Role of *IL-10* polymorphisms in susceptibility to hepatitis B virus-related hepatocellular carcinoma

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ABSTRACT. We conducted a case-control study to investigate the role of three common single nucleotide polymorphisms of *IL-10* (-592G/A, -819T/C, and -1082A/C) in the development of hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC). The study included 173 HBV-related HCC patients and 182 healthy controls. A polymerase chain reaction-restriction fragment length polymorphism assay was applied to assess the sequence variants of interest. Compared with control subjects, HCC patients were more likely to be older (t = 1.94, P = 0.03), have a family history of cancer (chi square = 17.86, P < 0.001), and exhibit higher alanine transaminase (t = 13.32, P < 0.001) and aspartate transaminase (t = 12.63, P < 0.001) levels. Using unconditional logistic regression analyses, we found that the GG genotype of -592G/A was associated with increased risk of HCC [odds ratio (OR) = 2.20, 95% confidence interval (CI) = 1.12-4.38], compared to the AA genotype. Under a dominant model, the AG+GG genotype correlated with HBV-related HCC susceptibility compared to the AA genotype, with an OR (95%CI) of 1.56 (1.02-2.48). Moreover, a recessive model showed the GG genotype to be associated with elevated risk of HCC compared to the

AA+AG genotype (OR = 1.85, 95%CI = 1.01-3.47). However, no significant association between the -819T/C and -1082A/C variants and development of HBV-related HCC was observed under codominant, dominant, and recessive models. We conclude that the *IL-10* -592G/A polymorphism does play a role in susceptibility to HBV-related HCC under codominant, dominant, and recessive models.

Key words: *IL-10*; Polymorphism; Hepatitis B virus; Hepatocellular carcinoma

INTRODUCTION

Hepatocellular carcinoma (HCC) counts among the most important health problems worldwide, especially in developing countries (Ferlay et al., 2013). The etiology of HCC is poorly understood, and its development involves many complex environmental and lifestyle aspects (Ma et al., 2014). Hepatitis B virus (HBV) is reported to be an important influence in the development of HCC (Yang et al., 2008). However, the morbidity of HCC varies substantially between similarly HBV exposed subjects, suggesting that genetic factors may contribute to its development. Indeed, previous studies have indicated that many genetic aspects can affect HCC susceptibility (Zhao et al., 2014; Labib et al., 2015; Liu et al., 2015; Sheu et al., 2015; Zhang et al., 2015a; Zhao et al., 2015; Zheng et al., 2015).

Interleukin-10 (IL-10) is a pleiotropic cytokine, and a wealth of evidence supports its regulatory role in carcinogenesis and tumor growth (Tanikawa et al., 2012; Holan et al., 2014). Observational studies have reported high serum levels of IL-10 in patients with a variety of solid tumors, including HCC (Rad et al., 2004; Szkaradkiewicz et al., 2010; Kim et al., 2012). It is therefore reasonable to consider the *IL-10* gene a candidate factor in cancer susceptibility. Previous studies have examined associations between *IL-10* polymorphisms and HCC risk, but with inconclusive results (Tseng et al., 2006; Wang et al., 2006; Truelove et al., 2008; Wei et al., 2011; Yu et al., 2013). Therefore, we conducted a case-control study to investigate the role of three common *IL-10* single nucleotide polymorphisms (SNPs; -592G/A, -819T/C, and -1082A/C) in the development of HBV-related HCC.

MATERIAL AND METHODS

Subjects

A case-control design was used in our study, which included 173 HBV-related HCC patients and 182 healthy controls. HCC patients were recruited between December 2012 and September 2014, and diagnoses were confirmed by pathologic findings. All HCC patients were verified as HBV-positive using cytological tests. Control subjects were diagnosed as being free of HBV, any malignant tumors, and other serious liver and kidney diseases.

Demographic and lifestyle data were collected from all HCC patients and control subjects with a self-designed questionnaire. These included gender, age, tobacco and alcohol consumption, and family history of cancer. Clinical information, comprising alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, tumor-node-metastasis (TNM) stage, and Child-Pugh classification were collected from medical records with patients' consent. A signed informed

consent form was obtained from all participants before their inclusion in the study. Furthermore, the Ethical Committee of the Wuhan University School of Basic Medicine approved the study protocols.

DNA extraction and SNP genotyping

A peripheral blood sample (5 mL) was obtained from each patient and control subject, and kept at -20°C until needed. DNA was extracted from these samples using the TIANamp Blood DNA Kit (TIANGEN Biotech, Beijing, China). A polymerase chain reaction (PCR)-restriction fragment length polymorphism assay was applied to assess *IL-10* -592G/A, -819T/C, and -1082A/C sequence variations. The PCR was performed with an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 45 s, and extension at 72°C for 60 s, before a final extension step at 72°C for 10 min. PCR products were visualized after electrophoresis on a 3% agarose gel stained with 0.1% ethidium bromide.

Statistical analysis

All statistical analyses were conducted using SPSS for Windows, Version 16.0 (SPSS Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) was tested by goodness-of-fit chi-square tests for each SNP in the patient and control groups. Unconditional logistic regression analysis was employed to analyze the correlation between *IL-10* -592G/A, -819T/C, and -1082A/C polymorphisms and risk of HCC, with results being expressed as odds ratios (ORs) and their 95% confidence intervals (95%CIs). A P value less than 0.05 was considered to indicate a significant difference.

RESULTS

The characteristics of HBV-related HCC patients and control subjects are reported in Table 1. The average participant age in these groups was 56.32 ± 7.55 and 54.70 ± 8.14 years for patients and controls, respectively. No significant difference was found between patients and controls in terms of gender (chi-square = 0.86, P = 0.35), smoking (chi-square = 1.54, P = 0.22), or drinking habit (chi-square = 1.11, P = 0.29). Compared with the control subjects, HCC patients were more likely to be older (t = 1.94, P = 0.03), have a family history of cancer (chi-square = 17.86, P < 0.001), and exhibit higher ALT (t = 13.32, P < 0.001) and AST (t = 12.63, P < 0.001) levels. Of the 173 patients with HBV-related HCC, 65 (37.57%) were at TNM stage I-II, while 108 (62.43%) were at stage III-IV. With respect to Child-Pugh classification, 42 (24.28%) patients were categorized as class A, 70 (40.46%) class B, and 61 (35.26%) class C.

Genotype frequencies of *IL-10* -592G/A, -819T/C, and -1082A/C polymorphisms are shown in Table 2. A goodness-of-fit chi-square test revealed the genotype distributions of these variants to conform to HWE. Using a chi-square test, we found that the distribution of *IL-10* -592G/A genotypes significantly differed between HBV-related HCC patients and control subjects (chi-square = 6.30, P = 0.04). However, no such difference was observed in regard to -819T/C (chi-square = 1.37, P = 0.51) and -1082A/C (chi-square = 1.29, P = 0.53) genotype distributions.

Table 1. Comparison of demographic and clinical data relating to hepatitis B-related hepatocellular carcinoma patients and control subjects.

Variable	Patients	%	Controls	%	Chi-square test	P value
Age (years)		56.32 ± 7.55		54.70 ± 8.14	1.94	0.03
Gender						
Female	110	63.58	107	58.79		
Male	63	36.42	75	41.21	0.86	0.35
Smoking						
No	108	62.43	125	68.68		
Yes	65	37.57	57	31.32	1.54	0.22
Drinking						
No	95	54.91	110	60.44		
Yes	78	45.09	72	39.56	1.11	0.29
Family history of cancer						
No	136	78.61	171	93.96		
Yes	37	21.39	11	6.04	17.86	< 0.001
ALT (mean ± SD)	55.85 ± 27.43		27.43 ± 8.54		13.32	< 0.001
AST (mean ± SD)	54.71 ± 25.53		29.57 ± 7.83		12.63	< 0.001
TNM stage						
I-II	65	37.57				
III-IV	108	62.43				
Child-Pugh classification						
A	42	24.28				
B	70	40.46				
C	61	35.26				

ALT = alanine aminotransferase; AST = aspartate aminotransferase; SD = standard deviation; TNM = tumor-node-metastasis.

Table 2. Genotype distributions of *IL-10* -592G/A, -819T/C, and -1082A/C gene polymorphisms in hepatitis B-related hepatocellular carcinoma patient and control groups.

Genotype	Patients (N = 173)	%	Controls (N = 182)	%	HWE P value		Chi-square	P value
					Patients	Controls		
-592A/C								
AA	57	32.95	79	43.41				
AG	81	46.82	81	44.50				
GG	35	20.23	22	12.09	0.53	0.86	6.30	0.04
-819T/C								
TT	74	42.77	86	47.25				
TC	77	44.51	78	42.86				
CC	22	12.72	17	9.34	0.78	0.91	1.37	0.51
-1082A/G								
AA	83	47.98	96	52.75				
AC	74	42.77	74	40.66				
CC	16	9.25	12	6.59	0.93	0.65	1.29	0.53

HWE = Hardy-Weinberg equilibrium.

We further analyzed the correlation between the three *IL-10* polymorphisms and susceptibility to HBV-related HCC using unconditional logistic regression analyses. We found that the -592G/A GG genotype was associated with increased risk of HCC (OR = 2.20, 95%CI = 1.12-4.38) compared to the AA genotype (Table 3). Under a dominant model, the AG+GG genotype correlated with susceptibility to HBV-related HCC compared to the AA genotype, with an OR (95%CI) of 1.56 (1.02-2.48). In addition, the use of a recessive model revealed the GG genotype to be associated with elevated risk of HCC compared to the AA+AG genotype (OR = 1.85, 95%CI = 1.01-3.47). However, we did not observe a significant association between *IL-10* -819T/C and -1082A/C polymorphisms and development of HBV-related HCC under codominant, dominant, or recessive models.

Table 3. Association between *IL-10* -592G/A, -819T/C, and -1082A/C gene polymorphisms and hepatitis B-related hepatocellular carcinoma risk.

Polymorphism	Patients (N = 173)	%	Controls (N = 182)	%	OR (95%CI)	P value
-592A/C						
Codominant						
AA	57	32.95	79	43.41	Reference	
AG	81	46.82	81	44.5	1.39 (0.85-2.25)	0.16
GG	35	20.23	22	12.09	2.20 (1.12-4.38)	0.01
Dominant						
AA	57	32.95	79	43.41	Reference	
AG+GG	116	67.05	103	56.59	1.56 (1.02-2.48)	0.04
Recessive						
AA+AG	138	79.77	160	87.91	Reference	
GG	35	20.23	22	12.09	1.85 (1.01-3.47)	0.03
-819T/C						
Codominant						
TT	74	42.77	86	47.25	Reference	
TC	77	44.51	78	42.86	1.15 (0.72-1.83)	0.54
CC	22	12.72	17	9.34	1.50 (0.70-3.26)	0.25
Dominant						
TT	74	42.77	86	47.25	Reference	
TC+CC	99	57.23	95	52.2	1.21 (0.78-1.88)	0.37
Recessive						
TT+TC	151	87.28	164	90.11	Reference	
CC	22	12.72	17	9.34	1.41 (0.68-2.93)	0.32
-1082A/G						
Codominant						
AA	83	47.98	96	52.75	Reference	
AC	74	42.77	74	40.66	1.16 (0.73-1.83)	0.51
CC	16	9.25	12	6.59	1.54 (0.64-3.79)	0.29
Dominant						
AA	83	47.98	96	52.75	Reference	
AC+CC	90	52.02	86	47.25	1.21 (0.78-1.88)	0.37
Recessive						
AA+AC	157	90.75	170	93.41	Reference	
CC	16	9.25	12	6.59	1.44 (0.62-3.45)	0.35

OR = odds ratio; CI = confidence interval.

DISCUSSION

In this hospital-based case-control study, we investigated the role of three important polymorphisms in the *IL-10* gene, -592G/A, -819T/C, and -1082A/C, in the risk of HBV-related HCC. We found the *IL-10* -592G/A variant to be associated with increased risk of this disease. Moreover, this significant relationship persisted even after adjusting for confounding variables.

The candidate gene approach is increasingly being adopted to distinguish susceptibility genes that may trigger the initiation and progression of various types of cancer. One such gene possibly associated with HCC is *IL-10*. Previous studies have reported associations between polymorphisms of this gene and the development of cancers, as well as several other diseases, including acute pancreatitis, coronary artery disease, and colorectal, gastric, oral, lung, esophageal, and breast cancers (Miteva et al., 2014; Qi et al., 2014; Hsu et al., 2015; Jia et al., 2015; Kumar et al., 2015; Vinod et al., 2015; Xu and Liu, 2015; Yang and Fa, 2015; Zhang et al., 2015b). For instance, Miteva et al. (2014) reported that the *IL-10* -1082A/G polymorphism has a significant impact on the development of colorectal cancer. Qi et al. (2014) conducted a meta-analysis of 12 studies, finding that the -592G/A variant correlates with increased risk of gastric cancer among Asians, while Hsu et al. (2015) reported that Taiwanese carriers of the -819T/C CT genotype have a 3.32-fold increased risk of oral cancer. Moreover, Zhang et al. (2015b) observed that the *IL-*

10 -592G/A and -819T/C polymorphisms are closely connected to lung cancer susceptibility in a Chinese population. However, an investigation conducted in an Indian population found that only the -819T/C SNP appears to play a role in the development of gastric cancer (Kumar et al., 2015). Yang and Fa (2015) indicated that *IL-10* -1082A/G constitutes a candidate biomarker for the prediction of susceptibility to esophageal cancer, while Vinod et al. (2015) suggested that the AA genotype of this SNP correlates with breast cancer oncogenesis in the South Indian population.

Several previous studies have examined the association between *IL-10* gene polymorphisms and development of HCC (Ben-Ari et al., 2003; Heneghan et al., 2003; Tseng et al., 2006; Truelove et al., 2008; Qiu et al., 2011; Wei et al., 2011). Tseng et al. (2006) demonstrated that the -592G/A variant affects HCC risk, while Qiu et al. (2011) reported that certain genotypes of the -819 and -592 SNPs increase and decrease susceptibility, respectively. Furthermore, Truelove et al. (2008) suggested that *IL-10* gene sequence variations influence HBV infection outcome, although Ben-Ari et al. (2003) reported that the polymorphisms investigated in the current study are not associated with development of chronic HBV infection and related diseases. Finally, Heneghan et al. (2003) conducted a study among the Hong Kong Chinese population, finding that *IL-10* polymorphisms do not contribute to HCC susceptibility. In our analysis, the *IL-10* -592G/A polymorphism was seen to be associated with increased risk of HBV-related HCC, but no such relationship was established in regard to the -819T/C and -1082A/C variants.

We conclude that the *IL-10* -592G/A gene polymorphism does play a role in susceptibility to HBV-related HCC under codominant, dominant, and recessive models. Future studies using larger sample sizes and employing either similar or different analytic strategies may help to elucidate the impact of *IL-10* gene polymorphisms on risk of HBV-related HCC.

Conflicts of interest

The authors declare no conflict of interest.

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