Int J Clin Exp Pathol 2016;9(2):2330-2334
www.ijcep.com /ISSN:1936-2625/IJCEP0011177

Original Article

Correlation between *BIRC5* -625G/C polymorphism and hepatocellular carcinoma

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Received June 8, 2015; Accepted July 21, 2015; Epub February 1, 2016; Published February 15, 2016

Abstract: Aim: This study aimed to discuss the association between *BIRC5* -625G/C polymorphism and hepatocellular carcinoma (HCC) susceptibility in south of China. Methods: We detected *BIRC5* -625G/C polymorphism in 106 HCC patients and 124 healthy persons in southern China and genotypes were separated with the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The correlation between *BIRC5* -625G/C polymorphism and HCC risk was evaluated by odds ratio (OR) with 95% confidence interval (CI) calculated using the chi-squared text. The genotypes distribution of *BIRC5* -625G/C in controls was checked based on Hardy-Weinberg equilibrium (HWE). Results: According to the data, we found that distributions of genotype CC and allele C in case group were higher than the control group, and the *BIRC5* -625G/C polymorphism could increase the HCC risk (CC vs. GG: OR=3.059, 95% CI=1.090-8.583; C vs. G: OR=1.547, 95% CI=1.025-2.335). HBsAg was associated with HCC risk and might make woke through the interaction with patients *BIRC5* -625G/C polymorphism (*P*=0.028). Conclusion: *BIRC5* -625G/C polymorphism might be an independent risk factor for the development of HCC and HBsAg is also associated with HCC.

Keywords: Hepatocellular carcinoma, *BIRC5*, polymorphism

Introduction

Hepatocellular carcinoma (HCC) has highly occurrence and mortality rate in south China [1]. It is rapidly multiplying and exceeds the speed of its blood supply, which leads to regional oxygen deficient and intratumoral hypoxia [2]. As we all known, the development of HCC is affected by genetic and environmental factors. Some previous studies have showed that environmental risk factors controlled HCC include chronic hepatitis b virus (HBV), hepatitis c virus (HCV) infection, alcohol consumption, hormone, AFB1 in food [3-5]. However, exposed to the same factors, such as AFB1 pollution in water and food, only a small part of people suffered from liver cancer. So genetic factors play vital roles in the development and progression of HCC, especially single nucleotide polymorphisms (SNPs). Recently, Studies find that apoptosis inhibiting gene may be associated with the risk of cancer [6].

BIRC5 is a newly-found member in inhibitor of apoptosis protein (IAP) family in recent years with the dual functions of inhibiting cell apoptosis and regulating cell proliferation [7-9]. Expression of *BIRC5* occurs embryonic tissues and developmental fetal normally, but not mature differentiated tissues [10]. Many reports showed that *BIRC5* recovered high expression in most tumor tissues, however, the expression was not checked in the corresponding paracarcinoma tissue [11-13]. So *BIRC5* plays an important role in the occurrence of cancers. What's more, SNPs in promoter region of *BIRC5* gene may influence the expression and function of *BIRC5* and then regulate the susceptibility to cancers. For example, *BIRC5* gene promoter polymorphisms -31G/C and -644C/T were identified that were associated with non-small cell lung cancer [14].

Recently, *BIRC5* -625G/C polymorphism has been found to influence the expression of *BIRC5* [8] However, so far, there is no report

about the association of *BIRC5* promoter -625G/C polymorphism with HCC risk in Chinese population. Therefore, we investigated the relationship between the *BIRC5* -625G/C polymorphism and the risk of HCC in Han population in southern China, which will help screen high-risk group with HCC and assess the risk of HCC in individuals and finally fulfill the early prevention, early diagnosis and early treatment of the disease.

Materials and methods

Study subjects

A population-based case-control design was perform in present study. We selected 106 cases with HCC diagnosed by histology from Bethune International Peace Hospital and 124 healthy adults as controls with normal liver functions, blood glucose and blood lipid concentrations in blood specimens. The controls frequency-matched with the cases by sex, age, ethnicity and region and had no statistical significance ($P>0.05$). The age gap should be less than 5 years in case and control groups. This research was supported by the Research Ethics Committee of six provinces in south of China and sample collecting was conducted according to the guidelines of the national human genome research ethics. Written consents were obtained from all subjects without related by blood each other.

DNA extraction and PCR primer design

2 ml venous blood was collected from each individual in control and case group, anti-freezing with EDTA-Na2. Next we extracted genome DNA by the saturation of phenol extraction-anhydrous ethanol precipitation. We chose SNPs of *BIRC5* gene based on references in database of http://hapmap.ncbi.nlm.nih.gov. We set the conditions as MAF ≥0.05, Han Chinese, and the data were from HapMap Data 24/phase II Nov08, Rel on NCBI B36 assembly, dbSNP b126.

This study determined *BIRC5* -625G/C as the research object. Primers were designed according to primer 5.0 software. The primer sequences: forward: 5'-GTTCATTTGTGCCTTCATGCGC-3'; reverse: 5'-GGCAGAGGGTGCAGTGAGC-3'. The design of primers was synthesized by TaKaRa Biology Co., Ltd. Amplified fragment length was 164 bp.

Genotying of BIRC5 -625G/C

Genotying of *BIRC5* -625G/C was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. PCR reaction system was 25 μl, which included genome DNA 1.5 μl, forward primer 0.5 μl, reverse primers 0.5 μl, 12.5 μl PCR Master Mix, sterile deionized water as a complement. PCR amplification was done under the following conditions: 94°C pre-degeneration for 4 min, 35 cycles with 94°C degeneration for 30 s, 60°C annealing for 30 s, and 72°C extension for 30 s and finally extension at 72°C for 10 min. The PCR amplified products were then digested with restriction endonuclease *BstUI* and were detected by 2% agarose gel electrophoresis and the results were analyzed by gel imaging and GelDocXR + BIO-RAD analysis system.

Statistical data

Hardy-Weinberg equilibrium (HWE) was tested by χ^2 test in the control group. Odds ratio (OR) with corresponding 95% confidence interval (CI) was used to assess the relevance strength between *BIRC5* -625G/C polymorphism and HCC. Frequencies of genotypes and alleles in each group were calculated by direct counting and tested by χ^2 test. 1) Formula used to calculate genotype frequency: Genotype frequency = number of genotype/total number of individuals; 2) Formula used to calculate allele frequency: G/C allele frequency = (2× the number of GG/CC genotype + the number of GC genotype)/(2× total number of individuals). Data analysis was conducted by SPSS 18.0 software and $P>0.05$ was statistically significant.

Results

HWE test

The genotypes frequencies of *BIRC5* -625G/C polymorphism in controls conformed to HWE. So our subjects in the control group had a good representativeness.

Statistics of genotype and allele frequency in BIRC5 -625G/C polymorphism

The result of the relevance between *BIRC5* -625G/C and HCC susceptibility was shown in **Table 1**. CC genotype frequency was higher in cases compared with the control group and

Table 1. Distributions of allele and genotype in *BIRC5* -625G/C polymorphism

Genotype/Allele	Cases (N, %)	Controls (N, %)	χ^2	OR (95% CI)	*P* value
-625G/C					
GG	51 (48.11)	72 (58.06)	-	1.000 (Ref.)	-
GC	42 (39.62)	46 (37.10)	0.817	1.289 (0.743-2.237)	0.366
CC	13 (12.27)	6 (4.84)	4.831	3.059 (1.090-8.583)	0.028
G	144 (67.92)	190 (76.61)	-	1.000 (Ref.)	-
C	68 (32.08)	58 (23.39)	4.338	1.547 (1.025-2.335)	0.037

Table 2. Relationship between *BIRC5* -625G/C polymorphism and clinical characteristics of HCC

Index		Cases (N, %)	Genotype (N, %)			χ^2	*P* value
			GG	GC	CC		
Clinical staging	I-II	63 (59.43)	25 (39.68)	30 (47.62)	8 (12.70)	0.982	0.612
	III-IV	43 (40.57)	17 (39.53)	23 (53.49)	3 (6.98)		
Differentiation degree	Well	59 (55.66)	25 (42.37)	26 (44.07)	8 (13.56)	0.934	0.627
	Poor	47 (44.34)	16 (34.04)	25 (53.19)	6 (12.77)		
Metastasis	Yes	62 (58.49)	24 (38.71)	31 (50.00)	7 (11.29)	0.496	0.780
	No	44 (41.51)	19 (43.18)	19 (43.18)	6 (13.64)		
HBsAg	Positive	59 (55.66)	17 (28.81)	29 (49.16)	13 (22.03)	7.163	0.028
	Negative	47 (44.34)	23 (48.94)	21 (44.68)	3 (6.38)		

was associated with the increased risk of HCC (OR=3.059, 95% CI=1.090-8.583). Allele C was 1.547 times higher in cases than controls and it might be also a risk factor for the occurrence of HCC (OR=1.547, 95% CI=1.025-2.335).

Relationship between BIRC5 -625G/C polymorphism and clinical characteristics of HCC

According to stratified analyses by clinical staging, degree of differentiation, metastasis and the status of HBsAg, we found that the genotype distribution in cases with positive HBsAg was more common than negative HBsAg (*P*=0.028). And there was no significant correlation between -625G/C polymorphism and clinical staging, degree of differentiation and metastasis (*P*>0.05). The data are shown in **Table 2**.

Discussion

Liver cancer is a kind of malignant tumor with the sixth incidence and the third mortality in the world. About 82% of death cases take place in developing countries and 55% of them are in China. Chronic HBV infection is the most important risk factor of HCC associated with 50% of HCC cases and HCV is also a major etiology of HCC [15, 16]. The occurrence of HCC is a complex biological process caused by both environmental and genetic factors [17]. The genetic background can determine the risk of HCC in a large extent. Therefore, the study of genetic susceptibility to HCC has gained extensive attention in recent years.

BIRC5 is a newly discovered IAP, which encodes the protein including 142 amino acids [18, 19]. Compared with the other members of IAP family, the structure of BIRC5 protein is unique. N-terminal domain only contains a structure domain of baculovirusIAP repeat (BIR), and there is an α helix structure instead of zinc finger structure on the C end [20]. The core mechanism of apoptosis is that Caspase cascade activates and dissolves proteins. BIRC5 exerts antiapoptotic by interfering with activation of Caspase-9 promotor on upstream of endogenous apoptosis signaling pathway and directly inhibiting endogenesis/exogenous apoptosis signaling pathways downstream of common factor Caspase-3, Caspase-7 [21].

Lots of researches have showed that increased expression level of *BIRC5* is found in majority of human malignant tumor tissues and *BIRC5* is also associated with poor prognosis, tumor progression and drug resistance [22-26]. Abnormal transcription of *BIRC5* gene is the primary

mechanism of high expression of *BIRC5* in tumor tissues. *BIRC5* -625G/C polymorphism is in the cell cycle dependent element (CDE) of promoter region/cell cycle homology region (CRH) and affects the genetic susceptibility to cancer by changing the CDE/CRH regulatory elements, strengthening the combination of repressor protein and CDE/CRH components and reducing the expression of *BIRC5* [27]. There is no report about *BIRC5* -625G/C polymorphism and tumor susceptibility.

In present study, we evaluated the effect of *BIRC5* -625G/C polymorphism on HCC risk. The result demonstrated that both CC genotype and C allele were associated with the development and occurrence of HCC and it might be a risk factor for populations in south of China. However, there was no significant correlation between *BIRC5* -625G/C polymorphism and clinical staging, degree of differentiation and metastasis of HCC, but HBsAg chronic infection factor was exception. Because of few reports on the association of *BIRC5* -625G/C polymorphism and HCC are published in the other populations, so the real connection remains to be further studied in different populations.

Studies have found that single mutation genotype may not obviously contribute to cancer risk, but the risk greatly increases if carriers with mutant gene are exposed to environmental risk factors. This study showed that there existed the interaction between genetic polymorphisms and HBsAg chronic infection and the risk of individuals with HBsAg chronic infection and mutant genotype CC of *BIRC5* -625G/C suffered from HCC was up to 15.5 times higher than those with CC genotype but not infected with chronic HBsAg. The result of interaction analysis was very important for primary prevention from liver cancer. Therefore, based on gene-environmental interactions, we may take corresponding measures to control environmental risk factors for susceptibility populations to HCC. Further study should be conducted with well-design and enough large sample size in the future to verify the relevance and provide effective methods for the diagnosis and treatment of HCC.

Disclosure of conflict of interest

None.

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