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RESEARCH

Low Frequency of *PIK3CA* Gene Mutations in Hepatocellular Carcinoma in Chinese Population

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Abstract PI3K/AKT constitutes an important pathway regulating the signaling of multiple biological processes and plays a critical role in carcinogenesis. *PIK3CA* gene missense mutations have been reported in many human cancer types. The mutation of it in hepatocellular carcinoma cases varies with different races and regions. In this study, we investigated *PIK3CA* mutation in Chinese hepatocellular carcinoma patients. A total 90 Chinese patients of hepatocellular carcinoma were recruited in this study. Exons 9 and 20 hotspots mutations of *PIK3CA* gene were detected by PCR-based DNA sequencing. Two point mutations (E542K and D549H) in exon 9 were found in only one patient (1/90; 1.11%), no mutation was found in exon 20 in any cases. 57 patients are associated with HBV infection (57/90; 63.3%), and 8 patients with HCV infection (8/90; 8.9%). The frequency of the *PIK3CA* mutations in hepatocellular carcinoma seems to be lower in Chinese hepatocellular carcinoma patients. These findings suggest that PI3K mutations may not play a major role in hepatic carcinogenesis in Chinese. HBV infection has close relationship with HCC in Chinese.

Keyword Hepatocellular carcinoma · *PIK3CA* · Gene mutation · Hepatitis B virus

Abbreviation

HCC	hepatocellular carcinoma
PI3K	phosphoinsitol 3-kinases
PIK3CA	phosphoinsitol 3-kinases catalytic subunit
HBV	hepatitis B virus
HCV	hepatitis C virus

Introduction

Hepatocellular carcinoma (HCC) is one of the world healthy problems, accounting for approximately 6% of all human cancers and the third leading cause of cancer mortality and is particularly prevalent in China [1, 2]. Genetic mutation is considered an important determinant for the development of cancer. PI3K/Akt constitutes an important pathway regulating the signaling of multiple biological processes such as cell proliferation, differentiation, apoptosis and motility. Abnormal activation of the pathway plays a critical role in carcinogenesis [3, 4]. The PI3Ks are heterodimers composed of a catalytic subunit (p110α, PIK3CA) and an adapter/regulatory subunit (p85), which is activated by tyrosine kinase growth factor receptors such as epidermal growth factor receptor (EGFR) and insulin-like growth factor-1 receptor (IGF-1R), cell adhesion molecules such as integrins, G-protein-coupled receptors (GPCRs), and oncogenes such as Ras. Somatic missense mutations in the *PIK3CA* gene have been reported in many human cancer types including cancers of the colon, esophagus,-breast, brain, stomach, and lung [4–16]. More-

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over, more than 75% of these mutations occurred in the helical (exon 9) and kinase domains (exon 20) of the gene and the mutant *PIK3CA* is likely to function as an oncogene in human cancers [4]. The mutation of it in HCC cases varies with different races and regions, which is 35.6% in Korean, 3.5% in French and 4% in Switzer, while *PIK3CA* hotspot mutations was not found in Japanese [17–20]. So far, *PIK3CA* mutation has not been investigated in Chinese hepatocellular carcinoma patients. Our research focused on the contribution of *PIK3CA* hotspot mutations in the pathogenesis of HCC in the Chinese population.

Patients, Materials and Methods

Patients

A total of 90 consecutive patients who had undergone surgery for HCC at the Department of General Surgery, The First Hospital of Lanzhou University (Lanzhou, China), from March 2009 and September 2010 were included in this study. All cases were histopathologically confirmed after operation. The research was approved by the ethical committees of The First Hospital of Lanzhou University. Patients gave informed consent to the work.

Extraction of Genomic DNA

DNA was extracted from fresh tissue samples using EZNA Tissue DNA kit Omega (USA), whereas the DNA of paraffin-embedded tissue was extracted using the FFPE DNA kit Omega (USA).

DNA Amplification and Sequencing

Two sets of primers were used for the detection of any point mutations in exons 9 and 20 of the PIK3CA gene. The following primers were used: exon 9 forward: 5'CAAAGCAATTTCTACACGAGATCC3', reverse: 5'GTAAAAACATGCTGAGATCAGCCACAT 3'; exon 20 forward: 5'TGGAATGCCAGAACTACAATCTTT3', reverse: 5'GGTCTTTGCCTGCTGAGAGTT3'. The polymerase chain reaction (PCR) reaction was carried out in a total volume of 20 μl. The mixture included 1x HotStarTaq buffer, 2.0 mM Mg^{2+}, 0.2 mM dNTP, 0.2 μM of each primer, 1U HotStarTaq polymerase (Qiagen Inc.) and 1 μl template DNA. The cycling program for exon 9 was initial denaturation at 95°C for 15 min, followed by 11 cycles at 94°C for 20 s, 62°C–0.5°C per cyrcle for 40 s, 72°C for 1 min, and cycling program for exon 20 was initial denaturation at 95°C for 15 min, followed by 27 cycles at 94°C for 20 s, 56°C for 30 s, 72°C for 1 min. Following purification of PCR products by SAP and Exo I, DNA sequencing was conducted using a Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) and analysed on an Applied Biosystems 3130XL Genetic Analyzer (Applied Biosystems).

Results

To investigate the frequency of *PIK3CA* mutations in Chinese patients with HCC, we sequenced the two hotspot-containing exons (9 and 20) in 90 HCC samples. The male to female ratio of the patients was 74:16, and their ages ranged from 31 to 75 years, with an average age of 53 years. In all, 57 patients had HCC associated with hepatitis B virus (HBV) infection (57/90; 63.3%), 8 patients had HCC associated with hepatitis C virus (HCV) infection, and 9 patients had HCC associated with alcohol (9/90; 10.0%), 10 patients had not clarified etiology. The concentrations of serum AFP of 16 patients were higher than 400 ng/ml. The Barcelona Clinic Liver Cancer (BCLC) staging classification was adopted for staging of HCC [21] (Table 1).

Using PCR amplification followed by direct sequencing of HCC DNA samples, we identified two point mutations in the *PIK3CA* gene. Both mutations were in exon 9 and the mutations occurred in the same one sample (1/90; 1.11%). One mutation was in E542K, one of the *PIK3CA* hotspot mutations (Fig. 1); the other was in D549H. The patient with two point mutations in exon 9 of *PIK3CA* is a 45 years old male, had chronic HBV infection and in stage C, AFP level is 650 ng/ml.

Discussion

PIK3CA gene mutations have been recognized as an significant risk factor for many cancer types, its mutation frequency has been reported at 3–4% in lung cancer [5, 6], 8–18% in colon cancer [7, 8], 2–11% in esophageal squamous cell carcinoma [9, 10] 32% to 39% in breast cancer [11, 12], 4% to 13% in gastric cancer [13, 14], and

Table 1 Clinical characteristics of HCC patients

	Sex		AFP(ng/ml)		Etiology			Stage				
	M	F	>400	≤400	HBV	HCV	alcohol	0	A	B	C	D
n	74	16	16	74	57	8	9	6	52	27	5	0

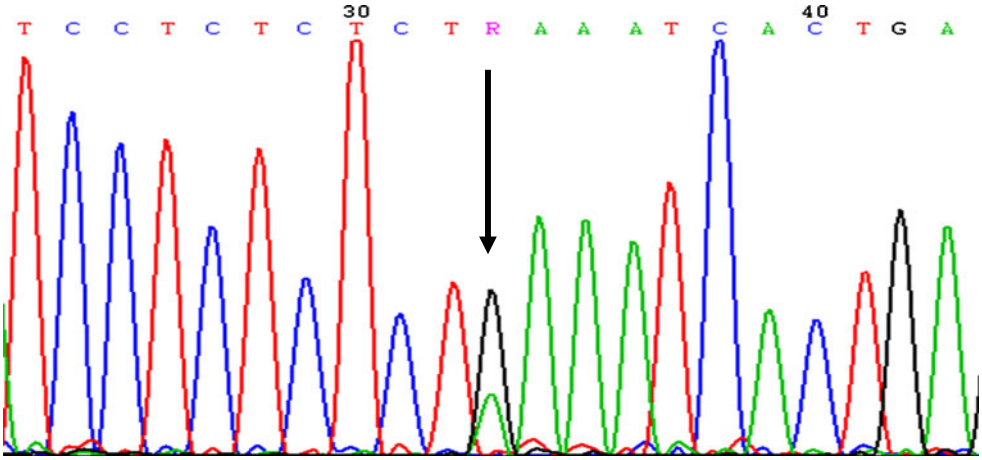

Fig. 1 PIK3CA-exon 9 E542K missense variant

35% in ovarian cancer [15, 16]. Lee et al detected *PIK3CA* gene highly mutated in approximately 35.6% of Korean HCC cases [17]. However, other studies of HCC patients showed *PIK3CA* gene mutations at a frequency of 3.5% in French [18] and 4% in Switzer [19]. In addition, Tanaka et al. did not find *PIK3CA* hotspot mutations in HCC in Japanese patients [20]. In this study, the *PIK3CA* gene mutations were identified in only one case (1.11%). This may suggest a lower frequency of the *PIK3CA* gene mutation in Chinese HCC patients. Hepatic carcinogenesis is a multifactor and multistep process, it seemed that *PIK3CA* gene mutation dose not play an important role in HCC for Chinese population (Fig. 2).

PIK3CA have five functional domains, included the p85 binding domain, Ras binding domain, C2 domain, helical domain, and kinase domain. It was reported that the percentage of mutations detected within helical domain, and kinase domain was 47% and 33%, respectively [3]. Both two point mutations (E542K and D549H) detected in our study were in helical domain. E542K is one of the *PIK3CA* hotspot mutations and it could increase lipid kinase activity in vitro [22–24]. D549H mutation has not been reported so far. There are three mutation sites (E542, E545, and Q546) nearby its position. They are clustered on an exposed face of this domain that could interact with another protein [25]. The definite function of D549H mutation needs to be studied further.

HBV and HCV infection are recognized as the major factors that increase the risk of HCC. Approximately, three fourths of all liver cancer deaths are attributed to HBV infection worldwide [26]. China is a hyperendemic area for HBV infection, the incidence of hepatitis B is 27–36 per 100,000 population [2, 27]. About 170 million Chinese are infected chronically with HBV and 10% suffer from chronic hepatitis. Around half a million Chinese die from hepatitis B caused hepatocellular carcinoma and endstage cirrhosis each year [28]. In the study, 57 patients are associated with HBV infection (57/90; 63.3%), and 8 patients with HCV infection (8/90; 8.9%), which reconfirmed that HBV infection has close relationship with HCC in China.

Due to the low frequency of the *PIK3CA* hotspot mutations, in this study, we conclude that those PI3K mutations seem not to contribute to Chinese HCC patients or they do but in a much lower degree. Other mechanisms of *PIK3CA* activation or mutations of other molecular pathways could be involved in the pathogenesis of HCC.

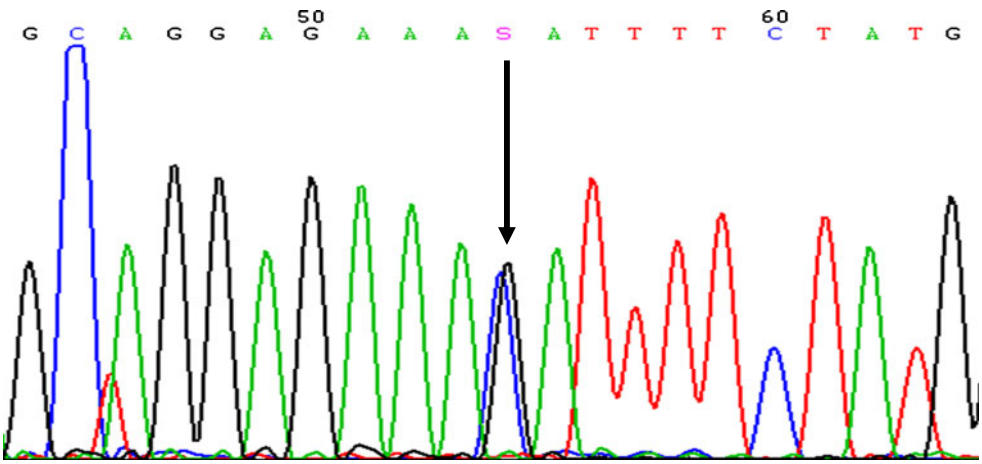

Fig. 2 PIK3CA-exon 9 D549H missense variant

Because of the small sample size used in this study, expanding the number of samples included may enhance our findings.

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Conflict of interest The authors declare that they have no conflict of interest.

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