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Original Article

Association of interleukin-6 polymorphisms with susceptibility to hepatocellular carcinoma

Xiaohuan Zheng[1], Cuiping Han[1], Rong Shan[1,2], Haitao Zhang[1], Zhaomin Zheng[1], Yuanshui Liu[1], Aiguang Wang[1]

[1]*Department of Oncology, Qianfoshan Hospital Affiliated to Shandong University, Jinan, Shandong, China;* [2]*Department of Function, Jinan Infectious Disease Hospital, Jinan, Shandong, China*

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Abstract: Target: Our study was to investigate the effects of interleukin-6 (*IL-6*) polymorphisms (rs2069837 and rs17147230) on the risk for hepatocellular carcinoma (HCC). Methods: A total of 226 HCC cases and 220 healthy controls were admitted into the study and genomic DNA was extracted from the peripheral blood. The genotyping was conducted by the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Odds ratio (OR) and 95% confidence interval (CI) were used to evaluate the relationship of *IL-6* rs2069837 and rs17147230 polymorphisms with HCC susceptibility. Results: The frequency of GG genotype of rs2069837 was higher in HCC patients, compared with controls ($P < 0.05$). Moreover, the results indicated that GG genotype was related with increased risk for HCC (OR = 2.303, 95% CI = 1.056-5.025). Similarly, the risk for G allele carriers was higher than that of A allele (OR = 1.392, 95% CI = 1.046-1.852). For rs17147230, TT genotype showed strong effect on HCC susceptibility (OR = 2.089, 95% CI = 1.135-3.845) and T allele appeared to be a risk factor for HCC (OR = 1.326, 95% CI = 1.010-1.740). Further analysis showed that G-T haplotype was associated with increased risk for HCC (OR = 3.125, 95% CI = 1.845-5.294, $P = 0.000$). Conclusion: *IL-6* rs2069837 as well as rs17147230 were associated with HCC susceptibility. In addition, G-T haplotype also served as a genetic-susceptibility factor for HCC.

Keywords: Interleukin-6, hepatocellular carcinoma, polymorphisms

Introduction

Primary liver cancer (PLC) commonly originates from hepatic parenchymal cells or intrahepatic biliary epithelial cells, which includes hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (ICC) and the mixed type of both. In most cases, PLC refers to HCC, because the majority of liver cancers occur in hepatocytes [1-3].

Prevalent in global scale, the incidence of HCC ranks the sixth among all the malignant tumors with about 620,000 new cases every year. In addition, ranking only second to lung cancer and gastric cancer, HCC becomes the third cause of death with the death toll of 600,000 annually. More seriously, up to 55% of new HCC cases (more than 300,000 cases) worldwide occur in China and over 350,000 cases die of HCC each year, which severely threatens human health and life [4, 5]. Multiple factors may be involved in the occurrence of HCC, such as virus infection including hepatitis b virus and hepatitis c virus, hepatic cirrhosis, aflatoxin, smoking, drinking and genes [6]. It has been demonstrated that genetic factor could affect individual susceptibility to HCC.

Interleukin-6 (*IL-6*), a multi-functional cytokine, has been intensively studied in recent years. *IL-6* gene locates on short arm of chromosome 7 and consists of 5 exons and 4 introns [7]. Up-regulation level of *IL-6* has been observed in serum of HCC patients, which suggested that *IL-6* might be related with the risk of HCC [8-11].

Moreover, studies have showed that the polymorphisms in promoter region of *IL-6* involved in the pathogenesis of several diseases [12-14]. However, there were few studies about the relationship of *IL-6* rs2069837 and rs17147230 polymorphisms with HCC risk. So we adopted the case-control design and explored the cor-

Table 1. Primer sequence of rs2069837 and rs17147230

Primer	Forward/Reverse	Primer sequence
rs2069837	Forward	5'-CTTCCTGCT GGAACATTCTATGGC-3'
	Reverse	5'-TTTCTGCCAGTGCCTCTTTGC-3'
rs17147230	Forward	5'-AAAAGGGCAAGGAAGGGAGGTA-3'
	Reverse	5'-CACGA GTCATTTGAGCCATCTTTG-3'

Table 2. Genotype and allele distribution of *IL-6* rs2069837 and rs17147230

Genotype/allele	Case (%)	Control (%)	χ^2	*P*	OR (95% CI)
rs2069837					
AA	92/40.7	111/50.5	-	-	1.00
AG	113/50	98/44.5	2.806	0.094	1.391 (0.945-2.048)
GG	21/9.3	11/5	4.565	0.033	2.303 (1.056-5.025)
A	297/65.7	320/72.7	-	-	1.00
G	155/34.3	120/27.3	5.151	0.023	1.392 (1.046-1.852)
rs17147230					
AA	80/35.4	90/44.5	-	-	1.00
AT	107/47.3	109/49.6	0.234	0.629	1.104 (0.739-1.651)
TT	39/17.3	21/5.9	5.717	0.017	2.089 (1.135-3.845)
A	267/59.1	289/65.7	-	-	1.00
T	185/40.9	151/34.3	4.150	0.042	1.326 (1.010-1.740)

relation of rs2069837 and rs17147230 polymorphisms and HCC susceptibility.

Materials and methods

Study objects

The age of 226 HCC patients including 140 men and 86 women ranged from 41 to 70, and the mean age was 57.6 ± 7.7. All the patients were confirmed with histopathological examination in our Hospital. Meantime, 220 unrelated healthy people aged from 32 to 76 (mean age 55.8 ± 7.2) were enrolled as controls, with normal blood routine, no tumors and hereditary diseases.

Genomic DNA extraction and genotyping

3 ml of fasting venous blood extracted from the subjects were shaken up in the EDTA anticoagulant tube and temporarily stored at -20°C in the fridge. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was adopted to detect rs2069837 and rs17147230 polymorphisms.

The primer sequence was shown in **Table 1**. PCR reaction mixture included ddH_2O 40.5 μl, 10× PCR Buffer (containing 1.5 mmol/L Mg^{2+}) 5 μl, 10 mmol/L dNTP 1 μl, Taq enzyme 0.5 μl (5 u/μL), forward primer 1 μl (20 μ mol/L), reverse primer 1 μl (20 μ mol/L), DNA template 1 μl (500 mg). The PCR reaction was conducted according to the following conditions: initial denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 40 s, annealing at 61.2°C for 40 s, annealing at 61°C for 40 s and extension at 72°C for 40 s; finally extension at 72°C for 10 min. 3 μL of PCR amplification products was detected by agarose gel electrophoresis.

The PCR products were mixed with 10× Buffer 0.5 μL, Mbi I restriction enzyme (Fermentas, Burlington, Canada) 0.5 μL and ultrapure water 4 μL. Then the digestion mixture was processed under 37°C for 6 h. Finally, the digestion products were observed and photographed with MKodak gel imaging analysis system. For genotyping analysis, we determined AA, AG and GG genotypes within rs2069837, and AA, AT and TT genotypes within rs17147230.

Statistical analysis

Statistical data were analyzed with SPSS18.0 software. Odds ratio (OR) with 95% confidence interval (95% CI), calculated by Chi-square test was applied to estimate the association of *IL-6* polymorphisms and HCC susceptibility. The significance level was set at $P < 0.05$.

Results

Correlation analysis

For rs2069837, the frequency of GG genotype in case group significantly differed from that of control group (9.3% vs. 5%, $P < 0.05$). The results showed that individuals with GG genotype suffered from higher risk of HCC, compared with AA genotype (OR = 2.303, 95% CI =

Table 3. LD and haplotypes of rs2069837 and rs17147230

Haplotype Locus	Case (n)	Control (n)	χ^2	*P*	OR (95% CI)
A-A	175	191	-	-	1.00
A-T	122	129	0.037	0.847	1.032 (0.748-1.424)
G-A	92	98	0.018	0.892	1.025 (0.721-1.455)
G-T	63	22	19.149	0.000	3.125 (1.845-5.294)
Total	452 (100.0)	440 (100.0)			

1.056-5.025, *P* = 0.033). Similarly, the G allele was also related with increased risk for HCC (OR = 1.392, 95% CI = 1.046-1.852, *P* = 0.023). For rs17147230, compared with AA genotype, the risk was increased by 2.541 folds in TT carriers (OR = 2.089, 95% CI = 1.135-3.845, *P* = 0.017). In the study, we also found that frequency of T allele was significantly higher in cases than controls (OR = 1.326, 95% CI = 1.010-1.740, *P* = 0.042) (**Table 2**).

Linkage disequilibrium test and haplotype analysis

With Haploview online software, we figured out the linkage disequilibrium (LD) coefficient of rs2069837 and rs17147230 (D' = 0.89, r^2 = 0.16), suggesting there was significant LD between the two polymorphisms, so four haplotypes (A-A, A-T, G-A, G-T) were constituted. As shown in **Table 3**, frequencies of G-T haplotype differed between groups with statistical significance (*P* < 0.05). Moreover, G-T haplotype was associated with increased risk for HCC (OR = 3.125, 95% CI = 1.845-5.294, *P* = 0.000). In contrast, no statistically significant results were found in A-A, A-T and G-A haplotypes (*P* > 0.05).

Discussion

IL-6 is a cytokine with complex biological functions in vivo and plays a vital role in immune response, acute phase response and hematopoiesis regulation, thus it is considered as a core member in cytokine family [15]. Recent studies have found disorder of *IL-6* expression was associated with some diseases, such as systemic lupus erythematosus, rheumatoid, multiple myeloma, and diabetes [16, 17]. Furthermore, *IL-6* exerts influence on occurrence and development of various tumors though interfering with adhesion and mobility of cells, thrombosis, expression of tumor specific antigens and proliferation of tumor cells [11, 18]. A large number of cells can synthesize and secrete *IL-6*, including activated lymphocytes, mononuclear macrophages, bone marrow cells as well as a few tumor cells. Besides, *IL-6* has been reported to regulate proliferation and differentiation of cells, immune defense system and hemopoiesis [19, 20].

The occurrence of HCC involves extremely complicated pathogenesis and its occurrence, development and metastasis are closely correlated with genetic mutations, cell signal pathways and abnormal proliferation of new vessels [21]. *IL-6*, also called hepatocyte stimulating factor, B cell stimulating factor or B cell differentiation factor, has been identified to have a vital part in regulating biological effects of hepatocytes [22]. As a hepatocyte stimulating factor, *IL-6* is able to induce the synthesis of acute phase response proteins in acute inflammatory reactions caused by infections or injuries [18, 23].

The present study explored the association of rs2069837 and rs17147230 polymorphisms within *IL-6* gene with HCC susceptibility and concluded that rs2069837 and rs17147230 polymorphisms both were related with increased risk for HCC. Further analysis indicated that G-T haplotype was also a genetic-susceptibility factor for HCC.

In summary, *IL-6* gene rs2069837 and rs17147230 polymorphisms were associated with HCC susceptibility. Due to the complicated pathogenesis of HCC, the definite mechanism though which *IL-6* influences susceptibility to HCC is still unknown. Given this, more large-scale and rigorous researches are necessary to investigate the issue.

Disclosure of conflict of interest

None.

Address correspondence to: Aiguang Wang, Department of Oncology, Qianfoshan Hospital Affili-

ated to Shandong University, NO. 16766 Jingshi Road, Jinan 250014, Shandong, China. E-mail: wag1974@126.com

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