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The influence of caveolin-1 gene polymorphisms on hepatitis B virus-related hepatocellular carcinoma susceptibility in Chinese Han population

A case-control study

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Abstract

This study aimed to explore the genetic association of polymorphisms in caveolin-1 gene (*CAV1*) with hepatitis B virus-related hepatocellular carcinoma (HBV-related HCC) susceptibility in a Chinese Han population.

The genotyping of polymorphism was conducted using polymerase chain reaction-restriction fragment length polymorphism method. Whether the genotype distribution of polymorphisms in the healthy controls was consistent with Hardy–Weinberg equilibrium (HWE) was detected. The genotype and allele frequency difference between the 2 groups was compared by chi-square test. Odds ratio (OR) and 95% confidence interval (95% CI) were calculated to show the relative risk of HCC which resulted from genetic variants in *CAV1*. Moreover, the linkage disequilibrium of *CAV1* polymorphisms was analyzed by Haploview.

The AG genotype and A allele of rs1049334 showed significantly higher frequency in HCC patients than that of chronic HBV patients and the healthy controls ($P < .05$); so their carriage obviously increased the susceptibility to HBV-related HCC, irrespective of the fact whether individuals were infected with hepatitis B virus or not (AG vs GG: OR 1.958, 95% CI 1.050–3.650, OR 1.899, 95% CI 1.034–3.487; A vs G: OR 1.667, 95% CI 1.033–2.689, OR 1.777, 95% CI 1.103–2.863). Additionally, A-G haplotype of rs3807989-rs1049334 showed the protective role for HBV-related HCC (OR 0.102, 95% CI 0.035–0.293; OR 0.135, 95% CI 0.046–0.395).

CAV1 rs1049334 polymorphism is significantly associated with the occurrence risk of HBV-related HCC, and the interaction of polymorphisms should not be neglected.

Abbreviations: 95% CI = 95% confidence interval, AFP = α-fetoprotein, AGE = agarose gel electrophoresis, CAV1 = caveolin-1, ELISA = enzyme-linked immunosorbent assay, eNOS = endothelial nitric oxide synthase, HBsAg = hepatitis B surface antigen, HBV = hepatitis B virus, HCC = hepatocellular carcinoma, HCV = hepatitis C virus, HWE = Hardy–Weinberg equilibrium, MRI = magnetic resonance imaging, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SNPs = single-nucleotide polymorphisms.

Keywords: caveolin-1, haplotype, hepatitis B virus, hepatocellular carcinoma, polymorphism

1. Introduction

Hepatocellular carcinoma (HCC) is a common malignant tumor in clinic, representing a leading cause of cancer-related deaths.[1] Epidemiology data find that 9.2% of people die of liver cancer in the world and about 85% of primary liver cancer cases are HCC.[2,3] The occurrence of HCC is a complex multiple-factor and multiple-step process, and the known influence factors include chronic hepatitis virus infection (HBV and HCV), aflatoxin B1 intake and alcohol abuse, and so on.[4–7] In China, HBV infection is a major cause for HCC.[8,9] However, multiple epidemiological studies show that not all the HBV-infected patients finally developed HCC, and the onset of HCC emerges from obvious familial aggregation and genetic predisposition, suggesting the individual differences in HCC susceptibility.

Caveolins are the major structural constitution of caveolae and participate in various cellular biological processes, including endocytosis, cholesterol transport, transmembrane signal transduction, and virus infections.[10] Alterations in caveolae and caveolins may contribute to human diseases, such as coronary heart disease, nervous system disorders, and cancers.[11,12] Caveolin-1 (CAV1) is an important member of caveolins and is mainly expressed in adipocytes, endotheliocytes, and fibroblasts of various tissues. It plays an important role in cell proliferation, differentiation, migration, and apoptosis, and its abnormal expression may be involved in initiation, progression, and metastasis of tumors.[13] Altered expression of CAV1 is observed in several malignancies, like breast cancer, colorectal cancer, renal cell carcinoma, and so on.[14–16] Certainly, the similar result is also obtained in HCC. The study carried out by

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Liu et al[17] reported that overexpression of CAV1, as an oncogene, could promote tumor growth and metastasis in HCC.

Recently, single-nucleotide polymorphisms (SNPs) in gene have widely attracted attentions because of huge numbers and stable heredity. They are associated with the individual susceptibility of diseases. Multiple SNPs in *CAV1* gene have been identified.[18]*CAV1* polymorphisms have been reported to be associated with several human cancers, including prostate cancer, breast cancer, and gastric cancer.[19–21] Rs3807989 and rs1049334 are 2 common polymorphisms in *CAV1* gene, and both of them are reported to be significantly associated with expression of CAV1.[16,22] Based on the related investigations, we speculated that *CAV1* rs3807989 and rs1049334 polymorphisms might be associated with HCC via controlling its gene expression profile. However, the relevant researches have been rarely reported in Chinese Han population.

In this study, we explored the association of *CAV1* rs3807989 and rs1049334 SNPs with the occurrence risk of HBV-related HCC in a Chinese Han population.

2. Materials and methods

2.1. Participants

In all, 338 subjects were recruited from Linyi Central Hospital from March 2014 to May 2016, including 225 chronic HBV patients and 113 healthy controls. HBV patients were divided into 2 groups: 118 HCC patients and 107 chronic HBV patients. HCC patients were all diagnosed by histopathology combined with magnetic resonance imaging (MRI) or computed tomography (CT) imaging examination in Oncology Department of the hospital. None of them had revived any treatments before blood collection, such as radiotherapy and chemotherapy, and surgery. Chronic HBV patients were confirmed by laboratory examinations and clinical diagnosis. For healthy controls, they had experienced the physical examination in the same hospital during the investigation period, with normal liver function. These people were not included in the control group who carried HBV, had the family history of HBV, and the history of cancer and immune disease. There were no statistically significant differences among these 3 groups for age and sex. The subjects were all of Chinese Han origin without any blood relationship with each other. This study was reviewed and supported by the Ethics Committee of Linyi Central Hospital. Before collecting blood sample, written consents were obtained from each subject.

The basic clinical characteristics of subjects in the 3 groups were investigated and recorded by trained professional doctors, and the detailed indexes included age, sex, smoking, alcohol consumption, serum α-fetoprotein (AFP), hepatitis B surface antigen (HBsAg), tumor grade, TNM stage, and metastasis. Smokers were defined that people smoked 1 or more cigarettes every day and continued more than half a year. People who drunk 2 or more times every week and kept on drinking for over 6 months were considered as a "drinker." HBV infection was confirmed via serological examination. AFP was examined by radioimmunoassay and HBsAg was tested by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.[23]

2.2. DNA extraction

After fasting for 8 to 10 hours, 2 mL peripheral venous blood was collected from each subject in the early morning and kept in blood collection tube, EDTA antifreezing. Blood leukocyte genemic DNA was extracted by the conventional methods of phenol-chloroform extraction and ethanol precipitation. The concentration of genomic DNA was tested by NanoDrop 2000c.

2.3. The genotyping of CAV1 polymorphisms

In this study, the genotyping of *CAV1* polymorphisms was conducted by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). First, PCR primers were designed by Primer Premier 5.0 software according to *CAV1* gene sequences published in NCBI web site and were synthesized in Sangon Biotech (Sangon, Shanghai). PCR primer sequences were as follows: rs3807989—5′-GCTATCGCTGGCCCTTCTG TGG-3′ (forward), 5′-GCTGTGAGCCGTCTGAGGGAAC-3′ (reverse); rs1049334—5′-ACGCTTTCCTGAATCCAAACTA A-3′ (forward), 5′-CAATGTTGAGCCACTAAACCACC-3′ (reverse). The PCR was performed in a total volume of 25.0 μL and the procedure was as follows: predenaturation at 95°C for 5 minutes, 40 cycles of denaturation at 95°C for 30 seconds, annealing at specific temperature for 30 seconds (60°C for rs3807989 and 56°C for rs1049334), extension at 72°C for 30 seconds, and the final extension at 72°C for 10 minutes. The quality of PCR products was detected by 1.0% agarose gel electrophoresis (AGE). PCR products were digested by restriction enzyme (*Hinf*I) and enzyme-digested products were separated by 2.0% AGE.

2.4. Statistical analysis

The genotype frequency of polymorphism was obtained by direct counting. Whether the genotype distribution of each polymorphism in the healthy controls conformed to Hardy–Weinberg equilibrium (HWE) was checked. The genotype distribution differences of each polymorphism were compared between groups by chi-square test. Odds ratio (OR) with the corresponding 95% confidence interval (95% CI) was calculated to express the relative risk of HCC caused by *CAV1* polymorphisms. The above data processing was completed by PASW Statics 18.0 software. Moreover, the linkage disequilibrium between rs3807989 and rs1049334 was analyzed by Haploview software. $P < .05$ was considered as the statistically significant difference.

3. Results

3.1. The clinical characteristics of subjects

The basic clinical information of subjects is shown in Table 1. The ratio of females and males was 63/55 in HCC patients, which was similar with the ratio in chronic HBV patients (54/53) and healthy controls (51/62) ($P > .05$ for both). Similarly, we could not detect the significant difference among the 3 groups in age (51.23 ± 9.86, 52.88 ± 10.23, and 48.62 ± 8.63; $P > .05$). We also compared percentages of smokers and alcohol abuse in HCC patients with that in chronic HBV patients and healthy controls. There were no significant differences ($P > .05$ for all). HCC patients with ≥400 μg/L AFP reached to about 80%, and two-thirds of HCC patients were in I to II according to pathological grade and TNM stage. In our study population, metastasis was observed in 16.10% of HCC patients.

3.2. The genetic association of CAV1 polymorphisms with HCC susceptibility

The genotype and allele frequencies of *CAV1* rs3807989 and rs1049334 polymorphisms among the 3 groups were displayed

Table 1

The clinical features of subjects in this study.

	HCC patients	The healthy controls	Chronic hepatitis B patients	P_1	P_2	P_3
Sex (n = female, %)	63 (53.39)	51 (45.13)	54 (50.47)	.661	.210	.428
Age, y	51.23 ± 9.86	48.62 ± 8.63	52.88 ± 10.23	.756	.211	.186
Smoking (n, %)	48 (40.68)	34 (30.09)	37 (34.58)	.346	.093	.476
Alcohol abuse (n, %)	56 (47.46)	32 (28.32)	44 (41.12)	.339	.210	.720
AFP, μg/L (n, %)						
<400	27 (22.88)					
≥400	91 (78.12)					
Pathological grade (n, %)						
I–II	87 (73.73)					
III–IV	31 (26.27)					
TNM stage (n, %)						
I–II	83 (70.34)					
III–IV	35 (29.66)					
Metastasis (n, %)	19 (16.10)					

AFP = α-fetoprotein, P_1 = HCC patients versus chronic hepatitis B patients, P_2 = HCC patients versus the healthy controls, P_3 = chronic hepatitis B patients versus the healthy controls.

in Table 2. The GG, AG, and AA genotype frequencies of rs3807989 were 57.63%, 34.74%, and 7.63% in HCC patients; 54.21%, 36.45%, and 9.34% in chronic HBV patients; and 50.44%, 38.94%, and 10.62% in healthy controls, respectively. There was no significant difference among the 3 groups according to our calculation criteria ($P > .05$). So was allele of rs3807989. For rs1049334, we found that the heterozygous AG genotype frequency was significantly higher in HCC patients than that of the healthy controls ($P = .037$) and chronic HBV patients ($P = .033$), which indicated that AG genotype carriers easily suffered from HBV-related HCC, whether individuals were infected with HBV or not (OR 1.958, 95% CI 1.050–3.650; OR 1.899, 95% CI 1.034–3.487). The A allele of rs1049334 was also detected the significant association with HBV-related HCC, no matter whether individuals were infected with HBV or not (OR 1.667, 95% CI 1.033–2.689; OR 1.777, 95% CI 1.103–2.863).

3.3. The haplotype analysis of CAV1 polymorphisms in HCC occurrence

The strong linkage disequilibrium was found between rs3807989 and rs1049334 polymorphisms (D' = 1.0, $r^2 = 0.601$) and 3 haplotypes were found: G-G, A-A, and A-G. The detailed frequencies are listed in Table 3. Compared with G-G haplotype, A-G haplotype showed obviously lower frequency in HCC patients than that in the healthy controls and chronic HBV patients ($P < .001$), indicating that A-G might be a protective factor of HBV-related HCC (OR 0.102, 95% CI 0.035–0.293; OR 0.135, 95% CI 0.046–0.395).

4. Discussion

Hepatocellular carcinoma is 1 of the major tumors leading to human death, and is characterized by high malignant degree, poor prognosis, and easily metastasis, which seriously influences human health. So far, although diagnosis and surgical techniques somewhat improve in clinic, most of HCC patients are still diagnosed in advanced stages with poor prognosis due to

Table 2

The genotype distribution of *CAV1* polymorphisms and the association with HCC.

	HCC patients		Chronic hepatitis B patients					The healthy controls				
	N = 118	%	N = 107	%	P_{HWE}	OR (95% CI)	P_1	N = 113	%	OR (95% CI)	P_2	OR (95% CI)	P_3
rs3807989					.186								
GG	68	57.63	58	54.21		Ref.		57	50.44	Ref.		Ref.	
AG	41	34.74	39	36.45		0.897 (0.512–1.572)	.703	44	38.94	0.781 (0.450–1.357)	.380	0.871 (0.495–1.533)	.632
AA	9	7.63	10	9.34		0.768 (0.292–2.017)	.591	12	10.62	0.629 (0.247–1.598)	.327	0.819 (0.328–2.046)	.669
G	177	75.00	155	72.43		Ref.		158	69.91	Ref.		Ref.	
A	59	25.00	59	27.57		0.876 (0.575–1.333)	.536	68	30.09	0.649 (0.426–0.987)	.042	0.884 (0.585–1.337)	.560
rs1049334					.051								
GG	72	61.02	80	74.77		Ref.		85	75.22	Ref.		Ref.	
AG	37	31.35	21	19.62		1.958 (1.050–3.650)	.033	23	20.35	1.899 (1.034–3.487)	.037	0.970 (0.499–1.888)	.929
AA	9	7.63	6	5.61		1.667 (0.565–4.912)	.350	5	4.43	2.125 (0.681–6.627)	.186	1.275 (0.374–4.342)	.697
G	181	76.69	181	84.58		Ref.		193	85.40	Ref.		Ref.	
A	55	23.31	33	15.42		1.667 (1.033–2.689)	.035	33	14.60	1.777 (1.103–2.863)	.017	1.066 (0.632–1.800)	.810

CI = confidence interval, OR = odds ratio, P_1 = HCC patients versus chronic hepatitis B patients, P_2 = HCC patients versus the healthy controls, P_3 = chronic hepatitis B patients versus the healthy controls.

Table 3

The haplotype analysis among *CAV1* polymorphisms.

rs3807989-rs1049334	HCC patients, %	Chronic hepatitis B patients, %	OR (95% CI)	P_1	The healthy controls, %	OR (95% CI)	P_2	OR (95% CI)	P_3
G-G	177 (75.00)	155 (72.43)	Ref.	—	158 (69.91)	Ref.	—	Ref.	—
A-A	55 (23.31)	33 (15.42)	1.460 (0.901–2.364)	.123	33 (14.60)	1.488 (0.919–2.409)	.105	1.019 (0.599–1.733)	.944
A-G	4 (1.69)	26 (12.15)	0.135 (0.046–0.395)	0	35 (15.49)	0.102 (0.035–0.293)	0	0.757 (0.435–1.317)	.324

CI = confidence interval, OR = odds ratio, P_1 = HCC patients versus chronic hepatitis B patients, P_2 = HCC patients versus the healthy controls, P_3 = chronic hepatitis B patients versus the healthy controls.

metastasis and recurrence.[24] Therefore, the precaution and early diagnosis of HCC remain great challenges for a number of medical scientists all the time. The occurrence of HCC results from a variety of factors, such as inflammation, tumor microenvironment, and oxidative stress, combined with some molecular alterations.[25,26] The activation of oncogenes and inactivation of antioncogenes are the 2 core events in tumor development.[27]

The human *CAV1* gene is located on chromosome 7q31.1–31.2, consisting of 3 exons and 2 introns. The expression profiles of *CAV1* exerted significantly different between cancer and para-carcinoma tissues, cancer patients, and healthy controls. Ning et al[28] reported that the expression of *CAV1* in high-grade osteosarcoma was obviously different compared with the normal controls. The report of Zhang et al[29] showed that the expression level of *CAV1* in the normal gastric tissues was significantly higher than that of gastric cancer tissues, and knockdown of the expression of *CAV1* would decrease E-cadherin expression, alter cell morphology, and enhance the migration of cancer cells. In most of the reported cancer cell lines, *CAV1* acted as an antioncogene, including sarcoma, breast, lung, colon, cervical cancers, and also HCC. In addition, hypoxia-induced expression of *CAV1* was closely correlated to the invasion and metastasis of HCC cells,[30] and *CAV1* could promote hepatoma cells' resistance to anoikis.[31]

With the development of molecular biology, various SNPs in gene are identified to be responsible for individual susceptibility to disease. A number of SNPs are also discovered in *CAV1* which are correlated with various diseases, especially cancers. Rs3807989 is a mutation located in intron 2 of *CAV1* with the replacement of A to G, and the A allele has been reported to be associated with the elevated expression of *CAV1* mRNA and protein.[32] Another common SNP in *CAV1*, rs1049334, is a mutation of G/A located on 3′untranslated region in *CAV1* and it also alters the expression level of *CAV1* mRNA.[16] rs4730751 is also a mutation in intron region of *CAV1* and is considered to alter the interaction of the corresponding gene products, CAV1, and some proteins, such as eNOS, which is frequently activated in caveolae.[33] But rs4730751 is very rare in the Chinese Han population.

In the current study, we investigated the genetic association of *CAV1* rs3807989 and rs1049334 polymorphisms with HBV-related HCC risk in the Chinese Han population. For polymorphisms, we did not find any significant association between rs3807989 polymorphism with the risk of HCC development. However, Hsu reports AA and AG genotypes of rs3807989 carriers show high risk to suffer from HCC, compared with GG genotype carriers,[18] and Zhao et al[34] suggest that A allele of rs3807989 is the protective factor of HCC. This discrepancy may derive from different study populations and ethnicity, inconsistent sample size, and different environmental factors. AG genotype of rs1049334 was significantly associated with the increased risk of HBV-related HCC development, whether individuals were infected with HBV or not. Moreover, the strong linkage disequilibrium between the 2 polymorphisms was found and A-G haplotype was a protective factor for HCC development in the study population.

5. Conclusions

In conclusion, *CAV1* rs1049334 polymorphism is significantly associated with the risk of HBV-related HCC and it may play roles in HCC via regulating the expression level of *CAV1*. The

exact mechanism still needs to be investigated in the next step. In this study, some limitations should be focused on, including relatively small sample size, only 1 population and race, and the interaction of environmental factors. Therefore, further well-designed studies with large sample size and multiple populations in different races are still needed to verify these results; meanwhile environmental factors should be considered in the future.

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