other haplotypes from apoptosis-related genes were recognized having any significant association with HCC prognosis. More comprehensive studies are needed to evaluate the association between genetic polymorphisms of apoptosis-related genes and prognosis of HCC.

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Disclosure of conflict of interest

None.

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