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TNF-α -863 polymorphisms and the risk of hepatocellular carcinoma

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Abstract. Hepatocellular carcinoma (HCC) is a common type of highly malignant tumor. Guangxi is an area of China characterized by a high incidence of HCC. Previous epidemiological studies have found that chronic infection with hepatitis B virus (HBV) is one of the major etiological risk factors for HCC in China. With the increased understanding of the host immune response against HBV and the pathogenesis of the virus, at present, greater attention is being given to the immune response of cytokine genes, as polymorphisms may have a major impact on the course and outcome of HBV infection. In the present study, we genotyped tumor necrosis factor-α (*TNF-α)* rs1800629 (-308G/A), rs1800630 (-863C/A); interleukin-1B rs1143627 (-31T/C); and transforming growth factor β1 (*TGF-β1*) rs1800469 (-509C/T) in a hospital-based study of 772 HCC cases and 852 cancer-free controls. The distribution of the frequency of *TNF-α* rs1800630 sites of CC, CA, AA were 65.67, 27.46 and 6.87% in the case group, respectively, as compared with 67.02, 29.58 and 3.40% in the controls, all with a statistical significance (P<0.05). The logistic regression analysis revealed that the variant rs1800630 AA genotypes were associated with a significantly increasing risk of HCC (OR=2.058, 95% CI 1.289-3.287), compared with the wild-type rs1800630 CC. Further stratified analyses showed that after stratification for history of alcohol drinking, in a subgroup of individuals without a history of drinking, the HCC risk in the group with the TNF-α rs1800630 A allele was 1.839 times higher than that in the group with TNF-α rs1800630 C (P<0.010). These findings suggest that TNF-α rs1800630 may contribute to the risk of HCC, however, these data require further validation.

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Abbreviations: TNF-α, tumor necrosis factor-α; HCC, hepatocellular carcinoma; IL-1B, interleukin-1B; TGF-β1, transforming growth factor-β1; SNP, single-nucleotide polymorphism

Key words: hepatocellular carcinoma, TNF-α, polymorphisms, single-nucleotide polymorphism

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide and the third most common cause of death from cancer. It is estimated that the number of new cases worldwide is 626,000 and deaths from liver cancer reached 598,000 in the year 2002 (1). An estimated 711,000 cases of primary liver cancer occurred worldwide in 2007; approximately three-quarters of these cases were HCC. More than 80% of cases occurred in developing countries; >55% occurred in China alone (2). Guangxi is an area in China characterized by a high incidence of HCC, and the statistical data indicate that in the period 2004-2005, the crude mortality from primary liver cancer was 34.39/100,000 individuals in Guangxi Province (3).

Previous epidemiological studies have found that chronic infection with hepatitis B virus (HBV) is one of the major etiological risk factors for HCC in China (4,5). Chronic HBV infection is an important cause of HCC in China and >90% of patients with HCC are associated with HBV infection (6). After initial infection with HBV, 90-95% of adults can rely on their own immune systems to clear the virus which presents as self-limiting hepatitis. Only 5-10% of those infected develop chronic hepatitis, and 10-25% of these patients eventually progress to HCC (7,8). This suggests that the body's scavenging ability for HBV is based on individual differences. That is, after hosts are infected with HBV disease, the progression and the outcome are largely determined by their antiviral immunity (or inflammation)-related gene polymorphisms. In fact, genetic polymorphisms that encode crucial immune-related genes have been identified as etiological factors in chronic inflammatory disorders (9). With an increasing understanding of the host immune response against HBV and the pathogenesis of the virus, greater attention has been paid to the immune response of cytokine genes, as polymorphisms may have a major impact on the course and outcome of HBV infection (10,11).

A large number of studies have revealed that tumor necrosis factor-α (TNF-α), interleukin-1B (IL-1B) and transforming growth factor-β1 (TGF-β1) are closely related to the development of HCC (7). As a proinflammatory cytokine, TNF-α at high levels has been associated with carcinogenesis.

Particularly in the promoter region of the TNF-α gene, TNF-α (-308) SNPs including TNF-α1 (-308G) and TNF-α2 (-308A) alleles induce expression of TNF-α and are associated with cancer susceptibility. In a case study in Taiwan, the authors concluded that carrying the TNF2 allele is a significant predictor of HCC independent of hepatitis B and C, and therefore that it may be used as a biomarker for susceptibility to HCC (12).

Several polymorphisms of proinflammatory IL-1B have been reported, and the IL-1B-31 the IL-1B-511 genotypes have been associated with enhanced IL-1B production. In a study of 274 Japanese patients with HCC with chronic HCV infection and 55 healthy individuals, the IL-1B-31 genotype T/T or IL-1B-511/-31 haplotype C/T was examined (13). The authors concluded that the IL-1B-31 genotype T/T or the IL-1B-511/-31 haplotype C-T was associated with the presence of HCC in Japanese patients with chronic HCV infection.

TGF-β1 SNPs may be associated with a reduced risk of developing viral hepatitis-mediated HCC. The relationship between polymorphisms of the TGF-β1 gene and the risk of HCC in chronic HBV patients was also tested in a cohort of 1,237 Korean patients: 1,046 with chronic HBV infection and 191 healthy individuals (14). In the Korean subjects, only two SNPs were found among the seven known polymorphisms of TGF-β1, at position -509 and in codon 10. The risk of HCC was significantly lower in patients with the T/T or C/T genotypes than in those with the C/C genotypes at position -509 ($P<0.02$), and the risk of HCC was also lower among the patients with the Pro/Pro or Leu/Pro genotypes than in those with the Leu/Leu genotypes in codon 10 ($P<0.007$). The results showed that the presence of the TGF-β1 -509C>T promoter or of the L10P polymorphism, and the combination of both (-509C>T; L10P) as a haplotype were strongly associated with a reduced risk of HCC in patients with chronic HBV infection. Another investigation to assess the -509C>T polymorphism of the TGF-β1 gene, which is associated with HBV-related HCC in Chinese patients, was carried out (15). In this study, a total of 575 patients with chronic HBV infection and 299 healthy volunteers with no evidence of recent or remote HBV infection were prospectively enrolled. The results of this study showed that the risk of HCC in Chinese patients with HBV infection was significantly lower for the TT genotypes than in patients with the CC genotypes at position -509 of the TGF-β1 gene ($P=0.01$).

In the present study, we hypothesized that TNF-α, IL-1B and TGF-β1 polymorphisms are associated with HCC risk. To test this hypothesis, we carried out a genotype analysis for rs1800629 (-308G/A), rs1800630 (-863C/A) in TNF-α; rs1143627 (-31T/C) in IL-1B; and rs1800469 (-509C/T) in TGF-β1 using a hospital-based study of 772 HCC patients as the case group, and 852 non-cancer patients as the control group.

Materials and methods

Study population. This hospital-based case-control study consisted of 772 patients with HCC and 852 cancer-free controls who were enrolled from June, 2007 to June, 2010. All cases were previously untreated (prior to chemotherapy or radiotherapy for cancer), clinical and/or histopathologically diagnosed with HCC at The First Affiliated Hospital of Guangxi Medical University, The First Affiliated Hospital of Guangxi Traditional Chinese Medicine University, 303 Hospital of Guangxi, and The First People's Hospital of Liuzhou. Controls were recruited from non-cancer patients admitted to the Spinal Orthopedic Department, Traumatic Department, and Departments of Aesthetic Plastic Surgery and Ophthalmology in the same region during the same period as the cases. Cancer-free, randomly selected controls were matched to the cases in relation to age (±5 years), gender and ethnicity and were unrelated ethnic Chinese. All subjects were personally interviewed by specially trained investigators. Each subject was interviewed to provide information on demographic data, disease history, personal history, tobacco smoking, and alcohol drinking history and family history of cancer (any reported cancer in first-degree relatives). All cases and controls were collected under an approved protocol followed by informed consent, and the present study was approved by the Chinese Administration Office of Human Genetic Resources. Two milliliters of fasting venous blood was collected in the morning on the second day, and genome DNA was extracted on the same day and used for genotyping assays.

Single-nucleotide polymorphism selection and genotyping. We searched functional SNPs from the HapMap database (HapMap data release 27 phase II+III, February 2009, on NCBI B36 assembly, dbSNP126, http://www.hapmap.org/index.html.zh), aiming criteria: minor allele frequency cut-off 0.05 in samples of Han Chinese in Beijing. As a result, we selected four SNPs: rs1800629 (-308G/A), rs1800630 (-863C/A) in TNF-α; rs1143627 (-31T/C) in IL-1B; and rs1800469 (-509C/T) in TGF-β1.

Genomic DNA was extracted from a leukocyte pellet by traditional phenol-chloroform extraction and ethanol precipitation. Genotyping was performed at the Laboratory of the College of Public Health (Medical University of Guangxi, China) with the ABI TaqMan platform, using an ABI 7500 real-time thermal cycler under standard conditions (Applied Biosystems, Foster City, CA, USA). The genotyping and recording of the results were carried out in a double-blinded manner without any personal information on the subjects, such as age and occupation.

Statistical analyses. Differences in the distributions of demographic characteristics, selected variables, and genotypes of the TNF-α and IL-1B variants between the cases and controls were evaluated by the χ^2 test or the Student's t-test. The associations between TNF-α and IL-1B genotypes and risk of HCC were estimated by computing the odds ratios (OR) and their 95% confidence intervals (CI) using logistic regression analyses. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test to compare the observed genotype frequencies to the expected ones among the control subjects. All the statistical analyses were carried out with SPSS Statistical Package (Statistical Package for the Social Sciences, version 14.0; SPSS, Inc., Chicago, IL, USA).

Results

Selected characteristics of the 772 HCC cases and 852 cancer-free controls are summarized in Table I. Compared with the control subjects, the HCC cases had a significantly higher

Table I. Demographic and selected variables in the hepatocellular carcinoma cases and controls.

Variables	Cases (n=772) n (%)	Control (n=852) n (%)	χ^2	P-value
Age (years)				
≤19	3 (0.40)	1 (0.10)	6.080	0.108
20-39	169 (21.90)	226 (26.50)		
40-59	462 (59.80)	489 (57.40)		
≥60	138 (17.90)	136 (16.00)		
Gender				
Male	623 (80.70)	656 (77.90)	1.906	0.167
Female	149 (19.30)	186 (22.10)		
HBV history				
No	383 (49.60)	820 (96.20)	458.645	<0.001
Yes	389 (50.40)	32 (3.80)		
HCC family history				
No	639 (83.00)	850 (99.8)	153.451	<0.001
Yes	133 (17.20)	2 (0.2)		
Tobacco smoking history				
No	496 (64.20)	729 (85.60)	99.287	<0.001
Yes	276 (35.80)	123 (14.40)		
Alcohol drinking history				
No	484 (62.70)	729 (85.60)	112.057	<0.001
Yes	288 (37.30)	123 (14.40)		
HBsAg				
(-)	165 (21.40)	591 (69.40)	374.983	<0.001
(+)	607 (78.60)	261 (30.60)		

frequency of a history of HBV (P<0.001), a higher frequency of a family history of HCC (P<0.001), a higher frequency of tobacco smoking history (P<0.001), and higher frequency of a history of alcohol drinking (P<0.001).

The genotype distributions of *TNF-α* rs1800629, rs1800630, *IL-1B* rs1143627, and *TGF-β1* rs1800469 in the cases and controls are shown in Table II. The observed genotype frequencies for these four polymorphisms in the controls were all in Hardy-Weinberg equilibrium (P=0.913, 0.854, 0.846 and 0.441 for rs1800629, rs1800630, rs1143627 and rs1800469, respectively). The distribution frequency of TNF-α rs1800630 sites of CC, CA, AA were 65.67, 27.46 and 6.87% in the case group, respectively, as compared with 67.02, 29.58 and 3.40% in the controls, all with a statistical significance (P<0.05). The logistic regression analysis revealed that the variant rs1800630 AA genotypes were associated with a significantly increased risk of HCC (OR=2.058, 95% CI 1.289-3.287), compared with the wild-type rs1800630 CC. However, no evidence of an association was observed between the *TNF-α* rs1800629, *IL-1B* rs1143627 and *TGF-β1* rs1800469 polymorphism and HCC risk (Table II).

Further stratified analyses showed that after stratification by alcohol drinking, in the subgroup without a history of drinking, the HCC risk for the group with TNF-α rs1800630 A allele was 1,839 times higher than that in the group with TNF-α rs1800630 C (0.010) (Table III). After stratification for history of hepatitis, tobacco smoking and HBsAg positivity, no risk-increasing genotype was found for TNF-α rs1800629, IL-1B rs1143627, and TGF-β1 rs1800469. However, due to the limited study sample size, all the results from the stratified analyses were preliminary.

Discussion

In this hospital-based case-control study, we investigated the association of TNF-α rs1800630 (-863C/A) polymorphisms with the risk of hepatocellular carcinoma. We found, for the first time, that *TNF-α* rs1800630 contributes to the risk of hepatocellular carcinoma in a Guangxi population. Stratified analyses indicated that the joint effects varied with drinking and *TNF-α* polymorphisms. These findings support our hypothesis that potentially functional polymorphisms in *TNF-α* may play a role in the etiology of hepatocellular carcinoma.

There is an increasing volume of literature published to date on the function of *TNF-α* rs1800630 (-863C/A). The TNF-α polymorphisms of 254 men of Swedish origin were

Table II. TNF-α, IL-1B and TGF-β1polymorphisms and hepatocellular carcinoma risk.

	Cases (n=772) n (%)	Controls (n=852) n (%)	χ^2	P-value	OR (95% CI)
TNF-α					
rs1800629					
G	1383 (89.57)	1526 (89.55)	0.000	0.986	1.000 (0.779-1.284)
A	161 (10.43)	178 (10.45)			
GG	619 (80.18)	683 (80.16)	0.002	0.999	0.981 (0.376-2.558)
GA	145 (18.78)	160 (18.78)			
AA	8 (1.04)	9 (1.06)			
GA+AA	153 (19.82)	169 (19.48)	0.000	0.993	0.999 (0.782-1.275)
rs1800630					
C	1226 (79.40)	1394 (81.81)	3.000	0.083	0.947 (0.762-1.179)
A	318 (20.60)	310 (18.19)			
CC	507 (65.67)	571 (67.02)	10.357	0.006	2.058 (1.289-3.287)
CA	212 (27.46)	252 (29.58)			
AA	53 (6.87)	29 (3.40)			
CA+AA	265 (34.33)	281 (32.98)	0.328	0.567	1.062 (0.864-1.305)
IL-1B					
rs1143627					
T	786 (50.91)	869 (51.00)	0.003	0.959	1.017 (0.804-1.288)
C	758 (49.09)	835 (49.00)			
TT	200 (25.91)	223 (26.17)	0.022	0.989	1.007 (0.765-1.326)
TC	386 (50.00)	423 (49.65)			
CC	186 (24.09)	206 (24.18)			
TC+CC	572 (74.09)	629 (73.83)	0.015	0.903	1.014 (0.812-1.266)
TGFβ1					
rs1800469					
C	966 (62.56)	1084 (63.62)	0.384	0.536	1.083 (0.877-1.337)
T	578 (37.44)	620 (36.38)			
CC	303 (39.25)	350 (41.08)	0.574	0.750	1.067 (0.789-1.444)
CT	360 (46.63)	384 (45.07)			
TT	109 (14.12)	118 (13.85)			
CT+TT	469 (60.75)	502 (58.92)	0.565	0.452	1.079 (0.885-1.316)

investigated (16). In 156 men, the -863C/A polymorphism was associated with the serum TNF-α concentration, and carriers of the rare A allele had a significantly lower TNF-α level (P<0.05). This report indicated that the -863C/A polymorphism in the promoter region of the TNF-α gene influenced TNF-α expression and suggested that it may be a useful genetic marker for resolving the issue of whether or not a causal relationship exists between TNF-α and human disease. This conclusion further confirms the function of the TNF-α -863C/A polymorphism. It has also been shown that the A allele at the -863 locus of the promoter region of the TNF-α gene predicts lower HBcAg-inducible TNF-α secretion. It is also associated with chronicity of HBV infection (17). In this study, polymorphisms in the TNF-α (-1031T/C, -863C/A, -857C/T, -308G/A and -238G/A) gene were evaluated in 274 chronic HBV-infected patients and 194 patients with resolved HBV infection. The peripheral blood mononuclear cells (PBMCs), which were isolated from 77 (28%) of the 274 chronic HBV-infected patients with negative HBeAg and positive antibody to HBeAg, were stimulated by HBcAg. The A allele in the -863 promoter region of the TNF-α gene was present in 154 (56.2%) chronic HBV-infected patients and 87 (44.8%) patients who recovered from HBV infection (OR=1.58, P<0.01). The TNF-α -863 A allele genotype predicted lower TNF-α production by PBMCs after *in vitro* HBcAg stimulation (P<0.02). Another study assessed the role of the TNF-α polymorphism on clearance of HBV and outcome of HBV-related chronic hepatitis (18). This investigation included 150 chronic HBV patients, 100 resolved hepatitis B and 150 healthy individuals with a similar ethnic background. Upon stratification of chronic HBV patients into patients without HCC and with HCC, the -863A allele was found to be significantly increased in the HCC group

Table III. Stratified analyses for history of alcohol drinking.

History of alcohol drinking	Gene	Genotype	Cases (n)	Controls (n)	P-value	OR (95% CI)
No	rs1800629	GG	383	586	0.594	1.081 (0.812-1.438)
		GA/AA	101	143		
Yes		GG	254	10	0.466	0.822 (0.485-1.392)
		GA/AA	58	2		
No	rs1800630	CC	335	481	0.240	0.863 (0.674-1.104)
		CA/AA	149	248		
Yes		CC	172	90	0.010	1.839 (1.157-2.923)
		CA/AA	116	33		
No	rs1143627	TT	121	197	0.433	1.111 (0.854-1.445)
		TC/CC	363	532		
Yes		TT	79	26	0.182	0.709 (0.428-1.174)
		TC/CC	209	97		
		AC/CC	208	91		
No	rs1800469	CC	202	299	0.803	0.971 (0.769-1.226)
		CT/TT	282	430		
Yes		CC	101	51	0.219	1.311 (0.851-2.022)
		CT/TT	187	72		

compared to the healthy controls (P_c=0.003, OR=2.61, 95% CI 1.49-4.60). Haplotype analysis (-863/-308/-238) revealed that the homozygosity of the haplotype (CGG/CGG) was a protective marker for HCC (OR= 0.37, 95% CI 0.17-0.79, P_c=0.02). Thus, the author proposed that carriers of the -863A genotype were associated with increased levels of TNF-α in the liver in response to HBV infection and hepatocyte damage was induced that finally led to HCC development. A meta-analysis was performed to examine the association between *TNF-α* promoter polymorphisms (-1031T/C, -863C/A, -857C/T, -308G/A and -238G/A) and chronic hepatitis B infection (19). Twelve studies were chosen in this meta-analysis, involving 2,754 chronic HBV infection cases and 1,630 HBV clearance cases. The data showed that the *TNF-α* -863CC genotype was significantly associated with HBV clearance (-863CC vs. AA: OR=0.64, 95% CI 0.42-0.97, P=0.04). The data revealed that polymorphism -863A in the *TNF-α* gene promoter region may be a risk factor for HBV persistence.

Our study showed that the variant -863AA genotypes were associated with a significantly increased risk of HCC (OR=2.058, 95% CI 1.289-3.287) compared with the wild-type -863 CC. Our results corroborated the above previous studies, and we proposed that carrying the -863AA genotype may reduce the excretion of TNF-α, which may influence the ability of immune clearance, thus increasing the risk of HCC.

There are three limitations to our study: the lack of measurement of local TNF-α expression levels, the small number of subjects and the scope of investigation confined to a hospital-based population. Therefore, the result of our study may not be representative for the target population, and our findings may not be generalizable to the general population. However, potential confounding factors may be minimized by matching the controls to the cases according to age and residential areas and by adjusting for some potential confounders in the final data analyses. Nevertheless, our findings need to be interpreted with caution, and the associations need to be replicated in larger, preferably population-based studies.

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