The role of ERCC1 and AFP gene polymorphism in hepatocellular carcinoma

Yu-Liang Huang, MD, Jun-Rong Wu, PD, Min Fang, PD, Hui-Liu Zhao, MD, Zhi-Min Liu, BD, Jian Ye, BD, Ling-Sha Huang, BD*, Bo Zhu, BD*

Abstract

The aim of this study was to evaluate the effects of polymorphisms in excision repair cross-complementation group 1 (ERCC1) and alpha-fetoprotein (AFP) genes and their haplotypes on the susceptibility to hepatocellular carcinoma (HCC), and to decipher the association between single-nucleotide polymorphisms (SNPs) and clinicopathologic characteristics of HCC.

Peripheral blood DNA was extracted from 206 subjects. SNaPshot technique was used for genotyping 5 SNP sites of the ERCC1 rs735482, rs1046282, rs3212948, and AFP rs737241, rs4024 genotypes. Chi-squared test and logistic regression model were used to analyze the relationship of different genotypes or haplotype and the susceptibility and clinicopathologic characteristics of HCC.

The frequency of GG.GA and AA genotypes at the AFP rs737241 site in the case and control groups showed statistically significant differences ($P < .05$). The risk of HCC in subjects carrying mutated allele A (GA+AA) was increased by 0.543-times ($P < .05$) compared to that in the subjects with the GG genotype. Significant differences were observed in the linkage disequilibrium between 2 of the five SNPs ($P < .05$); the frequency of ERCC1 C-C and AFP A-A haplotypes was significantly lower in the case group than in the control group ($P < .05$). The results of clinicopathologic analysis showed that A allele at the rs737241 locus could increase the expression level of AFP ($P = .007$), the rs1046282 mutation C allele could increase the AFP expression level ($P = .011$), rs4024 locus mutation A allele could reduce the risk of vascular invasion ($P = .013$), rs3212948 locus mutation T allele could reduce the differentiation of liver cancer ($P = .022$), rs1046282 locus C allele could reduce the DNA load of hepatitis B virus ($P = .035$), and rs735482 A allele could increase the tumor size in HCC ($P = .037$).

The SNPs in rs737241 for AFP gene may correlate with the occurrence of HCC. The SNPs in ERCC1 and AFP genes may affect the prognosis of HCC, offering reliable information for early prediction of tumor progression and diagnosis of HCC.

Abbreviations: AFP = alpha-fetoprotein, CI = confidence interval, ERCC1 = excision repair cross-complementation group 1, HBsAg = hepatitis B surface antigen, HCC = hepatocellular carcinoma, LD = linkage disequilibrium (D′), NCBI = National Center for Biotechnology Information, OR = odds ratio, PVTT = portal vein tumor thrombosis, SNP = single-nucleotide polymorphism.

Keywords: alpha-fetoprotein, excision repair cross-complementation group 1, hepatocellular carcinoma, single-nucleotide polymorphism

1. Introduction

Hepatocellular carcinoma (HCC) is the most important primary liver cancer, being the 6th most common type of tumor in the world. Identification of prognosis-related molecular markers for assessing the progression of HCC is an area of intense research and is important for its treatment. Excision repair cross-complementation group 1 (ERCC1) is an important gene involved in base excision repair of DNA. Single-nucleotide polymorphisms (SNPs) in ERCC1 have been related to tumor susceptibility and poor prognosis in gastric, lung, colorectal, and ovarian cancers.[1–4] Alpha-fetoprotein (AFP) gene consists of 15 exons and 14 introns; an enhancer located upstream of the gene has stimulatory effects on the expression of AFP protein. Studies have shown that AFP-692 rs10020432 polymorphism is associated with HCC and cirrhosis.[5,6] In this study, we analyzed the polymorphisms in ERCC1 and AFP genes and deciphered the relationships between the polymorphisms and haplotypes with the incidence of HCC and clinicopathologic parameters of HCC. We also investigated whether ERCC1 gene polymorphism is associated with the expression of AFP protein, with the aim of elucidating the molecular genetic mechanisms underlying HCC.

2. Materials and methods

2.1. Patients

A total of 206 subjects were selected for this study. The HCC group included 118 cases, which were diagnosed by imaging and pathologic examination between August 2017 and March 2018

* Correspondence: Bo Zhu, Ling-Sha Huang, Affiliated Tumor Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China (e-mails: m15077113895@163.com, ls_huang@163.com).

Table 1

Statistical analysis of population.

Variable	HCC (N = 118)	Control (N = 88)	χ^2	P-value
Gender			1.264	.261
Male	98	78		
Female	20	10		
Age, yr			3.051	.218
<40	27	24		
40–60	74	45		
>60	17	19		
Alcohol consumption			0.342	0.559
Yes	38	25		
No	80	63		
Smoking status			0.122	0.727
Yes	43	30		
No	75	58		
HBsAg			146.72	9.02×10^{-34}
+	102	1		
−	16	87		
Serum AFP levels, ng/mL			58.304	2.25×10^{-14}
<400	59	87		
≥400	59	1		

AFP = alpha-fetoprotein, HBsAg = hepatitis B surface antigen, HCC = hepatocellular carcinoma.

at the Affiliated Tumor Hospital of Guangxi Medical University, China. The cases with HCC were selected from among the newly admitted patients who were diagnosed with HCC, and blood samples were collected from them prior to the start of treatment (including surgical resection, radiotherapy, or chemotherapy). The control group included 88 cancer-free subjects (clinical and laboratory examinations of the subjects were normal and they had no family history of tumor), selected from the same hospital during the same period. There were 98 males and 20 females in the HCC group. The mean age of subjects in the HCC group was 47.625 years, whereas it was 49.022 years in the control group. All the characteristics of the studied subjects are shown in Table 1. Informed consent was obtained from all the patients and the study was approved by the Medical Ethics Committee of Guangxi Medical University, China.

2.2. DNA extraction

Peripheral blood (3–5 mL) was collected in anticoagulant (ethyl-enediaminetetraacetic acid)-coated tubes from all the subjects. Genomic DNA was extracted from the samples using TIANamp Blood DNA Kit DP348 (Tiangen, Beijing, China) following the manufacturer's instructions. The concentration of DNA was determined using NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE), and it was stored at −20°C until used.

2.3. Targeting of SNPs

Based on the Hapmap database and linkage disequilibrium (LD), the linkage imbalance coefficient (D') was determined, using 15% frequency as the boundary, and considering the function of SNP, the tapSNP sites, which might affect the expression of protein, were selected. Among the 3 tapSNPs in ERCC1 gene, rs1046282 and rs735482 are located in the 3′-untranslated region (UTR), and rs3212948 is located in exon 3, and these 3 are in LD. Of the 2 sites in the AFP gene, rs737241 is located in the 3′-UTR and rs4024 is located in the promoter region of the AFP gene.

2.4. Polymorphism genotyping

The sequences of the SNP loci present in the National Center for Biotechnology Information (NCBI) database were searched, and polymerase chain reaction (PCR) primers and extension primers were designed using Primer Premier 5.0 (Canada) (Table 2). PCR was performed in a total volume of 20 μL, which contained 1 μL genomic DNA template, 1 μL each of the 10 mmol/L upstream and downstream primers, 15 μL master mixture (Taq buffer [Mg^{2+} plus], dNTP mix [10 mM each], and Taq DNA polymerase), and 2 μL sterile water. The conditions for PCR were as follows: predenaturation at 95°C for 5 minutes; 10 cycles of denaturation at 95°C for 30 seconds, annealing at 62°C to 52°C (decreasing by 1°C per cycle) for 30 seconds, and extension at 72°C for 30 seconds; 40 cycles of denaturation at 95°C for 30 seconds, annealing at 52°C for 30 seconds, extension at 72°C for 30 seconds; extension at 72°C for 10 minutes; cooling to 4°C. The products were purified using the alkaline phosphatase-labeled streptavidin-biotin method (SAP)/exonuclease I (Exo I enzyme) method, and the final product was obtained using the SNaPshot kit (ABI). This product was sequenced using ABI 3730, and the sequence was analyzed by Gene Mapper 4.0 software; Liz120 was taken as the internal reference. To ensure the accuracy of genetic polymorphism, 20% of the specimens were randomly selected for resequencing, and the results were found to be consistent with those of the first experiment.

2.5. Serum AFP determination

The measurement of serum AFP concentration was done using the enzyme immunoassay method (Abbott Immunization i2000).

Table 2

PCR primers, single-base extension primer sequences of SRC sites of ERCC1 and AFP genes.

SNP	PCR primers	Single-base extension primer sequences
ERCC1rs1046282	F: 5′-GCCCCTACCTTTAGTGAGCT-3′ R: 5′-GATGCTGCTCGGTTCTCTTG-3′	Seq: 5′-ATAAGTAGAAATACTAACAAAGGGC-3′
ERCC1rs3212948	F: 5′-GCAAAATCCAACAGCATCA-3′ R: 5′-GCTACACCAATACAGCCACA-3′	Seq: 5′-GCTGTTCTAGGGATGACTCCAGTG-3′
ERCC1rs735482	F: 5′-CTCGGTTCTGTGCCTTTGG-3′ R: 5′-CGTCCCCTTTTGCCTCTTT-3′	Seq: 5′-TTTTTTGAGGAAGAAGCCCAAAGGGA-3′
AFPrs737241	F: 5′-TCTGCCCTGTTTGACTTGA-3′ R: 5′-CCTGTGCTGTACCCTATACC-3′	Seq: 5′-CTGAGCCAACTTTATATATC-3′
AFPrs4024	F: 5′-AGGACTTGGTTTATTAGGGC-3′ R: 5′-AGAAAAGACAGCAGATTTGG-3′	Seq: 5′-GTGTTTCTGAGCTAAACAATGACA-3′

AFP = alpha-fetoprotein, ERCC1 = excision repair cross-complementing group 1, PCR = polymerase chain reaction, SNP = single-nucleotide polymorphism.

2.6. Statistical analysis

Statistical analysis was performed using the SPSS 17.0 software (SPSS Inc, Chicago, IL). Hardy–Weinberg equilibrium was determined to confirm whether the study samples were representative of the population. The data from the 2 groups were compared by Chi-squared test and logistic regression analysis. Logistic regression model was used to calculate the odds ratios (OR) and 95% confidence interval (CI) of specific genotypes or variables of interest after adjusting for age, gender, alcohol consumption, and smoking. The haplotype analysis of gene was performed using SHEsis (http://analysis.bio-x.cn/myAnalysis.php).[7] All the P-values were 2-sided; statistical significance was defined as $P < .05$.

3. Results

3.1. Demographic statistics of the subjects

A pairwise comparison of the clinical characteristics of subjects in the HCC and control groups presented in Table 1 shows that sex, age, smoking status, and drinking status were not statistically different ($P > .05$); however, significant differences were observed in the levels of serum AFP and hepatitis B surface antigen between the 2 groups.

3.2. Genotype frequencies

The genotype distribution of ERCC1 (rs735482, rs1046282, and rs3212948) and AFP (rs4024 and rs737241) genes in the experimental and control groups was consistent with the Hardy–Weinberg equilibrium ($P > .05$), suggesting that the population had good representation. The results of Hardy–Weinberg equilibrium are shown in Table 3.

For AFP, the frequency for A allele at site rs737241 in the HCC group was significantly lower than that in the control group ($P < .05$). The individuals carrying the A allele were at 0.573-times lower risk of developing HCC than those carrying the G allele ($P = .008$, Table 4). The frequencies of GG, GA, and AA genotypes in the HCC and control groups were statistically significant ($P < .05$). After adjusting for age, gender, smoking, and drinking history, the risk of HCC was 0.543-times lower in individuals with at least one mute allele A (GA+AA) than in individuals with the GG genotype ($P = .037$, Table 5). There was no significant difference in the distribution of other SNP genotypes and alleles between the HCC and control groups ($P > .05$).

3.3. Analysis of haplotypes of ERCC1 and AFP

The LD of the 5 SNPs in ERCC1 and AFP genes was measured in terms of D' and r^2. The values of r^2 and D' were 0.277 and 0.696 for rs737241-rs4024, 0.031 and 0.493 for rs1046282-rs3212948, 0.184 and 0.574 for rs3212948-rs735482, and 0.169 and 0.655 for rs735482-rs1046282, respectively. These values demonstrate that the SNP sites in the two genes exhibit LD.

The haplotypes with a frequency <0.03 were excluded from the analysis. Three haplotypes were found to be associated with the HCC risk. Compared to the patients with other haplotypes, those with the C-C haplotype of ERCC1 rs1046282-rs3212948 showed a 0.259-fold decrease in the HCC risk ($P = .017$, 95% CI = 0.079–0.851), those with the A-A haplotype of AFP rs737241-rs4024 showed significant protection against HCC ($P = .044$, OR = 0.393, 95% CI = 0.155–0.997), whereas those with the G-G haplotype of AFP rs737241-rs4024 showed a 2.130-fold increase in the HCC risk ($P = .017$, 95% CI = 1.135–3.999) (Table 6).

3.4. Analyses of polymorphism in ERCC1 and AFP and clinicopathologic features of HCC

A stratified analysis of clinicopathologic features of HCC was performed. Because there are few wild-type genotypes, both wild-

Table 3

Distribution of SNP loci in ERCC1 and AFP genes and Harvard equilibrium test.

SNP	HCC N=118, n (%)	HWE	Control N=88, n (%)	HWE
AFPrs737241				
GG	59 (50.00)	0.494	31 (35.23)	0.904
GA	51 (43.22)		42 (47.73)	
AA	8 (6.78)		15 (17.05)	
AFPrs4024				
GG	24 (20.34)	0.221	19 (21.59)	0.132
GA	51 (43.22)		36 (40.91)	
AA	43 (36.44)		33 (37.50)	
ERCC1rs3212948				
GG	81 (68.64)	0.427	56 (63.64)	0.098
GC	32 (27.12)		25 (28.41)	
CC	5 (4.24)		7 (7.95)	
ERCC1rs1046282				
TT	45 (38.14)	0.759	33 (37.50)	0.316
CT	57 (48.31)		38 (43.18)	
CC	16 (13.56)		17 (19.32)	
ERCC1rs735482				
CC	13 (11.02)	0.056	12 (13.64)	0.495
CA	66 (55.93)		45 (51.14)	
AA	39 (33.05)		31 (35.23)	

AFP = alpha-fetoprotein, ERCC1 = excision repair cross-complementing group 1, HCC = hepatocellular carcinoma, HWE = Hardy–Weinberg equilibrium, SNP = single-nucleotide polymorphism.

Table 4

Distribution of alleles of ERCC1 and AFP gene SNP loci in case group and control group and its relationship with risk of liver cancer.

Variable	HCC (n=236)	Control (n=176)	OR (95% CI)	P
AFPrs737421				
G	169 (71.6)	104 (59.1)	1.000	
A	67 (28.4)	72 (40.9)	0.573 (0.379–0.865)	.008
AFPrs4024				
G	99 (41.9)	74 (42.0)	1.000	
A	137 (58.1)	102 (58.0)	1.004 (0.676–1.491)	.983
ERRC1rs3212948				
G	194 (82.2)	137 (77.8)	1.000	
C	42 (17.8)	39 (22.2)	0.761 (0.467–1.238)	.271
ERRC1rs1046282				
T	147 (62.3)	104 (59.1)	1.000	
C	89 (37.7)	72 (40.9)	0.875 (0.587–1.304)	.511
ERRC1rs735482				
C	92 (39.0)	69 (39.2)	1.000	
A	144 (61.0)	107 (60.8)	1.009 (0.677–1.506)	.964

AFP = alpha-fetoprotein, ERCC1 = excision repair cross-complementing group 1, HCC = hepatocellular carcinoma.

Table 5

Distribution of genotypes of ERCCl and AFP gene SNP loci in case group and control group and their relationship with risk of liver cancer.

SNP	HCC N=118	Control N=88	OR (95% CI)	P
AFPrs737241				
GG	59 (50.0)	31 (35.2)	1.000	
GA	51 (43.2)	42 (47.7)	0.631 (0.345–1.156)	.136
AA	8 (6.8)	15 (17.0)	0.297 (0.112–0.785)	.014*
GA+AA	59 (50.0)	57 (64.7)	0.543 (0.306–0.965)	.037*
AFPrs4024				
GG	24 (20.3)	19 (21.6)	1.000	
GA	51 (43.2)	36 (40.9)	1.043 (0.489–2.227)	.912
AA	43 (36.4)	33 (37.5)	1.004 (0.465–2.165)	.992
GA+AA	94 (79.6)	69 (78.4)	1.024 (0.5120–2.051)	.946
ERCC1rs3212948				
GG	81 (68.6)	56 (63.6)	1.000	
GC	32 (27.1)	25 (28.4)	0.947 (0.500–1.795)	.868
CC	5 (4.2)	7 (8.0)	0.461 (0.133–1.599)	.223
GC+CC	37 (31.3)	32 (36.4)	0.839 (0.461–1.525)	.564
ERCC1rs1046282				
TT	45 (38.1)	33 (37.5)	1.000	
CT	57 (48.3)	38 (43.2)	1.057 (0.569–1.964)	.861
CC	16 (13.6)	17 (19.3)	0.532 (0.225–1.259)	.151
TC+CC	73 (61.9)	55 (62.5)	0.896 (0.501–1.605)	.712
ERCC1rs735482				
CC	13 (11.0)	12 (13.6)	1.000	
CA	66 (55.9)	45 (51.1)	1.204 (0.495–2.929)	.682
AA	39 (33.1)	31 (35.2)	1.071 (0.423–2.712)	.885
CA+AA	105 (89.0)	76 (86.3)	1.149 (0.489–2.697)	.750

AFP = alpha fetoprotein, CI = confidence interval, ERCC1 = excision repair cross-complementing group 1, 1: HCC = hepatocellular carcinoma, 2: the benign liver disease, 3: the negative control, OR = odds ratio.
* Adjusted for age, sex, smoking, drinking.

Table 6

The association of haplotype of each gene with hepatocellular cancer risk.

Haplotype	HCC N=236	Control (%) N=176	P	OR (95% CI)
ERCC1 (1046282, 3212948, 735482)				
CCA	4.44 (1.9)	8.63 (4.9)	.08	0.369 (0.115–1.179)
CGA	75.57 (32.0)	51.48 (29.3)	.580	1.127 (0.737–1.724)
CGC	8.99 (3.8)	0.59 (6.0)	.289	0.614 (0.247–1.527)
TCA	28.67 (12.1)	19.43 (11.0)	.750	1.105 (0.599–2.038)
TCC	8.89 (3.8)	9.64 (5.5)	.398	0.670 (0.264–1.704)
TGA	35.32 (15.0)	27.46 (15.6)	.835	0.944 (0.549–1.623)
TGC	74.12 (31.4)	47.47 (27.0)	.353	1.227 (0.797–1.890)
ERCC1 (1046282, 3212948)				
CC	3.81 (1.6)	10.49 (6.0)	.017	0.259 (0.079–0.851)
CG	85.19 (36.1)	61.51 (34.9)	.810	1.051 (0.699–1.581)
TC	38.19 (16.2)	28.51 (16.2)	.996	0.999 (0.589–1.697)
TG	108.81 (46.1)	75.49 (42.9)	.516	1.139 (0.769–1.688)
ERCC1 (1046282, 735482)				
CA	80.20 (34.0)	59.32 (33.7)	.953	1.013 (0.670–1.53)
CC	8.8 (3.70)	12.68 (7.2)	.116	0.499 (0.206–1.208)
TA	63.80 (27.0)	47.68 (27.10)	.989	0.997 (0.643–1.5470)
TC	83.2 (35.3)	56.32 (32.00)	.490	1.157 (0.765–1.751)
ERCC1 (735482, 3212948)				
AC	34.46 (14.6)	26.63 (15.1)	.882	0.959 (0.554–1.660)
AG	109.54 (46.4)	80.37 (45.7)	.880	1.031 (0.697–1.525)
CC	7.54 (3.2)	12.37 (7.0)	.073	0.437 (0.173–1.104)
CG	84.46 (35.8)	56.63 (32.2)	.445	1.175 (0.777–1.7760
AFP (737241, 4024)				
AA	7.19 (3.0)	13.05 (7.4)	.044	0.393 (0.155–0.997)
AG	59.81 (25.3)	58.95 (33.5)	.070	0.674 (0.439–1.035)
GA	129.81 (55.0)	88.95 (50.5)	.370	1.196 (0.809–1.769)
GG	39.19 (16.6)	15.05 (8.5)	.017	2.130 (1.135–3.999)

AFP = alpha-fetoprotein, CI = confidence interval, ERCC1 = excision repair cross-complementing group 1, OR = odds ratio, 1 = HCC = hepatocellular carcinoma, 3 = the negative control.

type and heterozygous genotypes were combined for the analysis. We found that rs737241 and rs1046282 sites were significantly correlated with the AFP levels. The rs3212948 polymorphism was related to the pathologic grade of liver cancer and the rs4024 polymorphism was related to portal vein tumor thrombosis (PVTT) in HCC. The polymorphism at the rs1046282 locus was associated with the HBV DNA load whereas that at the rs735482 locus was significantly associated with tumor size in HCC. The 5 selected sites in the ERCC1 and AFP genes were not associated with Barcelona Clinic Liver Cancer (BCLC) staging, cirrhosis, foci, gross classification, and capsule invasion in HCC ($P > .05$), as shown in Table 7. Multivariate logistic regression showed that the mutant A allele of rs737241 increased the AFP expression ($P = .007$, OR = 2.982, 95% CI: 1.353–6.572), the mutant C allele of rs1046282 increased the AFP expression ($P = .011$, OR = 2.825, 95% CI: 1.274–6.263), the mutant A allele of rs4024 reduced the risk of PVTT in HCC ($P = .013$, OR = 0.273, 95% CI: 0.098–0.763), the mutant C allele of rs3212948 site decreased the differentiation and maturation of HCC cells ($P = .022$, OR = 2.749, 95% CI: 1.153–6.553), and the mutant C allele of rs1046282 reduced the DNA load of hepatitis B virus ($P = .035$, OR = 0.273, 95% CI: 0.098–0.763). The mutant A allele of rs735482 increased the tumor size of HCC ($P = .037$, OR = 3.859, 95% CI: 1.088–13.684), as shown in Table 8.

4. Discussion

In this study, we evaluated the significant association between the SNPs in the ERCC1 gene and partial AFP locus with the risk of developing HCC and clinicopathologic features of HCC after adjustment for age, gender, smoking, and drinking factors. The relationship between the mutation locus in the ERCC1 gene and the risk of HCC has been suggested in numerous studies. The 3′-UTR is one of the most important regulatory regions of the ERCC1 gene. Mutations in the 3′-UTR or coding region of ERCC1 gene may affect the coding of ERCC1. This might inhibit its binding to the XPF protein, and even affect the ability of nucleotide excision repair pathway, increase the genomic instability, lead to increased genomic instability, and thereby cause various cancers and malignant diseases. The 3′-UTR of the ERCC1 gene is associated with the risk of HCC. Li et al indicated that in the Guangxi Zhuang population, the rs3212986 (-8092) mutation was associated with an increased incidence of HCC.[8] Li et al reported that in the Liaoning Province, the rs3212961 mutation was associated with reduced risk of HCC in the population.[9] There are few reports on the polymorphism of these 3 loci and the risk of HCC. A study on the association between SNPs and haplotypes with the risk of liver disease revealed the involvement of rs735482 and rs1046282 locus in the 3′-UTR region and the rs3212948 locus in the coding region of the ERCC1 gene. Morris and Kaplan suggested that haplotype analysis is superior to single SNP analysis for several susceptibility genes.[10] However, they did not find the association of these 3 loci with the pathogenesis of liver cancer. We found that patients with CC haplotypes of ERCC1 rs1046282-rs3212948 have a 2.52-fold lower risk of HCC ($P = .017$, 95% CI = 0.079–0.851); these results differ from those obtained for an SNP. The reason for this difference is that polymorphic changes in the intron and

Table 7

Results of chi-square test between SNPs and clinic-pathologic features of HCC, n (%).

SNP	Serum AFP levels, ng/mL				EDSON stage (stage)				HBV DNA levels				PVTT				Tumor size (cm)			
	<400	≥400	χ^2	P	I–II	III–IV	χ^2	P	$<5\times10^2$	$\geq5\times10^2$	χ^2	P	No	Yes	χ^2	P	<5	≥5	χ^2	P
rs737241																				
GG	36 (30.5)	23 (19.5)	5.729	.017	19 (16.1)	40 (33.9)	2.864	.091	29 (24.6)	30 (25.4)	.851	.356	49 (41.5)	10 (8.5)	1.776	.138	29 (24.6)	30 (25.4)	2.827	.093
GA+AA	23 (19.5)	36 (30.5)			28 (23.7)	31 (26.3)			34 (28.8)	25 (21.2)			43 (36.4)	16 (13.6)			20 (16.9)	39 (33.1)		
rs4024																				
GG	12 (10.2)	12 (10.2)	0.000	1.000	13 (11.0)	11 (9.3)	2.580	.108	12 (10.2)	12 (10.2)	.139	.709	14 (11.9)	10 (8.5)	6.760	.009	12 (10.2)	12 (10.2)	0.891	.345
GA+AA	47 (39.8)	47 (39.8)			34 (28.8)	60 (50.8)			51 (43.2)	43 (36.4)			78 (66.1)	16 (13.6)			37 (31.4)	57 (48.3)		
rs1046282																				
TT	30 (25.4)	15 (12.7)	8.082	.004	22 (18.6)	23 (19.5)	2.490	.115	18 (15.3)	27 (22.9)	5.241	.022	39 (33.1)	6 (5.1)	3.205	.073	23 (19.5)	22 (18.6)	2.753	.097
TC+CC	29 (24.6)	44 (37.3)			25 (21.2)	48 (40.7)			45 (38.1)	28 (23.7)			53 (44.9)	20 (16.9)			26 (22.0)	47 (39.8)		
rs3212948																				
GG	39 (33.1)	42 (35.6)	0.354	.552	38 (32.2)	43 (36.4)	5.480	.020	42 (35.6)	39 (33.0)	.246	.620	62 (52.5)	19 (16.1)	.304	.581	36 (30.5)	45 (38.1)	0.906	.341
GC+CC	20 (16.9)	17 (14.4)			9 (7.6)	28 (23.7)			21 (17.8)	16 (13.6)			30 (25.4)	7 (5.9)			13 (11.0)	24 (20.3)		
rs735482																				
CC	8 (6.8)	5 (4.2)	0.778	.378	8 (6.8)	5 (4.2)	2.873	.090	5 (4.2)	8 (6.8)	1.803	.253	11 (9.3)	2 (1.7)	.376	.540	9 (7.6)	4 (3.4)	4.681	.032
CA+AA	51 (43.2)	54 (45.8)			39 (33.1)	66 (55.9)			58 (49.2)	47 (39.8)			81 (68.6)	24 (20.3)			40 (33.9)	65 (55.1)		

AFP = alpha-fetoprotein, PVTT = portal vein tumor thrombosis, SNP = single-nucleotide polymorphism.

Table 8

Logistic regression analysis of the relationship between AFP, ERCC1 gene polymorphism, and clinicopathologic features of hepatocellular carcinoma.

Clinicopathologic	No adjusted		Adjusted*	
	OR (95% CI)	P	OR (95% CI)	P
Serum AFP levels				
rs737241				
GA+AA	2.450 (1.169–5.134)	0.018	2.982 (1.353–6.572)	.007
GG	1.000		1.000	
rs1046282				
TC+CC	3.034 (1.395–6.601)	0.005	2.825 (1.274–6.263)	.011
TT	1.000		1.000	
EDSON stage				
rs3212948				
GC+CC	2.907 (1.175–7.192)	0.021	2.749 (1.153–6.553)	.022
GG	1.000		1.000	
HBV DNA levels				
rs1046282				
TC+CC	0.439 (0.206–0.938)	0.034	0.429 (0.196–0.940)	.035
TT	1.000		1.000	
PVTT				
rs4024				
GA+AA	0.287 (0.108–0.760)	0.012	0.273 (0.098–0.763)	.013
GG	1.000		1.000	
Tumor size				
rs735482				
CA+AA	3.656 (1.056–12.659)	0.041	3.859 (1.088–13.684)	.037
CC	1.000		1.000	

AFP = alpha fetoprotein, CI = confidence interval, ERCC1 = excision repair cross-complementing group 1, OR = odds ratio, PVTT = portal vein tumor thrombosis.
* Adjusted for age, sex, smoking, drinking.

3′-flanking sites do not result in a single functional change; they may interact with the expression of gene, as well as with the process of translation, affecting the function of the ERCC1 protein. We believe that the susceptibility of individuals with rs1046282-rs3212948 gene polymorphism to liver cancer deserves further investigation and requires more rigorous experimental design and increased sample size.

The AFP can promote the growth of tumor cells. The survival rate of patients with high levels of expression of this protein is lower than that of patients with low expression levels.[11] The mechanism through which the AFP protein promotes tumor cell growth might be the immune surveillance by AFP that helps cancer cells escape the host cells.[12,13] For studying the association of AFP gene polymorphism and the risk of liver cancer, the AFP-980 gene was sequenced in a Hong Kong population and it was found that -692 CG increased the risk of liver cancer.[5] In Indonesia, detection of 6 separate SNPs (rs3796678, rs3796677, rs3796676, rs28532518, and rs4646038) in AFP intron 1 and intron 2 (rs6834059) was associated with liver cancer, whereas rs2298839 and rs10020432 sites were associated with increased susceptibility to cirrhosis.[6] The rs737241 site is in the coding region of the AFP gene and rs4024 is located in the promoter region. The frequency of occurrence of rs737241 locus A allele found in the HCC group was lower than that in the control group, and the risk of HCC was 0.573-times lower in individuals carrying the A allele. Individuals with at least 1 mutant allele A (GA+AA) had a 0.543-fold lower risk of developing liver cancer compared to those with the GG genotype. We believe the reasons for this observation to be as follows: mutation in the rs737241 locus of the AFP coding region might affect mRNA synthesis, thereby, reducing the synthesis of

the AFP protein, weakening the AFP immune surveillance that helps cancer cells in escaping the host cells; thus, the body can effectively identify and remove tumor cells and tumor metastasis is reduced, leading to a certain degree of protection; Suriapranata et al reported that in an Indonesian population, the polymorphism at the rs4640638 locus was associated with reduced risk of liver cancer,[6] with both sites in the coding region of the gene. The polymorphism at the rs4014 locus has not been found to be associated with the pathogenesis of liver cancer. Suriapranata et al. did not find an association between rs4024 and liver cancer in the Indonesian population.[6] The 2 results suggest that the association of rs4024 polymorphism with the incidence of liver cancer is not relevant. We found that rs737241-rs4024 in patients with AFP haplotypes was significantly related to the protection against HCC (P = .044, OR = 0.393, 95% CI = 0.155–0.997), and GG haplotype showed a significantly increased risk of liver cancer (P = .017, OR = 2.130, 95% CI = 1.135–3.999). The results obtained for the AFP gene were consistent with the results of the allelic analysis. The risk of developing liver cancer in individuals carrying the A allele at the rs737241 locus was reduced, whereas it was increased in individuals carrying the G allele at this locus.

The HCC is highly malignant and has a poor prognosis. Even for radical resection, the 5-year disease-free survival rate in HCC is only 16% to 27.1%.[14] Pathologic features, such as vascular invasion, tumor thrombosis and cirrhosis, and AFP levels, are the main risk factors for postoperative recurrence.[15] The search for these factors mainly depends on imaging, surgical exploration, and laboratory testing; however, achieving accuracy with these methods is difficult. To find suitable indicators, molecular markers related to pathologic features of tumors need to be identified. We found that the presence of rs737241 site mutation A allele or the rs1046282 site mutation C allele increased the expression levels of AFP. High AFP levels were found to be related to poor prognosis in liver cancer.[16,17] However, the regulation of AFP expression is a complex process involving the interaction of transcription factors with promoters and enhancers of the AFP gene.[18,19] The results are different from those of a previous screening for HCC risk, suggesting that rs737241 does not necessarily regulate the expression of AFP. Nonetheless, we believe that the relationship between gene polymorphism and serum AFP levels are worthy of further study, but the number of samples needs to be expanded and there is a need for stratified analysis of experimentally determined AFP levels in different groups. The presence of A allele at the rs4024 site can reduce the risk of vascular invasion, which may be caused by the rs4024 mutation resulting in a decrease in the production of AFP protein. The high level of AFP expression found in the study is related to the formation of tumor thrombus.[19] Carrying the rs3212948 site mutation T allele can reduce the degree of differentiation of HCC, the C allele at rs1046282 locus can reduce the DNA load of HBV, and carrying the allele of rs735482 locus can increase the tumor size in liver cancer. It is suggested that the above 3 ERCC1 gene SNPs might have significance for the prognosis of liver cancer. The rs735482 and rs1046282 polymorphisms are located in the 3′-noncoding region (UTR), which is the major regulatory region for gene duplication and expression. The occurrence of T>C allele at the rs1046282 locus might affect the replication of HBV DNA in liver cancer patients. The C>A allele at rs735482 might affect the posttranscriptional modification of ERCC1 mRNA, which in turn affects the stability and function of mRNA, the ability of DNA repair is reduced, and cancer cells become prone to proliferation. Genes can be involved in increasing the tumor size in liver cancer. Rs3212948 is located in the coding region of the gene. When a base mutation occurs, the DNA repair ability of the cell is reduced, the cancer cell is less likely to differentiate and remains in a poorly differentiated state. A high degree of malignancy indicates a poor prognosis; however, further experiments are needed in this regard.

In summary, we believe that the polymorphism at rs737241 site is associated with the risk of liver cancer. Our results demonstrate that ERCC1 gene polymorphism can be correlated with the level of AFP protein, EDSON grade, tumor size, and HBV DNA load, whereas polymorphism of AFP gene is possibly associated with the level of its protein and tumor thrombosis. It is suggested that ERCC1 and AFP gene polymorphisms are important molecular markers for the occurrence and prognosis of liver cancer. The differences in the results between the present and previous studies might be because of the sample size, the research method, and differences in gene distribution in different geographical populations, or might be a matter of chance. It is possible that the negative result in the present study might be because of the relatively smaller sample size; however, this assumption needs to be experimentally verified. Recent studies have shown that polymorphisms in ERCC1 gene are associated with tumorigenesis and drug resistance in lung, colorectal, ovarian, and head and neck cancers.[20–22] AFP protein can be used as a prediction marker for postoperative recurrence of liver cancer.[23] Whether the above-mentioned locus is related to resistance or pathogenesis of liver cancer remains unclear. Moreover, determination of the level of gene mutation that actually causes the change in the level of AFP protein would need further research.

Author contributions

Conceptualization: Min Fang.
Formal analysis: Hui Liu Zhao.
Funding acquisition: Zhi Min Liu.
Investigation: Jian Ye.
Methodology: Ling Sha Huang.
Project administration: Bo Zhu.
Resources: Bo Zhu.
Software: Yu Liang Huang.
Supervision: Min Fang.
Validation: Jun Rong Wu.
Visualization: Yu Liang Huang.
Writing – review & editing: Yu Liang Huang.

References

[1] Mo J, Luo M, Cui J, et al. Prognostic value of ERCC1 and ERCC2 gene polymorphisms in patients with gastric cancer receiving platinum-based chemotherapy. Int J Clin Exp Pathol 2015;8:15065–71.
[2] Lee SM, Falzon M, Blackhall F, et al. Randomized prospective biomarker trial of ERCC1 for comparing platinum and nonplatinum therapy in advanced non-small-cell lung cancer: ERCC1 Trial (ET). J Clin Oncol 2017;35:402–11.
[3] Min Ni , Zhang WZ, Qiu JR, et al. Association of ERCC1 and ERCC2 polymorphisms with colorectal cancer risk in a Chinese population. Sci Rep 2014;4:4112.
[4] Qi BL, Li Y, Wang N, et al. Polymorphisms of ERCC1 gene and outcomes in epithelial ovarian cancer patients with platinum-based chemotherapy [in Chinese]. Zhonghua Fu Chan Ke Za Zhi 2013; 48:847–52.
[5] Chen GG, Ho RL, Wong J, et al. Single nucleotide polymorphism in the promoter region of human alpha-fetoprotein (AFP) gene and its

significance in hepatocellular carcinoma (HCC). Eur J Surg Oncol 2007;33:882–6.

[6] Suriapranata IM, Sudania WM, Tjong WY, et al. Alpha-fetoprotein gene polymorphisms and risk of HCC and cirrhosis. Clinica Chimica Acta 2010;411:351–8.

[7] Li Z, Zhang Z, He Z, et al. A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res 2009;19:519–23.

[8] Li Y, Ou C, Shu H, et al. The ERCC1-4533/8092, TNF-alpha 238/308 polymorphisms and the risk of hepatocellular carcinoma in Guangxi Zhuang populations of China: case-control study. Medicine (Baltimore) 2016;95:e5217.

[9] Wang B, Xu Q, Yang HW, et al. The association of six polymorphisms of five genes involved in three steps of nucleotide excision repair pathways with hepatocellular cancer risk. Oncotarget 2016;7:20357–67.

[10] Morris RW, Kaplan NL. On the advantage of haplotype analysis in the presence of multiple disease susceptibility alleles. Genet Epidemiol 2002;23:221–33.

[11] Li P, Wang SS, Liu H, et al. Elevated serum alpha fetoprotein levels promote pathological progression of hepatocellular carcinoma. World J Gastroenterol 2011;17:4563–71.

[12] Li MS, Ma QL, Chen Q, et al. Alpha-fetoprotein triggers hepatoma cells escaping from immune surveillance through altering the expression of Fas/FasL and tumor necrosis factor related apoptosis-inducing ligand and its receptor of lymphocytes and liver cancer cells. World J Gastroenterol 2005;11:2564–9.

[13] Takayama T, Sekine T, Makuuchi M, et al. Adoptive immunotherapy to lower postsurgical recurrence rates of hepatocellular carcinoma: a randomised trial. Lancet 2000;356:802–7.

[14] Kim J, Choi SJ, Lee SH, et al. Predicting survival using pretreatment CT for patients with hepatocellular carcinoma treated with transarterial

chemoembolization: comparison of models using radiomics. AJR Am J Roentgenol 2018;211:1026–34.

[15] Llovet JM, Bru C, Bruix J, et al. Prognosis of hepatocellular carcinoma: the BCLC staging classification. Semin Liver Dis 1999;19:329–38.

[16] Rungsakulkij N, Suragul W, Mingphruedhi S, et al. Prognostic role of alpha-fetoprotein response after hepatocellular carcinoma resection. World J Clinical Cases 2018;6:110–20.

[17] Silva JP, Gorman RA, Berger NG, et al. The prognostic utility of baseline alpha-fetoprotein for hepatocellular carcinoma patients. J Surg Oncol 2017;116:831–40.

[18] Nakabayashi H, Hashimoto T, Miyao Y, et al. A position-dependent silencer plays a major role in repressing alpha-fetoprotein expression in human hepatoma. Mol Cell Biol 1991;11:5885–93.

[19] Sawadaishi K, Morinaga T, Tamaoki T. Interaction of a hepatoma-specific nuclear factor with transcription-regulatory sequences of the human alpha-fetoprotein and albumin genes. Mol Cell Biol 1988; 8:5179–87.

[20] Borchiellini D, Etienne-Grimaldi MC, Bensadoun RJ, et al. Candidate apoptotic and DNA repair gene approach confirms involvement of ERCC1, ERCC5, TP53 and MDM2 in radiation-induced toxicity in head and neck cancer. Oral Oncol 2017;67:70–6.

[21] Dai Q, Luo H, Li XP, et al. XRCC1 and ERCC1 polymorphisms are related to susceptibility and survival of colorectal cancer in the Chinese population. Mutagenesis 2015;30:441–9.

[22] Han JY, Lee GK, Lim KY, et al. ERCC1 expression-based randomized phase II study of gemcitabine/cisplatin versus irinotecan/cisplatin in patients with advanced non-small cell lung cancer. Cancer Res Treat 2017;49:678–87.

[23] Cai Z-Q, Si S-B, Chen C, et al. Analysis of prognostic factors for survival after hepatectomy for hepatocellular carcinoma based on a Bayesian Network. PLoS One 2015;10:e0120805.