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Original Article

PIK3CA polymorphisms associated with susceptibility to hepatocellular carcinoma

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Abstract: Purpose: Our study was carried out to explore the relationship of *PIK3CA* rs17849071 and rs17849079 polymorphisms with the susceptibility to hepatocellular carcinoma (HCC) in Chinese Han population. Methods: 150 HCC patients and 152 healthy individuals were recruited in the case and control groups respectively. The genotypes of *PIK3CA* rs17849071 and rs17849079 polymorphisms were detected with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The linkage disequilibrium and haplotypes were analyzed with Haploview software. Differences in frequencies of genotypes, alleles, and haplotypes between the case and control groups were checked with χ^2 test. The controls were matched with the cases in age and gender. The relative risk of HCC was represented by odds ratio (OR) and 95% confidence interval (95% CI). Results: Significant difference in frequencies of GG genotype and G allele in *PIK3CA* rs17849071 polymorphism existed between the two groups (*P*=0.040; *P*=0.028), indicating that rs17849071 was closely related to the increased risk of HCC (OR=2.919, 95% CI=1.007-8.460; OR=1.642, 95% CI=1.051-2.564). Furthermore, TT genotype also significantly increased the susceptibility to HCC (OR=3.438, 95% CI=1.050-11.250) and so was T allele (OR=1.521, 95% CI=1.052-2.199). The haplotype analysisshowed that G-T haplotypes were higher in cases than that of controls (*P*=0.030), which suggested that G-T might be a susceptible haplotype to HCC. Conclusions: The *PIK3CA* rs17849071 and rs17849079 polymorphisms may increase the risk of HCC either independently or synergistically.

Keywords: *PIK3CA*, polymorphism, hepatocellular carcinoma, haplotype

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors all over the world. There are about 500,000-600,000 new HCC cases every year and one half of them are in China, of which Guangxi Province is one of the high-incidence areas. HCC patients are often diagnosed in advanced stage due to the lack of effective early diagnosis. With a short disease course and quick development, HCC possesses the peculiarity of hardly treatment and prone to relapse. Until now, there are still no effective therapies for the disease, and the 5 years survival rate is only 3%~5% [1]. In China, the incidence of HCC ranks the fourth place among malignant tumors; and the mortality is second only to lung cancer. The occurrence and development of HCC are related to various genetic and environmental factors, and the disease itself involves multiple factors and stages. However, the specific molecular mechanism of HCC is still not very clear.

Phosphatidylinositol 3-kinase (PI3K) belongs to the lipid kinase family. PI3K-AKT is an important signal pathway for the adjustment of cellular functions, and its functions are closely related to cell proliferation, survival, activity, adhesion and differentiation as well as the restructure of cell framework and the intracellular transportation [2]. *PIK3CA*, PI3K catalytic alpha polypeptide gene, encodes p110α catalytic subunit of PI3K [3]. Current studies have shown that *PIK3CA* gene amplifies and overexpresses in ovarian cancer, and has a high mutation rate (average 25%-30%) in colon, cerebral, breast, gastric, and liver cancers [4-7]. Kang et al. have found that *PIK3CA* is an oncogene related to many tumors [8]. Many recent reports have indicated that the mutations of *PIK3CA* gene commonly occur in thyroid cancer, breast

cancer, lung cancer, gastric cancer, HCC, pancreatic cancer, endometrial cancer, and ovarian cancer [9-17].

PIK3CA gene mutations in different types of tumors show the important role. However, few of the studies at home investigated the genetic changes of *PIK3CA* in HCC. To explore the relationship between the onset of HCC and heredity, we studied the association of two single nucleotide polymorphisms (SNPs) in *PIK3CA* with HCC occurrence from the perspective of genetic polymorphisms.

Materials and methods

Research subjects

In this case-control study, 150 HCC patients (89 males and 61 females) hospitalized in Shandong Provincial Hospital Affiliated to Shandong University during 2010 to 2014 were selected in the case group. Their age was among 37-65 years old with an average age of 53.52±9.86. 152 healthy individuals (95 males and 57 females) aged 35-62 were enrolled in the control group with an average age of 51.69±10.32. The HCC patients confirmed by pathology and underwent no radiotherapy or chemotherapy before operation. The 150 healthy individuals selected from the health examination center of the hospital had normal liver functions, blood sugar concentration, and blood lipid concentration, and had no family history of tumors or such liver diseases as hepatitis and cirrhosis. The individuals in the control group were randomly collected with the same ethnicity, place and duration of residence, and the age difference between two groups was less than five years. Differences in age, gender, ethnicity, and native place between two groups were not significant through statistical test ($P>0.05$). All participants were not related by blood. Our study obtained the approval from the Ethics Committee of Shandong Provincial Hospital affiliated to Shandong University and the written informed consensus from every subject. The sample collection was conducted in accordance with the national ethical principles of human genome research.

DNA extraction and PCR amplification

DNA extraction: HCC tissues surgically removed were preserved at -80°C. Sample DNA was extracted with standard methods strictly according to the instructions of the kit (Beijing Tiangen Biotech Company). The extraction was carried out on ice.

PCR amplification: The following forward and reverse primers of each SNP were synthesized by Shanghai Sangon Biotech Co., Ltd.: rs17849071 primers: forward: GATTGGTTCTTT-CCT-GTCTCTG and reverse: CCACAAATATCA-ATTTACAAC-CATTG; rs17849079 primers: forward: 5'-CCAGAACTACAATCTTTTGATGACA-3' and reverse: 5'-CCAGAACTACAATCTTTTGATGA-CA-3.

The PCR amplification was conducted using high-fidelity DNA polymerase 2 Mixster Taq enzyme (Dalian Takara Biotech Company). The PCR for *PIK3CA* rs17849071 and rs17849079 polymorphisms was performed in a total volume of 25 μl solution, containing 12.5 μl PCR Master Mix, each of 0.5 μl forward and reverse primers, 1.0 μl template DNA, and the rest volume of tri-distilled water. Different amplification conditions were adopted for the two SNPs: (1) rs17849071: initial degeneration at 95°C for 3 min, 30 cycles of 95°C for 40 s, 54°C for 40 s and 72°C for 40 s, followed by extension at 72°C for 5 min; (2) rs17849079: initial degeneration at 95°C for 5 min, 30 cycles of 95°C for 30 s, 54°C for 30 s and 72°C for 45 s; and extension at 72°C for 5 min. The PCR amplification products were bi-directionally sequenced with DNA automatic sequencer.

Statistical analyses

The χ^2 test was performed with PASW Statistics 18.0 software to compare the differences in genotypes and alleles frequencies of *PIK3CA* rs17849071 and rs17849079 polymorphisms between the case and control group (statistical significance existed only when $P<0.05$). Linkage disequilibrium and haplotypes were analyzed with Haploview software. Hardy-Weinberg Equilibrium (HWE) test was performed in the control group with PLINK 1.07 software. The relative risk of HCC was represented by odds ratio (OR) and 95% confidence interval (95% CI) calculated by the χ^2 test.

Results

Basic characteristics of research subjects

The distributions of *PIK3CA* rs17849071 and rs17849079 polymorphisms accorded with

Table 1. Genotype and allele distributions of *PIK3CA* rs17849071 and rs17849079 polymorphisms

Genotype/Allele	Cases (n=150) (%)	Controls (n=152) (%)	χ^2	*P* value	OR (95% CI)
rs17849071					
TT	106 (70.7)	119 (78.3)	-	-	1.000 (Ref.)
TG	31 (20.7)	28 (18.4)	0.552	0.457	1.243 (0.700-2.207)
GG	13 (8.6)	5 (3.3)	4.206	0.040	2.919 (1.007-8.460)
T	243 (81.0)	266 (87.5)	-	-	1.000 (Ref.)
G	57 (19.0)	38 (12.5)	4.813	0.028	1.642 (1.051-2.564)
rs17849079					
CC	72 (48.0)	90 (59.2)	-	-	1.000 (Ref.)
CT	67 (44.7)	58 38.2)	2.368	0.124	1.444 (0.904-2.307)
TT	11 (7.3)	4 (2.6)	4.601	0.032	3.438 (1.050-11.250)
C	211 (70.3)	238 (78.3)	-	-	1.000 (Ref.)
T	89 (29.7)	66 (21.7)	5.010	0.025	1.521 (1.052-2.199)

Table 2. Analysis of linkage disequilibrium and haplotypes of rs17849071 and rs17849079

Haplotype site1-site2	Cases (2n=300)	Controls (2n=304)	χ^2	*P* value	OR (95% CI)
T-C	176	212	-	-	1.000 (Ref.)
T-T	67	54	3.705	0.054	1.495 (0.991-2.253)
G-C	35	26	3.056	0.080	1.622 (0.940-2.798)
G-T	22	12	4.697	0.030	2.208 (1.063-4.588)

Note: site1: rs17849071; site2: rs17849079.

HWE in both the case and control group. The goodness of fit of HWE was fine in the control group ($P>0.05$), which showed that the controls were in an equilibrium state and had good representativeness.

Relationship between genotypes and alleles of PIK3CA polymorphisms and risk of HCC

The genotype distributions of *PIK3CA* rs17849071 and rs17849079 polymorphisms (**Table 1**) showed that the frequency of GG genotype in rs17849071 polymorphism was significantly higher in the case than control groups, and that the difference was statistically significant ($P=0.040$), indicating that GG genotype might be a susceptible genotype to HCC (OR=2.919, 95% CI=1.007-8.460). Meanwhile, TT genotype in rs17849079 also increased HCC risk (OR=3.438, 95% CI=1.050-11.250). Furthermore, the rare alleles G and T of rs17849071 and rs17849079 polymorphisms respectively were more frequent in cases than in controls, which suggested that they were closely related to HCC onset (OR=1.642, 95% CI=1.051-2.564; OR=1.521, 95% CI=1.052-2.199).

Haplotype analysis of PIK3CA polymorphisms

Linkage disequilibrium analysis of *PIK3CA* rs17849071 and rs17849079 (**Table 2**) was conducted with Haploview software showed that 4 haplotypes were formed by two SNPs. The association analysis between 4 haplotypes and HCC risk with SPASS 18.0 showed that there was significant difference in G-T haplotype frequency between the case and control groups ($P=0.030$), so G-T haplotype increased HCC risk (OR=2.208, 95% CI=1.063-4.588), however, the other haplotypes had no significant relationship with HCC occurrence.

Discussion

55% of new HCC cases across the world occur in China, that is, more than 300,000 Chinese people are diagnosed with HCC each year. With a hidden onset, HCC is usually diagnosed at an advanced stage, and there are no effective treatments for it yet. Therefore, it is especially important to carry out studies on HCC pathogenesis in China. Previous studies have shown that HCC may be related to various factors, including the infection of hepatitis B virus (HBV) and hepatitis C virus (HCV), cirrhosis, aflatoxin, smoking, drinking and other environmental factors [18]. However, among different individuals who are similarly exposed to the above risk factors, only a part of people suffer from HCC, which suggests that the occurrence of HCC may be associated with individual sensitivity to risk factors.

PIK3CA gene located on chromosome 3q26.3, is an oncogene obtained from yeast artificial

chromosome (YAC) DNA with in situ hybridization technique. It has many important physiological functions such as regulating the proliferation, differentiation and survival of cells. The gene commonly exists in an inactive state, and usually cannot be easily detected. It has been shown that *PIK3CA* gene expresses in such human organs as brain, oral cavity, lung, breast, liver, esophagus, gastrointestine, prostate, cervix, and ovary [19-22]. PI3K is a heterodimer consisting of regulatory subunit p85 and catalytic subunit pllo, and is divided into type I, II, and III according to its structure and substrate specificity [23]. Among the three types of PI3K, the type I PI3K, activated by cell surface receptors, is the most widely researched type and plays a very important role in the development of cancers. The type I PI3K is a heterodimer constituted by a catalytic subunit p110 and a regulatory subunit p85 which plays a role in stabilizing the catalytic subunit p110 and inhibiting the activity of PI3K in dormant cells [24]. Studies show that the mutations of *PIK3CA* gene can not only enhance the catalytic activity of PI3Ks, but also cause cell canceration [25].

This case-control study investigated the relation between *PIK3CA* rs17849071 and rs17849079 polymorphisms and HCC risk. According to the results, the GG and TT genotypes in rs17849071 and rs17849079 respectively all increased the risk of HCC. Additionally, the rare alleles G and T in these two SNPs had significant differences between two experimental groups, increasing the occurrence of HCC.

Haplotype analysis and linkage disequilibrium test were conducted for *PIK3CA* rs17849071 and rs17849079 polymorphisms respectively. The frequencies of G-T haplotype were higher in the case group than in the control group, which indicated G-T haplotype might be the susceptible haplotype to HCC. Combined with the above results, the fact that haplotype G-T included alleles T and G further showed that the G and T allele of rs17849071 and rs17849079 might be the susceptible alleles to HCC.

In conclusion, *PIK3CA* rs17849071 and rs17849079 polymorphisms are closely associated with the onset of HCC and these two polymorphisms play the role on HCC not only independently but synergistically. However, the sample size of the study was relatively small, so the results need to be repeatedly verified by further studies with larger sample sizes in other independent races or regions in the future so as to provide a more scientific basis for the prevention and diagnosis of HCC.

Disclosure of conflict of interest

None.

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