Original Article

SNP rs2240688 in *CD133* gene on susceptibility and clinicopathological features of hepatocellular carcinoma

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Background: CD133 is one of the important cancer stem cells (CSCs) markers of hepatocellular carcinoma (HCC). The aim of this study was to explore the relationship between CD133 single-nucleotide polymorphisms (SNPs) and risk factors associated with HCC susceptibility and clinicopathological features in HCC cases and healthy controls from the Guangxi region of southern China.

Methods: A case control study was conducted, including 565 HCC patients and 561 control subjects. The genotyping of rs2240688 was performed using the SNaPshot method. Unconditional logistic regression was used to correct for gender, age, and other confounding factors. Odds ratio (OR) and its 95% confidence interval (CI) were calculated to analyze the relationship between allele and genotype frequency and the risk of HCC.

Results: The distribution frequencies of CD133 alleles and genotypes in the HCC case group and the control group were statistically significant (P<0.05). The CA heterozygous (P=0.003, OR =1.463, 95% CI: 1.134–1.887) and CC homozygous genotypes (P=0.036, OR =1.910, 95% CI: 1.044–3.493), as well as C carrier status (P=0.004, OR =1.465, 95% CI: 1.136–1.889) and C alleles (P=0.004, OR =1.465, 95% CI: 1.136–1.889), were associated with an increased risk of HCC. Additionally, in the subgroup analysis of CD133 rs2240688 polymorphism and clinical characteristics, the results showed that the genotype distribution of CD133 rs2240688 was significantly different in genotype distribution of metastasis and alanine aminotransferase (ALT).

Conclusions: the expression of miRNA binding site rs2240688 of tumor stem cell marker gene *CD133* in HCC may be a promising marker for the prediction of HCC, but larger studies are still needed.

Keywords: Hepatocellular carcinoma (HCC); single-nucleotide polymorphisms (SNPs); cancer stem cells (CSCs); CD133; rs2240688

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Introduction

Primary liver cancer has the fifth highest incidence rate of malignant tumors worldwide, and is the second leading cause of male mortality (1). The estimated annual global incidence of primary liver cancer is 841,000, and the number of deaths is estimated to be 782,000 (1). In China, liver cancer has the second highest mortality rate of malignant tumors, and new liver cancer cases account for more than 50% of the world's total, increasing year by year (2). There are several risk factors for liver cancer, including viral infection, heredity, aflatoxin contamination,

carcinogen exposure, non-fatty alcoholic hepatitis, and various single-nucleotide polymorphisms (SNPs) (1-3). Despite the advancements in technology and improvements in treatments including surgery, radiotherapy, chemotherapy, and the use of other biological agents, the prognosis of hepatocellular carcinoma (HCC) is poor due to recurrence and metastasis, with a 5-year disease-free survival rate of 16% to 27.1% (4).

Several studies have demonstrated that cancer stem cell (CSCs) subgroups, whose functions are responsible for tumor persistence and recurrence, metastasis, drug resistance, and radiation tolerance, may drive tumorigenesis (5,6). CD133 (prominin-1) is a 5-transmembrane glycoprotein expressed on a subset of hematopoietic stem cells derived from fetal liver and bone marrow. CD133 is considered to be a CSC marker for a variety of cancer types, including HCC (7,8), colon cancer (9), gastric cancer (10), and ovarian cancer (11). It is associated with higher colony formation efficiency, a greater proliferation rate, and higher tumor incidence (12). Studies have found that HCC patients with elevated CD133 levels have a lower overall survival rate and higher recurrence rates than patients with lower CD133 expression levels. Although there are several studies on certain susceptibility genes for HCC (13-15), studies on CD133 SNPs in the context of HCC susceptibility and clinical features are still lacking. Furthermore, polymorphisms in the *CD133* gene have been associated with a variety of human diseases (16-18). Given the limited number of studies examining CD133 polymorphisms in HCC, we investigated the association between SNP rs2240688 and the demographics, clinical features, and prognosis of HCC in a Chinese population.

Methods

Study population

Subjects for this case control study were recruited from the Affiliated Tumor Hospital of Guangxi Medical University between September 2016 to December 2018. All participants received a relevant questionnaire in order to collect information on the history of environmental exposure after signing written informed consent. The demographic data collected included medical record number, gender, age, drinking status, smoking status, histological tumor type, tumor-node-metastasis stage, related biochemical indicators, and other information. In order to avoid selection bias, inclusion criteria, such as age and gender, were matched between the control group and the case group. The control group was recruited continuously from December 2018 to February 2019 from the physical examination center of the First Affiliated Hospital of Guangxi Medical University. Meanwhile, the control group comprised healthy subjects who had good daily life function, and no heart disease, cerebrovascular disease, infectious disease, autoimmune disease, abnormal physical examination indexes, or a personal or family history of cancer. Written informed consent was provided by all subjects in the study. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of the Affiliated Tumor Hospital of Guangxi Medical University (approval ID: LW2020007).

DNA extraction and genotyping assays

Blood samples (2 mL) from each subject were collected and placed in an EDTA-K2 anticoagulant tube, thoroughly mixed, and stored at –20 ℃. Genomic DNA was isolated using a commercial kit (Adelaide, Beijing, China) according to the manufacturer's instructions. The genotyping of CD133 rs2240688 was performed using the SNaPshot method (19), and, in order to ensure the accuracy of genotype evaluation, a negative control was used for each test. The forward primer sequence for CD133 rs2240688 polymorphism was 5'-CTCATGTTAGCTGCACTCCAAT-3', and the reverse primer sequence for CD133 rs2240688 polymorphism was 5'-ACCATTGACTTCTTGGTGCTG-3' (328 bp).

Statistical analysis

In order to confirm the representativeness of the population of study samples, a Chi-square test was used to determine whether the samples conformed to the Hardy-Weinberg Equilibrium (HWE) law. When the P value >0.05, the samples were considered to be representative of the population. Two independent sample Chi-square tests were used to test the difference between the two groups. Differences in genotype and allele frequency between the HCC and control groups were assessed using a Chi-square test with Bonferroni correction. Chi-square testing and logistic regression analyses were used to compare the distribution data of alleles and genotypes, and the relative risk was expressed as an odds ratio (OR) and its 95% confidence interval (CI). The logistic regression method

was applied to correct for the effects of confounding factors such as gender and age. All statistical tests were performed using SPSS 24.0 (SPSS Inc., Chicago, IL, USA), and were two-sided, with a P value <0.05 considered to be statistically significant.

Results

Baseline characteristics of the study population

Initially, 593 subjects were enrolled in the HCC group, and 561 subjects were enrolled in the control group. There were 21 cases without pathological reports and 7 cases with liver metastases records, which were excluded from the HCC group. Ultimately, 565 HCC patients and 561 controls were included in this study, and their clinical parameters are presented in *Table 1*. We analyzed the demographic characteristics of the subjects and found that the mean age, gender, and body mass index (BMI) classification of the two groups of patients were matched. The average age of patients with HCC was 53.62 years, ranging from 10–89 years. Similarly, the average age of the control group was 52.15 years, ranging from 22–78 years. Interestingly, the majority of patients were male (86.19%). After statistical analysis, the age and gender of HCC patients were not significantly different from those of the control group (P>0.05).

CD133 rs2240688 polymorphism and HCC risk

The genotype frequency of CD133 rs2240688 was consistent with the Hardy–Weinberg equilibrium law, indicating that the samples selected in this study were representative of the population of interest. The most frequently distributed allele in the controls and recruited HCC patients was AA heterozygous. The genotype frequencies of the CD133 rs2240688 locus in the HCC group were 333 (58.9%) for AA, 202 (35.7%) for CA, and 30 (5.3%) for CC. Similarly, the genotype frequency distribution of this locus in the control group was 384 (68.4%) for AA, 159 (28.3%) for CA, and 18 (3.2%) for CC. The frequency distribution of the AA, CA, and CC genotypes between the two groups was statistically significant (P<0.001). In the overall analysis, multiple comparisons using a Chi-square test with Bonferroni correction found that the distribution of the AA genotype was different from that of the CA and CC genotype, and the distribution of the A allele also differed from that of the C genotype (P=0.0167). We then used the AA genotype and A allele as a reference to analyze the risk of HCC. For comparing genotypes and alleles of HCC susceptibility, the logistic regression model of the two categorical variables was used to correct for the influence of confounding factors such as gender and age, and the OR value and 95% CI of rs2240688 on the risk of liver cancer were calculated. Individuals carrying the rs2240688 CA + CC genotype had a 1.508-fold higher risk of developing HCC than individuals carrying the AA genotype (P<0.001, OR =1.910, 95% CI: 1.181–1.926). Similarly, individuals carrying the homozygous CC genotype were 1.910 times more likely to develop HCC than individuals carrying the AA genotype (P=0.036, OR =1.910, 95% CI: 1.044–3.493). Individuals carrying the heterozygous CA genotype were 1.463 times more likely to develop HCC than individuals carrying the AA genotype (P=0.003, OR =1.463, 95% CI: 1.134–1.887). In addition, individuals carrying the C allele were at 1.442 times greater risk of HCC than those carrying the A allele (P<0.001, OR =1.442, 95% CI: 1.772–1.774), indicating that the C allele mutation was associated with an increased risk of HCC. The detailed results are summarized in *Table 2*.

We further investigated whether there was a difference in the distribution of the rs2240688 genotype between the clinical subgroups (*Table 3*). Results showed that the genotype distribution of CD133 rs2240688 was significantly associated with metastasis (P=0.008). However, the results also showed that the genotype distribution of CD133 rs2240688 was not significantly associated with factors such as age, gender, alcohol consumption, and smoking status (P>0.05).

We also analyzed common pathological markers of HCC that are routinely tested for, including alpha-fetoprotein (AFP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyl transpeptidase (GGT). Results showed that the genotype distribution of CD133 rs2240688 was significantly associated with ALT (P=0.023).

Discussion

Recent research has suggested that CSCs contribute to tumor initiation, metastasis, relapse, and resistance to chemotherapy or radiotherapy (20). SNPs represent the largest proportion of genetic variation in the human genome, and their contribution to cancer susceptibility has been extensively explored (21,22). The *CD133*-encoding gene is located on human chromosome 4p15, a region

Table 1 General characteristics of HCC patients and the normal controls

Characteristics	Cases (n=565)	Controls (n=561)	χ^2	P value
Age (year)				
Range	10–89	22–78		
Mean	53.62	52.15		
<40	95	102	0.365	0.546
>40	470	459		
Gender				
Male	487	488	0.562	0.453
Female	78	73		
BMI (kg/m^2)				
≤18.5	62	51	1.805	0.406
18.5–23.9	366	359		
≥24	137	151		
BCLC stage				
A + B stage	259			
C + D stage	306			
Metastasis				
No	470			
Yes	95			
Smoking status				
No	344			
Yes	221			
Alcohol drinker				
No	374			
Yes	191			
Family history of cancer				
No	487			
Yes	78			
Liver cirrhosis				
Absent	172			
Present	393			
HBV infection				
HbsAg (–)	57			
HbsAg (+)	497			
HCV infection	11			

HCC, hepatocellular carcinoma; BMI, body mass index; BCLC, Barcelona clinic liver cancer; HBV, hepatitis B virus; HCV, hepatitis C virus.

Table 2 Comparison of genotype and allele distributions of CD133 rs2240688 in HCC group and controls group

Parameter	Case, n (%)	Controls, n (%)	OR (95% CI)	P_{OR}	OR_{adj} (95% CI)	P_{adj}
CD133 rs2240688						
All						
AA	333 (58.9)	384 (68.4)	1.00		1.00	
CA	202 (35.7)	159 (28.3)	1.465 (1.136–1.889)	0.004	1.463 (1.134–1.887)	0.003
CC	30 (5.3)	18 (3.2)	1.922 (1.052–3.511)	0.034	1.910 (1.044–3.493)	0.036
CA + CC	232 (41.0)	177 (31.5)	1.511 (1.180–1.924)	0.001	1.508 (1.181–1.926)	0.001
Alleles						
A	868 (76.8)	927 (82.7)	1.00		1.00	
C	262 (23.2)	195 (17.3)	1.443 (1.173–1.775)	0.001	1.442 (1.772–1.774)	0.001

HCC, hepatocellular carcinoma; OR, odds ratio; CI, confidence interval.

considered closely related to cancer susceptibility (18,23,24). CD133 is considered an important marker molecule for tumor cells (17,25,26), and O'Brien *et al.* demonstrated that $CD133^+$ tumor cells have stem cell characteristics (27). A growing number of studies have also demonstrated that CD133 is highly expressed in CSCs of pancreatic ductal adenocarcinoma (PDAC), glioma, colon cancer, gastric cancer, malignant melanoma, non-small cell lung cancer, and other tumors, which suggests that CD133 may play a multifaceted role in tumor development (9,28-32). The prognostic and clinicopathological value of CD133 protein and mRNA expression have also been demonstrated in other studies (33-35). For example, in HCC, subjects with greater CD133 mRNA levels also showed greater invasiveness than subjects with lower CD133 mRNA levels (36). Although it is widely believed that CD133 plays an important role in cancer, the relationship between CD133 polymorphisms and the clinical features of HCC are noticeably lacking. Therefore, in this case control study, we investigated the association of the CD133 SNP rs2240688 with the patient demographics, clinical features, and susceptibility to HCC.

We found that the variant genotypes (AC/CC) of rs2240688 A>C in the miRNA binding site of the stem cell marker gene *CD133* were associated with a higher susceptibility to HCC. The distribution frequency of rs2240688 alleles and genotypes in the HCC case group and control group was statistically significant, which is consistent with the results of Liu *et al.* (37) in lung cancer and Wang *et al.* (38) in gastric cancer. We found that the CA heterozygous and CC homozygous genotypes, along with C carrier status and C alleles, were associated with an increased risk of HCC. This may be attributed to the fact that CD133 expression is closely related to cell proliferation, apoptosis, invasion and metastasis, and angiogenesis (39-42). Furthermore, it has been shown that SNPs located in the 3'untranslated region (3'-UTR) region of the *CD133* gene are associated with a variety of human tumors (38,43,44). SNPs in the 3'-UTR have also been shown to have functional effects on the control of mRNA stability and efficiency through the regulation of miRNA, including miR-34a, -101, -128, -137 and -1385 (45-47). It has been shown that SNPs in a target-binding site can alter the miRNA-mRNA interaction and thus affect the expression of miRNA targets (48,49). Additionally, studies have confirmed that rs2240688 A-to-C transition gains a new binding site of the microRNA has-miR-135a/b, which may play a pivotal role in modulating the effect of the SNP on CD133 expression (38). Interestingly, rs2240688 is located at the 3'-UTR region of the *CD133* gene. SNP rs2240688 has been associated with an increased risk of HCC, consistent with the corresponding role of CD133 in promoting the development of liver cancer through other signaling pathways such as G protein-coupled receptor 87 and CXCL3 (50,51). Additionally, in the subgroup analysis of CD133 rs2240688 and clinical characteristics, our results showed that the genotype distribution of CD133 rs2240688 was significantly associated with metastasis and ALT. Considering the promotional capability of CSCs on tumor growth and metastasis, the present study suggests that CD133 might modify the metastasis competence of HCC via miRNA binding site polymorphisms, which could be a putative target for improved HCC treatment.

Table 3 Association of CD133 rs2240688 genotype with clinical characteristics in HCC patients

Characteristics	rs2240688				rs2240688			rs2240688		
	AA	CA	CC	P value	CA	CC	P value	AA	CA + CC	P value
Age (year)										
Range	19–87	10–89	35–76		10–89	35–76		19–87	10-89	
Mean	52.3	52.7	55.6		52.7	55.6		52.3	53.04	
Gender										
Female	48	27	3		27	3		48	30	
Male	285	175	27	0.778	175	27	0.608	285	202	0.615
BCLC stage										
A + B stage	183	105	18		105	18		183	123	
C + D stage	150	97	12	0.643	97	12	0.412	150	109	0.649
Smoking status										
No	202	121	20		121	20		202	141	
Yes	131	81	10	0.778	81	10	0.479	131	91	0.978
Alcohol drinker										
No	222	128	24		128	24		222	152	
Yes	111	74	6	0.191	74	6	0.074	111	80	0.776
Metastasis										
No	272	181	17		181	17		272	198	
Yes	71	21	3	0.008	21	3	0.545	71	24	0.002
Family history of cancer										
No	288	175	23		175	23		288	198	
Yes	45	25	7	0.271	25	7	0.110	45	32	0.892
Liver cirrhosis										
Absent	101	62	8		62	8		101	70	
Present	232	140	22	0.904	140	22	0.654	232	162	0.968
HBV infection										
HbsAg (–)	28	21	6		21	6		28	27	
HbsAg (+)	297	178	24	0.128	178	24	0.135	297	202	0.218
HBV infection	8	3	0		3	0		8		
AST										
Negative	178	97	18		97	18		178	115	
Positive	155	105	12	0.312	105	12	0.221	155	117	0.363
ALT										
Negative	211	110	23		110	23		211	133	
Positive	122	92	7	0.023	92	7	0.022	122	99	0.148
GGT										
Negative	116	71	14		71	14		116	85	
Positive	217	131	16	0.426	131	16	0.222	217	147	0.660
AFP										
Negative	135	78	13		139	13		135	91	
Positive	198	124	17	0.843	124	17	0.323	198	141	0.753

HCC, hepatocellular carcinoma; BCLC, Barcelona clinic liver cancer; HBV, hepatitis B virus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase; AFP, alpha-fetoprotein.

In summary, this study was the first to explore the relationship between rs2240688 and the risk of HCC. We found that rs2240688 was associated with an increased risk of HCC and may play an important role in tumor progression, thus providing a basis for the search for novel therapeutic targets. Due to the small sample size of this study, and the inability to obtain more accurate data from the control group, the applicability of these results may be limited. Therefore, future studies investigating more CD133 SNPs, with larger sample sizes and more clinical information, are needed to determine the relationship between CD133 polymorphisms and the risk of developing HCC.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tcr-19-2690). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). This study was approved by the ethics committee of the Affiliated Tumor Hospital of Guangxi Medical University (approval ID: LW2020007). Written informed consent was provided by all subjects in the study.

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