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ORIGINAL ARTICLE

Promoter polymorphism of MRP1 associated with reduced survival in hepatocellular carcinoma

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Abstract

AIM: To investigate the effect of the G-1666A polymorphism in the multidrug resistance related protein-1 (*MRP1*) on outcome of hepatocellular carcinoma (HCC).

METHODS: A cohort of 162 patients with surgically resected HCC who received no postsurgical treatment until relapse was studied. Genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism analysis. Electrophoretic mobility shift assay (EMSA) was used to evaluate the influence of the G-1666A polymorphism on the binding affinity of the *MRP1* promoter with its putative transcription factors.

RESULTS: Kaplan-Meier analysis showed that patients with GG homologues had a reduced 4-year disease-free survival compared with those carrying at least one A allele (P = 0.011). Multivariate Cox regression analysis indicated that the -1666GG genotype represented an independent predictor of poorer disease-free survival [hazard ratio (HR) = 3.067, 95% confidence interval (CI): 1.587-5.952, P = 0.001], and this trend became worse in men (HR = 3.154, 95% CI: 1.604-6.201, P = 0.001). A similar association was also observed between 4-year overall survival and the polymorphism in men (HR = 3.342, 95% CI: 1.474-7.576, P = 0.004). Moreover, EMSA suggested that the G allele had a stronger binding affinity to nuclear proteins.

CONCLUSION: The *MRP1* -1666GG genotype predicted a worse outcome and was an independent predictor of poor survival in patients with HCC from Southeast China.

Key words: Multidrug resistance related protein-1; Single nucleotide polymorphism; Hepatocellular carcinoma; Prognosis

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third leading cause of cancer death[1]. Optimal surgical resection is regarded as the best treatment for a curative outcome of HCC. However, long-term survival remains poor because of high rates of tumor recurrence or progression. Substantial effort has been made to identify prognostic factors

that can be used for improving therapeutic regimens and survival prediction. However, only a few factors, such as TNM stage or patient performance status, are consistent predictors, and their accuracy remains limited. Therefore, molecular markers that can accurately predict patient outcome are urgently needed.

The human multidrug resistance protein-1 (MRP1), also known as ABCC1, belongs to the ATP-binding cassette superfamily of cell-surface transport proteins. It participates in the transport of a wide variety of endogenously produced and exogenously administered molecules in an adenosine-triphosphate (ATP)-dependent manner[2,3]. Besides its well-known roles in drug resistance, MRP1 is proposed to contribute to the cellular antioxidative defense system by actively extruding glutathione (GSH)-conjugated xenobiotics and GSH-conjugated metabolites from cells[4]. Recent studies have also revealed that MRP1 is involved in inflammatory reactions, such as, dendritic cell differentiation and function[5]. MRP1 is expressed at moderate levels in most normal tissues, including lung, muscle, and kidney, but is barely detectable in normal liver[6-8]. However, in several liver diseases including HCC, its expression in the basolateral membrane is upregulated, which suggests a significant role for this transport protein during carcinogenesis[8,9].

Single nucleotide polymorphisms (SNPs) in the *MRP1* gene have been extensively studied in the past few years, and several genetic variants in the coding region have been shown to affect the function of MRP1[10-13]. For example, G2168A (Arg723Gln) can affect patients' sensitivity to chemotherapy in ovarian cancer[11]. G1299T (Arg433Ser) confers resistance to doxorubicin by reducing intracellular drug accumulation in HeLa cells that stably express mutant MRP1, whereas the G3173A (Arg1058Gln) variation increases the response to etoposide in HEK293 and CHO-K1 cells[12,13]. Recently, it has been observed that SNPs in the gene promoter can affect expression by disturbing the binding affinity of transcription factors, and are associated with disease prognosis[14]. However, whether SNPs in the *MRP1* promoter region have any clinical significance remains obscure. The expression level of *MRP1* is upregulated in HCC, therefore, we hypothesized that sequence variants in the promoter region potentially affect the expression of the *MRP1* gene and the prognosis of cancer, by modulating the efflux of toxins. To test this hypothesis, we investigated the potential of the *MRP1* G-1666A polymorphism (rs4148330) as a prognostic marker in a cohort of patients with HCC in Guangdong province of Southeast China.

MATERIALS AND METHODS

Study population

The study included 162 patients with HCC at the Cancer Center of Sun Yat-sen University (Guangzhou, China) from 2001 to 2005. All patients underwent hepatectomy as initial therapy, and did not receive chemotherapy or radiotherapy as follow-up treatment before recurrence. All samples were histologically confirmed. After surgical resection, the tissue samples were immediately frozen in liquid nitrogen and then stored at -80℃ until use.

Clinicopathological details and follow-up information were obtained from hospital records. The patients enrolled in the study were residents of Guangdong Province. Infection with hepatitis B virus (HBV) or hepatitis C virus (HCV) was diagnosed when HBV surface antigen or HCV antibody was detected by enzyme linked immunosorbent assay in the serum isolated from peripheral blood. The TNM criteria and the Edmondson and Steiner grading system were used to classify tumor stages and differentiation grades, respectively. Informed consent was obtained from each patient. This study was approved by the Clinical Research Ethics Committee of Sun Yat-sen University Cancer Center.

DNA isolation and genotyping

Total genomic DNA was isolated with a standard protocol that included proteinase digestion, phenol-chloroform extraction, and ethanol precipitation. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to detect the genotype. A 160-bp fragment that covered the G-1666A polymorphism was generated using sense primer 5'-GCAACAGCATAACTGGCATT-3' and reverse primer 5'-GAGACCTCCCCCCAATCA-3'. PCR was performed as follows: 20 ng genomic DNA was amplified in a 20-μL reaction mixture that contained 2 mmol/L $MgCl_2$, 0.4 mmol/L dNTPs, 0.2 μmol/L each primer, and 0.5 U *Taq* polymerase (Promega, Madison, WI, USA). After a total of 36 cycles of amplification at an annealing temperature of 58℃, 3 μL PCR products was then incubated overnight at 37℃ with 15 U *Hpa*Ⅱ (MBI Fermentas, Hanover, MD, USA). Digested products were analyzed by 2% agarose gel. PCR fragments that demonstrated altered electrophoretic patterns were purified and characterized by direct DNA sequencing. Results represent two independent experiments.

Cell lines and nuclear protein extraction

Liver cancer cell lines Huh7 and Hep3B were obtained from the American Type Culture Collection (Manassas, VA, USA) and grown in Dulbecco's Modified Eagle's Medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum in a humidified environment of 37℃ that contained 50 mL/L CO_2. Nuclear protein extracts from Hep3B and Huh7 cells were prepared according to the manufacturer's protocol (NucBuster Protein Extraction Kit; Novagen, Darmstadt, Germany).

Electrophoretic mobility shift assay

Electrophoretic mobility shift assay (EMSA) was performed with the Gel Shift Assay System (Promega), according to the manufacturer's instructions. The following oligonucleotides that corresponded to the promoter region of *MRP1* and covered the G-1666A polymorphism were synthesized (underline letters indicate polymorphism): -1666A allele, 5'-GGGGGACCCGGGCCAATAAAAAAATCA-3'; -1666G allele, 5'-GGGGGACCCAGGCCAATAAAAAAATCA-3'; nonspecific (scrambled) oligonucleotide, 5'-GAAGCGGTGACACGGAACATCACGAAA-3'. Oligonucleotides were annealed and end-labeled with [γ-^{32}P]-ATP. Five micrograms of Hep3B or

Huh7 nuclear extracts were added in each binding reaction. For the competition assay, a 10-, 50- or 100-fold molar excess of unlabeled oligonucleotide was added to the binding reaction mixture as a competitor. The products were separated on pre-electrophoresed 5% polyacrylamide gels at 4℃. The gels were then dried at 80℃ for 4 h and exposed to a Storage Phosphor Screen (Amersham Bioscience, Sunnyvale, CA, USA), which was subsequently read with a Typhoon Phosphor Imager (Amersham Bioscience). The putative transcription factors that recognized the sequences that overlapped the G-1666A site were predicted with Alibaba2.1 (http://www.gene-regulation.com/pub/programs/alibaba2/index.html) and the transcription element search software (TESS, http://www.cbil.upenn.edu/cgi-bin/tess/tess).

Statistical analysis

The χ^2 and Fisher's exact tests were used for the analysis of the relationship between the genotypes and clinicopathological characteristics. Disease-free survival (DFS) was calculated from the day of surgery to either relapse or death without relapse, and it was censored only for patients who were alive and recurrence-free at the last follow-up. Overall survival (OS) was measured from the date of hepatectomy to the time of death or the last follow-up. Survival curves were obtained by the Kaplan-Meier method, and the statistical significance of the differences in survival among subgroups was evaluated with the log-rank test. The Cox proportional hazards model was employed to assess the independent prognostic values of the polymorphisms. Statistical analyses were all performed with SPSS software package (version 13.0; SPSS, Inc., Chicago, IL, USA). All statistical tests were two-sided, and $P < 0.05$ was considered to be statistically significant.

RESULTS

Patient characteristics and genotype

Demographic and clinicopathological characteristics of the 162 patients with HCC are summarized in Table 1. The mean age at first diagnosis of HCC was 48 years. Consistent with our previous study[15], most patients showed excessive γ-glutamyl transpeptidase and α-fetoprotein, along with liver cirrhosis, and > 80% of the enrolled patients were infected with HBV (140/161, 87.0%), which implicated HBV infection as a leading cause of HCC in Southeast China. In contrast, only a small number of patients were infected with HCV.

Genotyping was performed by PCR-RFLP. A 160-bp *MRP1* promoter region that covered the G-1666A variant was digested with *Hpa*Ⅱ. After full digestion of the amplified PCR products, those from AA homozygotes still existed as a single 160-bp fragment, whereas those from the GG homozygotes had been divided into two fragments of 71 bp and 89 bp, respectively. The allele frequency of patients with HCC was 0.61 for *MRP1* -1666A and 0.39 for -1666G. However, no significant correlations were found between the nucleotide variants and clinical variables (data not shown).

Table 1 Physiological characteristics of hepatocellular carcinoma patients (n = 162)

	n (%)
Sex	
Female	15 (9.3)
Male	147 (90.7)
Age (yr)	
< 48	77 (47.5)
⩾ 48	85 (52.5)
HBV infection[1]	
-	21 (13.0)
+	140 (86.4)
HCV infection	
-	158 (97.5)
+	4 (2.5)
GGT (U/L)[2]	
< 50	45 (27.8)
50-99	48 (29.6)
⩾ 100	67 (41.4)
AFP (ng/mL)	
< 20	53 (32.7)
20-399	45 (27.8)
⩾ 400	64 (39.5)
Tumor size (cm)	
< 5	53 (32.7)
⩾ 5	109 (67.3)
Ascites[3]	
-	148 (91.4)
+	14 (8.6)
Cirrhosis	
Total	19 (11.7)
Mild	80 (49.4)
Moderate	49 (30.2)
Severe	14 (8.6)
Edmondson grade	
Ⅰ	11 (6.8)
Ⅱ	71 (43.8)
Ⅲ	77 (47.5)
Ⅳ	3 (1.9)
TNM stage	
Ⅰ	106 (65.4)
Ⅱ	7 (4.3)
Ⅲ	49 (30.3)
G-1666A genotype	
AA	55 (34.0)
AG	89 (54.9)
GG	18 (11.1)

[1](-) absence, (+) presence, one case unconfirmed; [2]Two cases unconfirmed; [3](-) absence, (+) presence. HBV: Hepatitis B virus; HCV: Hepatitis C virus; GGT: γ-glutamyl transpeptidase; AFP: α-fetoprotein.

Association analysis between the G-1666A polymorphism and survival

Growing evidence suggests that SNPs are closely related to the risk and outcome of cancer[14,16,17]. To investigate the impact of the G-1666A polymorphism on the prognosis of patients with HCC, we next analyzed the 4-year DFS and OS of patients with different genotypes. A significant correlation between the -1666 polymorphism and postoperative survival was found. The mean survival times of patients with the AA, AG and GG genotypes were 30.4 ± 18.2, 30.7 ± 17.4 and 24.8 ± 17.3 mo, respectively. The survival curves showed that the 4-year rate of DFS among patients who carried GG decreased significantly compared with those who carried the AA or AG allele (P = 0.031,

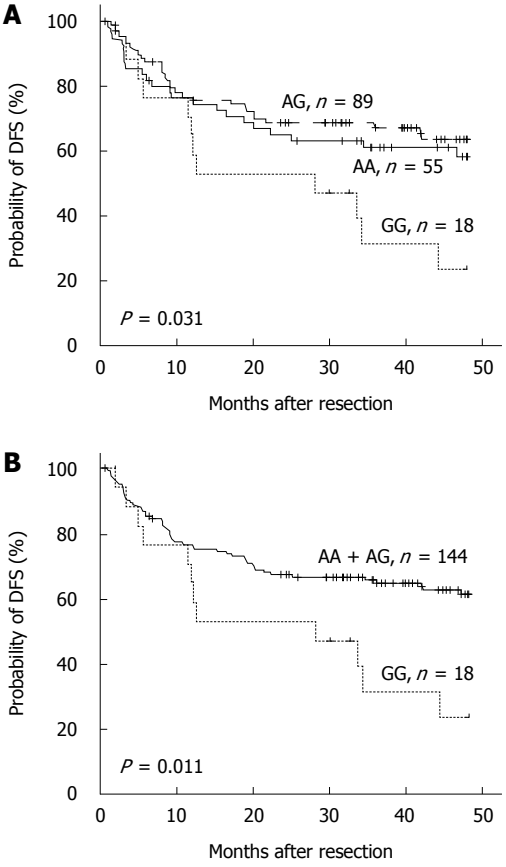

Figure 1 Kaplan-Meier disease-free survival curves for hepatocellular carcinoma patients who carried different *multidrug resistance related protein-1* -1666 genotypes. A: Comparison between three genotypes; B: GG genotype compared with the other two genotypes. Log-rank *P* values are indicated. Tick marks represent censored data. DFS: Disease-free survival.

Figure 1A). Moreover, if the patients with AA and AG genotypes were combined, the discrepancy became more obvious ($P = 0.011$, Figure 1B). Further analysis revealed a similar, albeit non-significant, trend between the -1666 polymorphism and 4-year OS (Table 2). Multivariate Cox proportional hazard analysis was then performed, and the variables that showed significance by univariate analysis were adopted as covariates (Table 2). The results revealed that the *MRP1* G-1666A polymorphism was an independent prognostic factor for 4-year DFS [hazard ratio (HR) = 3.067, 95% confidence interval (CI): 1.587-5.952, $P = 0.001$, Table 3].

One of the key features of HCC is the much higher incidence in men than in women[1]. In our study cohort, the male/female ratio was 9.8:1. Further stratification of the patients by sex revealed an even more pronounced association of the -1666GG genotype with poorer survival ($P < 0.05$, Figure 2). Multivariate analysis suggested that the -1666GG genotype was an especially powerful independent prognostic factor of 4-year DFS (HR = 3.154, 95% CI: 1.604-6.201, $P = 0.001$) and OS (HR = 3.342, 95% CI: 1.474-7.576, $P = 0.004$) in the men with HCC (Table 3).

Influence of the G-1666A polymorphism on the affinity of binding with nuclear proteins

We performed EMSA to evaluate the influence of the

Table 2 Determination of prognostic factors for disease-free survival and overall survival of patients with hepatocellular carcinoma, by univariate analysis ($n = 162$)

Variable	DFS		OS	
	HR (95% CI)	*P*[a]	HR (95% CI)	*P*[a]
Sex				
Female	1		1	
Male	4.231 (1.035-17.299)	0.045	7.522 (1.041-54.337)	0.045
GGT (U/L)[1]				
< 50	1		1	
50-99	2.064 (0.920-4.632)	0.079	1.771 (0.734-4.275)	0.203
⩾ 100	3.639 (1.761-7.519)	0.001	3.728 (1.734-8.014)	0.001
AFP (ng/mL)				
< 20	1		1	
20-399	1.852 (0.954-3.595)	0.068	2.004 (0.989-4.061)	0.054
⩾ 400	1.997 (1.070-3.725)	0.030	2.102 (1.079-4.094)	0.029
Tumor size (cm)				
< 5	1		1	
⩾ 5	2.230 (1.233-4.030)	0.008	2.089 (1.126-3.875)	0.019
Ascites[2]				
-	1		1	
+	2.562 (1.301-5.044)	0.007	2.81 (1.375-5.741)	0.005
Cirrhosis				
No	1		1	
Mild	2.005 (0.710-5.661)	0.189	2.505 (0.763-8.225)	0.130
Moderate	1.832 (0.623-5.387)	0.271	2.287 (0.670-7.806)	0.187
Severe	3.230 (0.993-10.505)	0.051	4.796 (1.297-17.738)	0.019
TNM stage				
Ⅰ	1		1	
Ⅱ + Ⅲ	3.165 (1.940-5.163)	< 0.001	3.424 (2.038-5.752)	< 0.001
Genotypes				
AA	1		1	
AG	0.830 (0.479-1.439)	0.507	0.818 (0.463-1.447)	0.490
GG	1.988 (0.982-4.024)	0.056	1.491 (0.682-3.258)	0.317
AA + AG	1		1	
GG	2.223 (1.185-4.172)	0.013	1.678 (0.822-3.422)	0.155

[a]Hazard ratio (HR) and *P* values were calculated using univariate Cox regression. $P < 0.05$ was considered to indicate statistical significance; [1]Two cases unconfirmed; [2](+) presence, (-) absence. DFS: Disease-free survival; OS: Overall survival; CI: Confidence interval; GGT: γ-glutamyl transpeptidase; AFP: α-fetoprotein.

G-1666A polymorphism on the binding affinity of the *MRP1* promoter with putative transcription factors. The radiolabeled -1666G probe showed strong DNA-protein binding ability in the presence of nuclear proteins extracted from the Hep3B cell line, whereas the -1666A probe barely showed any interaction (Figure 3A, lane 2 and lane 10, respectively). In order to assess the binding specificity and the differences in binding affinity between the G and A alleles, competition assays were performed with unlabeled -1666A and -1666G oligonucleotides. A 50-fold excess of unlabeled -1666A oligonucleotides only partially disrupted the binding of the radiolabeled -1666G probe with nuclear extracts (Figure 3A, lane 7 and Figure 3B, lane 6), whereas this amount of unlabeled -1666G oligonucleotides almost completely abolished the binding (Figure 3A and B, lane 4). In contrast, a non-specific competitor had no effect (Figure 3B, lane 8). Similar results were obtained when using the nuclear extracts from Huh7 cells (data not shown). These data suggested that the G-1666A polymorphism could affect the binding affinity of the *MRP1* promoter with transcription factors, and

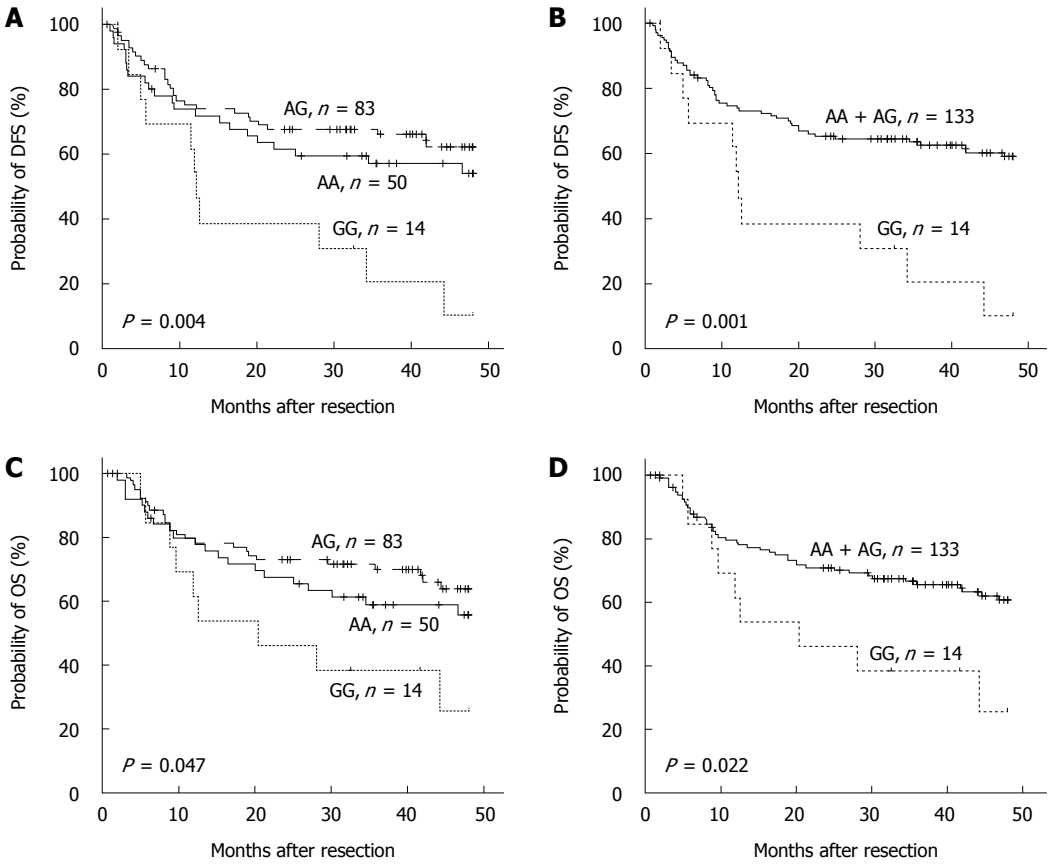

Figure 2 Kaplan-Meier curves for disease-free survival (A and B) and overall survival (C and D) for male patients with hepatocellular carcinoma and different *multidrug resistance related protein-1* -1666 genotypes. A: Comparison of disease-free survival (DFS) between three genotypes; B: AA and AG grouped together and compared to GG genotype; C: Comparison of overall survival (OS) between three genotypes; D: OS of AA and AG genotypes compared with GG genotype. Log-rank *P* values are indicated. Tick marks represent censored data.

Table 3 Multivariate analysis for prognostic value of *multidrug resistance related protein-1* G-1666A polymorphism in patients with hepatocellular carcinoma

Genotypes	DFS[1]		OS[2]	
	HR (95% CI)	*P*[a]	HR (95% CI)	*P*[a]
All (*n* = 162)				
AA+AG	1			
GG	3.067 (1.587-5.952)	0.001		
Men (*n* = 147)				
AA + AG	1		1	
GG	3.154 (1.604-6.201)	0.001	3.342 (1.474-7.576)	0.004

[a]Hazard ratio (HR) and *P* values were calculated using multivariate Cox regression. $P < 0.05$ was considered to indicate statistical significance; [1]Multivariate analysis of disease-free survival (DFS) in all patients was adjusted for sex, γ-glutamyl transpeptidase (GGT), α-fetoprotein (AFP), tumor size, ascites, and TNM stage; in male patients, it was adjusted for GGT, tumor size, and TNM stage; [2]Multivariate analysis of OS in male patients was adjusted for GGT, tumor size, ascites, cirrhosis, and TNM stage. OS: Overall survival; CI: Confidence interval.

that the G allele had a stronger binding affinity than the A allele.

DISCUSSION

The multidrug resistance protein family transports a wide range of physiological substrates and diverse therapeutic agents. In the past decade, much effort has been focused on MRP1-mediated drug resistance[18,19]; and emerging evidence indicates that SNPs within the *MRP1* gene have prognostic value in predicting the response to chemotherapy in different cancers[11,20]. Notably, MRP1 takes part in the transport of aflatoxin B1, a well-know human liver carcinogen that can induce a characteristic mutation in *p53* at codon 249[21,22]; and previous studies have shown that *p53* mutations are significantly associated with a poor prognosis for patients with HCC[15,23]. In addition, MRP1 also plays important roles in cellular antioxidant defense and immune cell function[4,5]. These observations and the upregulated expression level of *MRP1* gene in several liver diseases, including HCC, suggest the possibility that this protein is involved in tumorigenesis and progression[8,9]. Our present study examined the role of the *MRP1* G-1666A polymorphism as a prognostic factor in patients with HCC who were treated only with curative surgery, and proved that the GG genotype was an independent predictor of poor survival, especially in men with HCC. Furthermore, specific binding of nuclear proteins to G allele was found, which suggested a difference in transcription activity between different genotypes.

In the present study, there were 147 men (90.7%) and 15 women with HCC (9.3%), with a male-to-female ratio

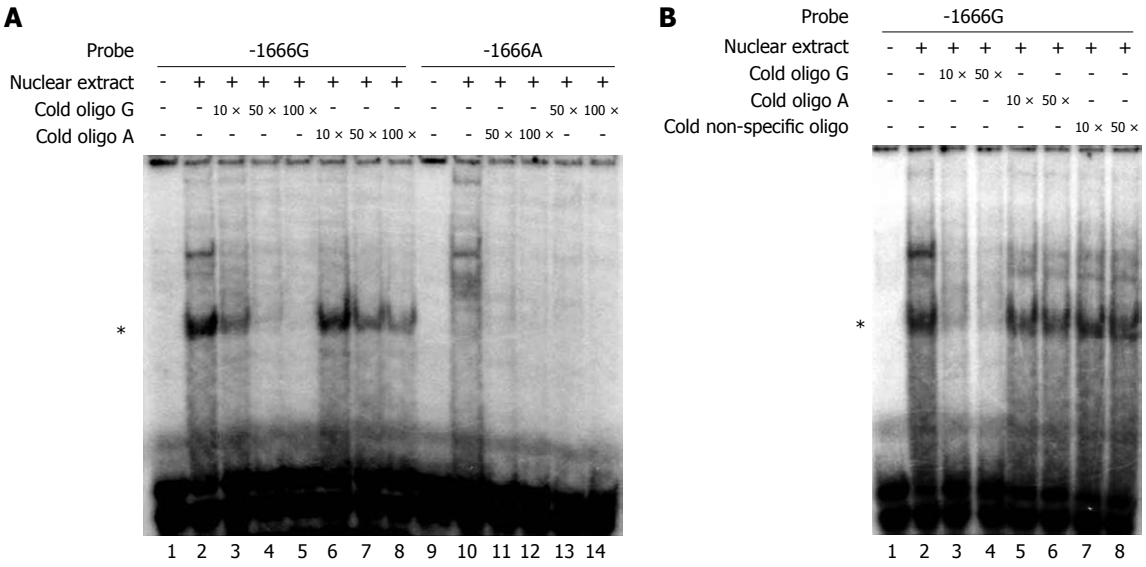

Figure 3 Electrophoretic mobility shift assay of the *multidrug resistance related protein-1* promoter region that contained the G-1666A site. A: Analysis was performed in the presence (+) or absence (-) of Hep3B nuclear extract. Each binding reaction contained γ-^{32}P-labeled -1666G (lanes 2-8) or -1666A (lanes 10-14) probes. A 10-, 50-, or 100-fold (as indicated) excess of unlabeled (cold) -1666A or G oligonucleotides (lanes 6-8, 11, and 12 or 3-5, 13, and 14) were included in the binding reactions as specific competitors. Labeled oligonucleotides incubated without the nuclear extracts were included as negative controls (lanes 1 and 9); B: In the present of Hep3B nuclear extract, 10- or 50-fold more excess of unlabeled -1666G oligonucleotides (lanes 3 and 4) or -1666A oligonucleotides (lanes 5 and 6) or non-specific oligonucleotides (lanes 7 and 8) were used as competitors. Lane 1 was the negative control. Lane 2 indicated the labeled -1666G oligonucleotides incubated with the nuclear extracts only. The asterisks indicated the DNA-protein complex.

of 9.8:1. We observed a remarkably significant association of the *MRP1* G-1666A polymorphism with 4-year OS in men with HCC, but not in the entire cohort. This phenomenon might result from the interaction between the polymorphism and sexual hormones during carcinogenesis, which has been demonstrated in the example of *MDM2* SNP309[24]. Therefore, the correlation between the *MRP1* G-1666A polymorphism and the survival of women with HCC requires further investigation to generate a definite conclusion.

SNPs in the promoter region of a gene can potentially alter the affinity of interactions between DNA and nuclear proteins and, in turn, affect the efficiency of transcription. We found that the G allele of the *MRP1* G-1666A polymorphism had a stronger binding affinity for nuclear proteins in hepatoma cells than the A allele had. This finding accords with our presumption that the G-1666A polymorphism might dominate the pumping ability of MRP1 by affecting the expression of the protein. Although a G-1666A polymorphism located 1.5 kb upstream of the core promoter of *MRP1*, and two major regulatory domains had already been found in tandem upstream of the core promoter[25], recent studies have revealed that distal regions (enhancer or suppressor) can influence gene transcription through physical association with the transcription start site[26]. Furthermore, allele G of the G-260C polymorphism could lead to lower activity of the *MRP1* promoter in cell lines, which suggests that nucleotide variants in the *MRP1* gene account, in part, for inter-individual variations and population differences in cellular efflux[27]. These data suggest that the G-1666A polymorphism functions as a distal element through the folding of the DNA strand.

The three transcription factors Sp1, NF-1 and CTF were predicted to bind to the promoter region, including the G-1666A site, by Alibaba2.1 software, but only the Sp1 consensus motif could partially disrupt the DNA-protein binding in a competition assay, whereas the other two did not show significant influence on the binding (data not shown). Sp1 antibody failed to reveal any super-shift band when added to the EMSA reaction complex (data not shown), which suggested that Sp1 binding to the cis-element, including the G-1666A site, required an interaction between Sp1 and other nuclear proteins. Future work will be required to identify such nuclear proteins.

In summary, our present study shows that the *MRP1* G-1666A polymorphism is an independent prognostic factor for patients with HCC, which implies a role for MRP1 in tumor progression. Clearly, much more work remains to be done to confirm our findings and overcome the limitations in our work before this SNP can be used as a marker for poor outcome in HCC.

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COMMENTS

Background

Hepatocellular carcinoma (HCC) is the fifth most common malignancy worldwide and the third leading cause of cancer death, and long-term survival remains poor because of high rates of tumor recurrence or progression. Therefore, markers that can be used for improving therapeutic regimens and survival prediction are urgently needed.

Research frontiers

The finding that human multidrug resistance protein-1 (MRP1) is expressed

in unusually large amounts in HCC suggests it has a role in the growth and progression of this cancer. Expression of MRP1 is affected by the genetic sequence in its promoter region, therefore, the authors of this study examined the potential of different sequences (polymorphisms) in the *MRP1* promoter to serve as indicators of prognosis and outcome in patients with HCC.

Innovations and breakthroughs

Recent studies have demonstrated that mutations within the *MRP1* gene have value in predicting the response to chemotherapy in different cancers, but the clinical significance of such mutations in the *MRP1* promoter for patients with HCC is unknown. This is believed to be the first study to identify a polymorphism in the promoter of *MRP1* that is an independent prognostic factor for 4-year overall survival in men with HCC. The correlation between the *MRP1* polymorphism and the survival of women with HCC requires further investigation. The authors also demonstrated that the polymorphism altered the affinity of nuclear proteins for the DNA in the HCC cells, which might explain the mechanism by which the expression of MRP1 was reducing.

Applications

The genetic sequence identified in this study can be used to test tissue samples from patients with HCC to help predict their outcome after therapy. This information can be used to guide treatment decisions and improve therapeutic regimens for individual patients.

Terminology

MRP1 is one of a family of proteins found on the surface of cells. These proteins transport a wide variety of substances and can contribute to resistance to chemotherapy by transporting anticancer drugs out of cancer cells. The promoter region of a gene is a sequence of nucleotides that regulates whether and how much of a protein is synthesized from that gene.

Peer review

This is a study of an important area of cancer genomics. The authors found that a single nucleotide polymorphism of *MRP1* promoter region is an independent prognostic factor for HCC patients. The study design was well-organized and they reached a conclusion by making full use of the data.

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