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ORIGINAL ARTICLE

Correlation between the DEPDC5 rs1012068 polymorphism and the risk of HBV-related hepatocellular carcinoma

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KEYWORDS
DEPDC5;
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Summary
Introduction: HBV and/or HCV infection is the main cause of hepatocellular carcinoma (HCC), but the molecular mechanisms by which HBV promotes HCC are not clear. In 2011, the result of a GWAS revealed a common variant of DEPDC5 affected HCC susceptibility in patient with chronic HCV infection in Japan. This study investigated the correlation between DEPDC5 polymorphism and HBV-related HCC.
Materials and methods: 1289 samples of Han population were involved in northern China and peripheral blood samples were obtained, including 506 healthy controls, 217 Hepatitis B chronic (CHB) and 258 liver cirrhosis (LC), and 308 HBV-related HCC patients. SNPs in the DEPDC5 rs1012068 were detected by MALDI-TOF-MS.
Results: After controlling for the influence of sex, smoking and drinking, this study showed a significant relationship between the polymorphism of DEPDC5 rs1012068 and HBV-related HCC. Healthy participants with CC genotype showed 2.008 (95% CI = 1.145, 3.520; $P = 0.015$) times more likely to develop HCC; CHB cases with CC genotype showed 2.241 (95% CI = 1.226, 4.461; $P = 0.022$) times more likely to develop HCC; LC cases with CC genotype showed 2.706 (95% CI = 1.371, 5.340; $P = 0.004$) times more likely to develop HCC; and individuals with AC genotype showed 1.615 (95% CI = 1.110, 2.352; $P = 0.012$) times more likely to develop HCC.
Conclusions: There was a significant correlation between DEPDC5 rs1012068A/C and HBV-related HCC in the Han Chinese population. A to C mutation increased the risk of the developing of HBV-related HCC.

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Introduction

As one of the common cancers, Hepatocellular carcinoma ranked the third leading cause of cancer death world-wide [1]. There are about 750,000 new diagnoses of HCC annually all over the world, and China accounts for nearly a half [2—4]. Previous studies on the etiology of HCC have suggested that the pathogenesis of this disease may have relation to various factors, and among these factors, HBV and/or HCV infection is the major reason [5], and HBV-related liver disease or HCC caused more than 0.5 million deaths annually [6]. In addition, liver cirrhosis, smoking, alcohol drinking and other factors also contribute to the incidence of HCC. However, only a small fraction of the people with these factors exposure developed HCC, which likely owning to individual differences in sensitivity to cancer [7]. The pathogenesis of HBV inducing the development of HCC is a crucial research subject that has attracted more and more attention. Grounded on the current understanding of the molecular mechanisms of cancer, single nucleotide polymorphisms (SNPs) was regarded to be an important factor in tumor development.

In 2011, a GWAS reported a common variant in the Dishevelled Egl-10 and Pleckstrin domain-containing 5 (DEPDC5) loci on chromosome 22, which affected the susceptibility of chronic HCV infection to HCC in Japan [8]. Until now, no studies have reported that DEPDC5 is associated with HCC, but It has been reported the absense of DEPDC area in malignant brain glioblastomas [9]. Although the function of DEPDC5 is still unclear [10], notably, DEPDC1, containing a DEP domain similar to DEPDC5, has been revealed to affect the development of bladder carcinogenesis [11,12]. However, we have not fully understood whether DEPDC5 polymorphisms could affect the development HBV-related HCC in Chinese populations.

In this study, we carried out a case-control study to probe the association of DEPDC5 rs1012068 polymorphisms with HBV-related HCC. The study of hereditary factors may provide us a better comprehending of the molecular biology mechanisms of HCC, and supply important information for the research and development of new therapies for HBV-related HCC.

Materials and methods

Subjects

A total of 1,289 samples involved in the Han population in northern China were enrolled from the First and Second Affiliated Hospital of Hebei Medical University and the Fifth Hospital of Shijiazhuang in China from January 2010 to January 2012, including 506 healthy controls, 217 Hepatitis B chronic (CHB) and 258 liver cirrhosis (LC), and 308 HBV-related HCC patients. All subjects were non-related and ethnically Han Chinese individuals who had been residents in Hebei for six months or longer.

Healthy participants had to meet following criteria: Negative for hepatitis B surface antigen (HBsAg), hepatitis B e-antibody (anti-HBe), antibody to hepatitis B virus core antigen (anti-HBc) and other HBV biomarkers. Based on the inclusion and exclusion criteria [13—16], patients of CHB, LC and HBV-related HCC were selected. In addition, patients were excluded if they had positive laboratory tests for HIV or/and HCV or alcoholic liver disease. A sample of 2 mL of venous blood was collected from each individual. All subjects signed the consent form and the study was approved by the ethics committee of Hebei Medical University (No. 2016009).

Data collection

Complete clinical information of all subjects were collected from a unified questionnaire and medical records. including general information (sex, age, history of smoking and drinking, etc.), historical disease background (medical history and course of disease, other medical history, family medical history), and the results of medical records (quantity of HBV-DNA, concentrations of ALT, AST, etc.).

DNA extraction and genotyping

Genomic DNA was extracted from venous blood samples by a genomic DNA purification kit purchased from Promega (Promega Biotech Co.Ltd., USA). SNP Genotyping was executed using MassARRAY® Assay system (Sequenom Inc., San Diego, CA, USA) by MALDI-TOF MS. Additional, the Hardy-Weinberg equilibrium of the allele frequencies of SNP genotypes was examined.

Statistical analysis

All statistical analysis was conducted by SPSS 16.0. Continuous variables of normal and abnormal distribution were described as Means $\pm$ SD and M (QR), respectively. Categorical variables are expressed as frequencies. The evaluation of the differences in continuous data between groups was performed by Student's *t*-test or Wilcoxon test. The comparation of the differences in categorical variables between groups was performed by Pearson's Chi^2 test. Multiple logistic regression was used to calculate OR and OR 95% CI, controlling for the effects of gender, smoking and alcohol drinking. A *P*-value of 0.05 was regarded to be significant statistically.

Results

General information of the subjects

All subjects were individed into 4 groups, the general information of them are shown in Table 1. There were significant differences in age, sex, smoking, and drinking when HBV infection occurred. The HCC group had higher mean age than the CHB group significantly (55.96 ± 9.06 vs. 38.25 ± 14.28, $P = 6.74 \times 10^{-4}$). Compared with health controls, CHB and LC group, the HCC group showed a significant higher ratio of male, and a higher ratio of smoking, and drinking (all $P < 0.05$).

Table 1 The general information of study subjects.

Variable	Health control ($n=506$)	CHB ($n=217$)	LC ($n=258$)	HCC ($n=308$)	P-value
Age(years, mean ± SD)	49.31 ± 14.15	38.25 ± 14.28	51.74 ± 11.34	55.96 ± 9.06	< 0.001
Sex(Male/Female)	296/210	136/81	147/111	245/62	< 0.001
Tobacco smoking(Yes/No)	141/365	93/127	84/174	176/132	< 0.001
Alcohol drinking(Yes/No)	170/336	95/122	100/158	191/117	< 0.001
DEPDC5 rs1012068 (AA/CA/CC)	264/211/31	124/79/14	158/85/15	145/130/33	

CHB: chronic hepatitis B; LC: liver cirrhosis; HCC: hepatocellular carcinoma.

Table 2 Genotype and minor allele frequencies of DEPDC5 rs1012068 [n (%)].

Genotype	Health control ($n=506$)	CHB ($n=217$)	LC ($n=258$)	HCC ($n=308$)
AA	264(52.2)	124(57.1)	158(61.2)	145(47.1)
AC	211(41.7)	79(36.4)	85(32.9)	130(42.2)
CC	31(6.1)	14(6.5)	15(5.8)	33(10.7)
MAF (A allele)	0.189	0.767	0.432	0.633

MAF: minor allele frequency.

Table 3 Distribution of DEPDC5 rs1012068 in Non-HCC and HCC groups.

Genotype	Control vs. HCC		CHB vs. HCC		LC vs. HCC	
	P-value[a]	OR (95% CI)	P-value[a]	OR (95% CI)	P-value[a]	OR (95% CI)
AA						
AC	0.450	1.122 (0.833, 1.511)	0.069	1.407 (0.974, 2.034)	0.005	1.667 (1.169, 2.375)
CC	0.013	1.938 (1.140, 3.294)	0.028	2.016 (1.032, 3.937)	0.007	2.397 (1.251, 4.595)

[a] $P \leq 0.05$ was considered to be statistically significant.

Genotype distribution of DEPDC5 rs1012068

A Hardy-Weinberg test was performed for SNP of DEPDC5 rs1012068, all cases complied with the Hardy-Weinberg equilibrium ($P > 0.05$). The results are shown in Tables 2.

Compared with health controls, CHB and LC group, the SNP of DEPDC5 rs1012068 was significantly associated with the development of HBV-related HCC. Patients with HBV-related HCC were taken as case group, the health subjects and CHB groups and LC groups as the control group, respectively, we discovered that the DEPDC5 rs1012068 CC genotypes in HCC group was more than in health subjects (OR = 1.938, $P = 0.013$), and the percentage of DEPDC5 rs1012068 CC genotypes were significantly higher in HCC group than in CHB group (OR = 2.016, $P = 0.038$). Meanwhile, the percentage of DEPDC5 rs1012068 AC and CC genotypes were significantly higher in HCC group than in LC group (OR = 1.667, $P = 0.005$ for AC; OR = 2.397, $P = 0.007$ for CC). The results are shown in Tables 3.

Multivariate analysis for SNP and HCC susceptibility

The SNP of DEPDC5 rs1012068 was revealed to be associated with the development of HCC. After adjusting the effects of sex, drinking, and smoking by multiple logistic regression model, the final result revealed that the variant DEPDC5 rs1012068 genotypes were independent risk factors of HBV-related HCC.

Controlling for the effects of gender, smoking and alcohol drinking, the comparisons between HCC group and healthy control, HCC group and CHB, HCC group and LC group are shown in Tables 4, 5 and 6, respectively. Compared with the wild-type, healthy individuals with the DEPDC5 rs1012068 CC genotype showed 2.008 (95 % CI = 1.145, 3.520; $P = 0.015$) times more likely to develop HBV-related HCC; CHB cases with the DEPDC5 rs1012068 CC genotype showed 2.241 (95 % CI = 1.226, 4.461; $P = 0.022$) times more likely to develop HCC; LC cases with the DEPDC5 rs1012068 CC genotype showed 2.706 (95 % CI = 1.371, 5.340; $P = 0.004$) times more likely to develop HCC; and LC individuals with the AC genotype showed 1.615 (95% CI = 1.110, 2.352; $P = 0.012$) times more likely to develop HCC.

Discussion

In the present study, we determined that the SNP of DEPDC5 rs1012068A/C was correlated with the development of HBV-related HCC significantly, and the A to C mutation significantly increased the risk of developing HBV-related HCC for healthy individuals, CHB and LC patients in the Han Chinese population.

The *DEPDC5* gene was located at 22q12.3, and encoded a cytoplasmic protein. Recently, a research reported that

Table 4 Multivariate analysis[1] for SNP and HCC susceptibility between health control and HCC.

Variable	Coefficient estimate	*SE*	OR (95% CI)	*P*-value
Male	0.323	0.205	1.381 (0.923, 2.065)	0.116
Drink	0.695	0.184	1.964 (1.368, 2.819)	< 0.001
Smoke	0.727	0.192	2.069 (1.420, 3.017)	< 0.001
DEPDC5 rs1012068[a]	0.157	0.162	1.170 (0.851, 1.609)	0.334
DEPDC5 rs1012068	0.697	0.286	2.008 (1.145, 3.520)	0.015

1 Logistic regression model: Hosmer-Lemeshow goodness-of-fit test ($P = 0.014 < 0.05$).
[a] The DEPDC5 rs1012068 SNP has the AA, CA and CC genotypes, whereas the AA genotype is the wild type and was taken as the reference group.

Table 5 Multivariate analysis[1] for SNP and HCC susceptibility between CHB and HCC.

Variable	Coefficient estimate	SE	OR (95% CI)	*P*-value
Male	0.686	0.246	1.986 (1.226, 3.219)	0.005
Drink	0.565	0.256	1.760 (1.065, 2.907)	0.027
Smoke	−0.141	0.270	0.868 (0.512, 1.473)	0.600
DEPDC5 rs1012068[a]	0.334	0.195	1.397 (0.953, 2.049)	0.087
DEPDC5 rs1012068	0.807	0.351	2.241 (1.226, 4.461)	0.022

1 Logistic regression model: Hosmer-Lemeshow goodness-of-fit test ($P = 0.881 > 0.05$).
[a] The DEPDC5 rs1012068 SNP has the AA, CA and CC genotypes, whereas the AA genotype is the wild type and was taken as the reference group.

Table 6 Multivariate analysis[1] for SNP and HCC susceptibility between LC and HCC.

Variable	Coefficient estimate	SE	OR (95% CI)	*P*-value
Male	0.725	0.231	2.065(1.312,3.249)	0.002
Drink	0.369	0.236	1.447(0.911,2.298)	0.118
Smoke	0.412	0.246	1.510(0.932,2.445)	0.094
DEPDC5 rs1012068[a]	0.480	0.192	1.615(1.110,2.352)	0.012
DEPDC5 rs1012068	0.995	0.347	2.706(1.371,5.340)	0.004

1 Logistic regression model: Hosmer-Lemeshow goodness-of-fit test ($P = 0.514 > 0.05$).
[a] The DEPDC5 rs1012068 SNP has the AA, CA and CC genotypes, whereas the AA genotype is the wild type and was taken as the reference group.

DEPDC5 gene played a crucial role in central nervous system epilepsy [17]. A GWAS, which involved 212 chronic HCV carriers with HCC (cases) and 765 chronic HCV carriers without HCC (controls) in Japan, discovered the SNP of DEPDC5 rs1012068 A/C was significantly correlated with HCV-related HCC. The interesting thing is that the more subjects (710 cases of HCV-related HCC and 1625 HCV carriers without HCC) studied, the stronger correlation between the polymorphism of DEPDC5 rs1012068 and HCV-related HCC [8]. Although the virological of HBV and HCV are completely different [18], we discovered the correlation between DEPDC5 rs1012068 and HBV-related HCC. This study analyzed the role of *DEPDC5* gene polymorphisms on susceptibility to HBV-related HCC for the first time.

The mechanisms of DEPDC5 in the development of HCC has not been determined yet. However, It had been demonstrated that DEPDC1 affected the development of bladder carcinogenesis, which contained a DEP domain similar to DEPDC5 [11,12]. A previous study reported that the DEPDC5 deletion mutation was associated with the occurance of malignant brain glioblastomas [9], we suspected that DEPDC5 might have similar effects with DEPDC1. Compared with non-tumor tissues, the relative expression of DEPDC5 mRNA increased in tumor tissues [8]. DEPDC5 was reported to be a multi-functional protein involved in a variety of cellular systems, including inflammation, cell growth, and tumorigenesis, such as hepatocarcinogenesis, by blocking the effects of mammalian target of rapamycin (mTOR) [19—21]. A previous study has revealed that the DEPDC5 variant stimulated fibrosis progression in subjects with chronic HCV infection in Europe, suggested that the down-regulation of DEPDC5 might lead to HCV-related fibrosis by promoting MMP2 synthesis [22]. The present study revealed that the genovariation of *DEPDC5* rs1012068 (A/C) significantly increased the risk of developing HBV-related HCC. We speculated the pathogenic mechanism of HCC might involve in that, the gene mutation of *DEPDC5* rs1012068 stimulated liver fibrosis progression by decreasing the expression of DEPDC5, but the exact pathogenesis needs to be further determined by animal experiments.

The rs1012068 is an intronic variant of DEPDC5 without definite function, but our study revealed rs1012068 was

a significantly susceptibility locus to HBV-related HCC. On the basis of the characteristics of SNP, the region where rs1012068 is located is a susceptible region. Thus, in the functional region of the coding genes or other non-coding regulatory genes (such as lncRNA and miRNA, etc.) within 5-100 KB within 5-100 KB around rs1012068, other functional SNP loci or rare mutations are likely to exist, which are associated with HBV-related HCC. Consequently, we will explore the functional SNP or rare mutation around DEDPC5 rs1012068 by second-generation sequencing, and investigate the remote regulatory function of rs1012068, e.g., regulation of some miRNA or lncRNA by eQTL analysis. Nevertheless, this study identified the susceptible regions for us and provided evidence for subsequent functional mutation excavation.

From this study, we have understood that the polymorphism locus within DEPDC5 rs1012068 was correlated with the progress of HBV-related HCC. Due to the allele frequency of rs1012068 varies among different nationalities, it is unclear whether the associations is established in other ethnic groups, even among individual population within Asia. Therefore, these findings are significant for global comparisons of different races.

Disclosure of interest

The authors declare that they have no competing interest.

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