RESEARCH COMMUNICATION

Genetic Variation in the Fat10 Gene is Associated with Risk of Hepatocellular Carcinoma in a Chinese Population

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Abstract

Background and Objectives: This study examined whether variation in exonic and flanking sequences of the human HLA-F adjacent transcript 10 (FAT10) gene might be associated with susceptibility and clinicopathological development of hapatocellular carcinoma (HCC). Methods: A total of 522 subjects, including 268 healthy controls and 254 patients with HCC, were recruited. Genotyping was accomplished using DNA sequencing. Haplotypes were determined through genotypic and disequilibrium analysis of identified single nucleotide polymorphisms (SNPs). Results: Ten SNPs in FAT10 were identified, namely -143 A/G (rs362535), -121 A/G (rs2272991), +3446 C/T, +3476 T/C (rs2076484), +3527 T/C (rs2076485), +3607 T/C (rs2076486), +3620 C/G (rs2076487), +3803 C/G (rs8337), +3809 G/T (rs7757931), +3833 G/C (rs444013). +3446 C/T is a novle polymorphism. The -143 A/G, -121 A/G, +3476 T/C, +3607 T/C, +3620 C/G and +3809 G/T genotypes were associated with a decreased risk for HCC (all P-values <0.05). No SNPs were associated with disease clinicopathology (all P-values > 0.05). Furthermore, under the analysis of haplotype, GGCTCGT and AGCTCGT were related to reduced HCC risk (OR=0.41, 95% CI=0.24, 0.70, P<0.05 and OR=0.43, 95% CI=0.22, 0.983, P<0.05, respectively), while AATTTCG was associated with an increased risk (OR= 1.64, 95% CI=1.24-2.17, P<0.05). 10-million permutation testing also indicated the AATTTCG and GGCTCGT haplotypes to be associated with HCC susceptibility (both P-values <0.05). Patients carrying AATTTCG were in higher tumor and clinical stages (P<0.05), while GGCTCGT appeared protective in this context (P <0.05). Conclusion: This study provides first evidence that FAT10 gene genetic variants may be involved in the susceptibility and clinicopathological development of HCC in the Chinese han population.

Keywords: FAT10 - polymorphisms - haplotype - susceptibility - hepatocellular carcinoma

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Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide with about 600,000 patients dying from the disease annually (Schutte et al., 2009) Development of hepatocarcinoma is a multi-step process, with multiple risk factors that include chronic HBV and HCV infection, cirrhosis, carcinogen exposure, and a variety of genetic variants (Farazi et al., 2006; Firpi and Nelson, 2006; Yano et al., 2006; Weng et al., 2010)

Several studies have identified genes with genetic variations that are associated with the risk for HCC and may be involved in HCC development. For example, HCC tumor related mutations have identified in HFE (Ropero et al., 2007), PRKAR1A (Gennari et al., 2008), β-catenin (Miyoshi et al., 1998; Elmileik et al., 2005; Zucman-Rossi et al., 2007), AXIN1 (Satoh et al., 2000; Zucman-Rossi et al., 2007) and TCF1 (Boyault et al., 2007). SNPs that are predictive of high risk for HCC have been found in the insulin-like growth factor (IGF-2), IGF-2R, and plasminogen activator inhibitor (PAI-1) (Weng et al., 2010a; 2010b). Identification of additional genetic variants associated with HCC may identify other genes involved in HCC and help to elucidate the complex process of hepatocarcinogenesis and improve the deveolopment of preventive interventions for the disease.

Ubiquitin is a highly conserved 76-residue protein in all eukaryotic cells (Hochstrasser, 1996), which plays a role in cell-cycle control, apoptosis, and transcription. Misexpression of ubiquitin can lead to cancer (Nakayama and Nakayama, 2006). An growing group of related low-molecular-weight proteins known as ubiquitin-like proteins have been identified within the recent years that are implicated in diverse cellular processes. To date, two families of ubiquitin-like proteins have been identified;

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ubiquitin-like modifiers (UBLs) and ubiquitin-domain proteins (UDPs) (Jentsch and Pyrowolakis, 2000). UBLs have similar functions as ubiquitin and may play an important role HCC and other malignant tumor development by regulating cell cycle and Wnt signaling (Jentsch and Pyrowolakis, 2000; Wu and Mo, 2007; Yam et al., 2010; Wei et al., 2009).

The Human HLA-F adjacent transcript 10 (FAT10) belongs to the UBL class of ubiquitin-like proteins(Fan et al., 1996). FAT10 is an18-kDa protein consisting of two ubiquitin-like domains in a head-to-tail arrangement, and plays an important role in cancer biology by influencing the cell cycle, apoptosis, and tumorigenesis (Fan et al., 1996; Ren et al., 2006; Canaan et al., 2006; Lee et al., 2003). The expression of FAT10 gene was up regulated in 90% of human hepatocellular carcinomas (Lee et al., 2003). FAT10 was also reported to qualify as a marker for an interferon response in HCC (Lukasiak et al., 2008) ADDIN EN.CITE ADDIN EN.CITE.DATA . Another study found that FAT10 is an epigenetic marker for liver preneoplasia in a drug-primed mouse model of tumorigenesis (Oliva et al., 2008). Furthermore, FAT10 was localized within some nuclei in the tumor forming mouse livers and in human hepatocellular carcinomas. Preclinical analysis indicated that the levels of FAT10 influence mitosis, chromosome stability, and that FAT10 expression is regulated by P53 (Ren et al., 2006; Zhang et al., 2006) ADDIN EN.CITE ADDIN EN.CITE.DATA. These findings suggest that FAT10 may be involved in the development of HCC but the specific molecular mechanism is unclear.

In the present study, a case-control association study in a Chinese Han population was performed for variations in the flanking and exonic sequences of FAT10 to assess whether genetic variations in this gene may be involved in the HCC susceptibility.

Materials and Methods

Study Population

This was a hospital-based case-control study in a Chinese Han population, which comprised of 254 patients with HCC and 268 healthy unrelated Han controls. Patients were recruited from second Affiliated Hospital of Nanchang University between January 2007 and December 2010. HCC was confirmed by histopathology. The healthy control group comprised persons of the same geographical origin as the patients. Individuals with secondary, recurrent cancers and who had had blood transfusion from others were excluded from the study.
The study was carried out in accordance with the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the College of Medicine of Nanchang University. All participants gave written informed consent.

Genotypic Analysis

From each participant, 5 ml blood was collected in tube containing EDTA and stored at -20˚C. Genomic DNA was extracted using standard procedures and stored -60˚C. FAT10 sequences were amplified using the primers: 5'-TAGACATAGAGATCTGCAGC-3' and 5'-TTCTCCTGAAGGATGCCTGC-3' that amplified a 520 bps including exon1and 5'-UTR;

5'-TCCTAGGTAACTGTCTTGTG-3' and 5'-TCCATTGCAAGTCACAATC-3' that amplified a 416 bp fragment that includes part of exon 2;

5'-CAGCTCAGTGGCACAAGT-3' and 5'-ACAAGGTATCAAGACAGA-3' that amplified a 472 bp fragment containing a fragment of exon 2 and 3'-UTR. The FAT10 fragments were amplified by multiplex polymerase chain reaction using an ABI premix. PCR was performed in a 16 ul total reaction volume containing 8.75μl 2×Master Mix (Tiangen Biotech, Beijing, China), 0.5μM each primer, 50 ng of genomic DNA, and 5.75μl ddH_2O. The cycling conditions were 5 minutes at 95 oC; followed by 35 cycles of 95˚C for 30 seconds, 56-58˚C for 30 seconds, and 72˚C for 45 seconds; and a final extension at 72˚C for 10 minutes. PCR products were separated on a 1% agarose gel and purified using exonuclease I (TaKaRa Biotechnology, Dalian, China) and SAP (TaKaRa Biotechnology, Dalian, China). Fragments were sequenced with an ABI 9700 genetic analyzer (Applied Biosystems, Foster City, CA) using the BigDye Terminator (v3.1) Cycle Sequencing Kit (Applied Biosystems, Austin City, Minnesota). Potential polymorphic alleles were confirmed by resequencing using different primers.

SNP selection and HCC association

An algorithm was used to identify the relevant SNPs in the exons and flanking sequences. These SNPs were used to assess the association of SNPs with HCC. The criteria for selecting the relevant SNPs (1) had an minor allele frequency of > 5%; (2) were in Hardy-Weinberg P-value > 0.001; (3) had heterozygosity; and (4) were compatible with the genotyping platform.

LD and haplotype analyses

The Haploview software (http://www.broad.mit.edu/haploview/haploview) was used to assess the pair-wise linkage disequilibrium (LD) by the lewontin coefficient D' (D'=D/Dmax) and r2 among the polymorphisms within FAT10 (Barrett et al., 2005). The estimation of haplotype frequency and association analyses were performed using the Haploview program. Haplotypes with a frequency of < 0.01 were not further analyzed.

Statistical analysis

The $\chi 2$ test was used to assess the difference in the distributions of categorical variables and allele frequencies between patients and controls. Hardy–Weinberg equilibrium in experimental and controls was assessed by using the chi-square test. Unconditional logistic regression was done to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) of the individual SNPs or haplotypes.

The main effects of a single loci or haplotype with adjustment for multiple testing was assessed using the permutation step-down procedure by the Haploview program. For this analysis, 10-million permutations were performed. $P < 0.05$ being considered statistically significant. Analysis of data was conducted using SPSS version 11.5 (SPSS, Chicago, IL).

Results

Characteristic of the study group and an overview of the detected SNPs

Patients and controls were similar with respect to age, gender distribution, smoking, and alcohol consummation (Table 1). These parameters were matching variables (all P-values >0.05). We identified 10 SNPs in both HCC patients and control subjects, namely -143 A/G (rs362535), -121 A/G (rs2272991), +3446 C/T, +3476 T/C (rs2076484), +3527 T/C (rs2076485), +3607 T/C (rs2076486), +3620 C/G (rs2076487), +3803 C/G (rs8337), +3809 G/T (rs7757931), +3833 G/C (rs444013) (Figure 1). The +3446 C/T SNP had not been previously identified. SNPs -143 A/G and -121 A/G were locate in the promoter region of 5'-UTR (Lim et al., 2006). The SNPs +3446 C/T, +3476 T/C, +3527 T/C, +3607 T/C, +3620 C/G, +3803 C/G, +3809 G/T SNPs are located in exon 2, and +3833 G/C SNP is in 3'-UTR (Figure 2).

The selected SNPs

The SNPs that were selected for further analysis were chosen based on the minor allelic frequency, heterozygosity, and whether they were in Hardy-Weinberg equilibrium in both HCC patients and control subjects. The SNPs, -143 A/G, -121 A/G, +3476 T/C, +3527 T/C, +3607 T/C, +3620 C/G, +3809 G/T were included in this study (Table 2). The +3446 C/T , +3803 C/G and +3833

Table 1. General Information of Controls and HCC Groups

Group	Controls (N = 268)	HCC (N = 254)	χ^2 value	P*-value
Age group				
<30	11 (4.10)	9 (3.54)		
30-60	212 (79.1)	210 (82.7)		
>60	45 (16.8)	35 (13.8)	1.09	0.58
Gender				
Male	207 (77.2)	213 (83.9)		
Female	61 (22.8)	41 (16.1)	3.63	0.06
Smoking status				
No	166 (62.0)	152 (60.0)		
Yes	102 (38.0)	102 (40.0)	0.24	0.62
Drinking status				
No	198 (74.0)	179 (70.5)		
Yes	70 (26.1)	75 (29.5)	0.76	0.39

*χ^2, test were applied for the age group, gender, smoking status and drinking status

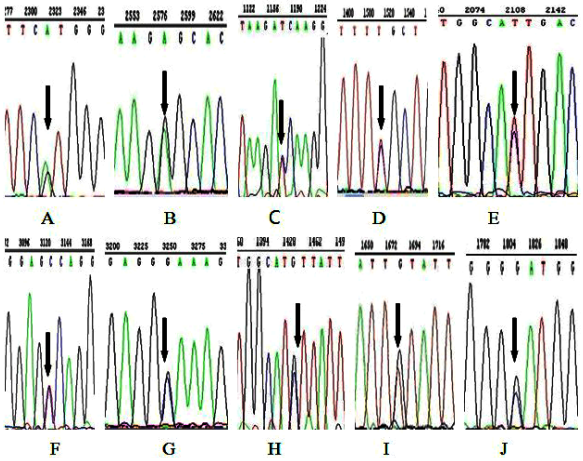
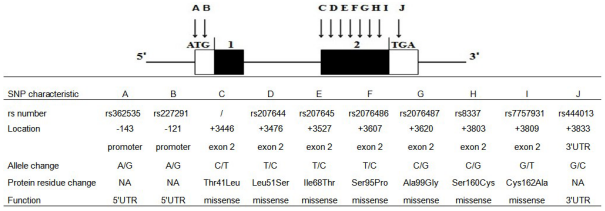


Figure 1. DNA Sequencing Genotyping. 10 genetic polymorphisms in 254 HCC patients and 268 healthy control subjects. A. -143A/G (rs362535), B.-121A/G (rs2272991), C. +3446 C/T, D. +3476 T/C (rs2076484), E. +3527 T/C (rs2076485), F. +3607 T/C (rs2076486), G. +3620 C/G (rs2076487), H. +3803 C/G (rs8337), I. +3809 G/T (rs7757931), J. +3833 G/C (rs444013)



SNP characteristic	A	B	C	D	E	F	G	H	I	J
rs number	rs362535	rs227291	/	rs207644	rs207645	rs2076486	rs2076487	rs8337	rs7757931	rs444013
Location	-143	-121	+3446	+3476	+3527	+3607	+3620	+3803	+3809	+3833
	promoter	promoter	exon 2	exon 2	exon 2	exon 2	exon 2	exon 2	exon 2	3'UTR
Allele change	A/G	A/G	C/T	T/C	T/C	T/C	C/G	C/G	G/T	G/C
Protein residue change	NA	NA	Thr41Leu	Leu51Ser	Ile68Thr	Ser95Pro	Ala99Gly	Ser160Cys	Cys162Ala	NA
Function	5'UTR	5'UTR	missense	missense	missense	missense	missense	missense	missense	3'UTR

Figure 2. Exon and Intron Structure of FAT10 Gene in Homo Sapiens and the Identified SNPs in Both HCC Patients and Control Subjects. Exons (filled boxes) are numbered from 1 to 2 from the 5'- to 3'-end of the gene; introns (thin lines); the untranslated regions (open boxes); and the detected SNPs are indicated (black arrows) are labeled A to J. The start codon (ATG) and stop codon (TGA) are indicated in exons 1 and 2, respectively.

G/C SNPs were excluded: due to the a minor allelee frequency for both of +3446 C/T and +3803 C/G (minor allele frequency < 5%), and +3833 G/C SNP not being in Hardy-Weinberg equilibrium (P < 0.001) (Table 2).

Individual single-nucleotide polymorphism association analysis

The genotype and allele frequencies of the selected FAT10 gene SNPs in HCC patients and healthy controls

Table 2. List of the Information of the Detected SNPs and the Minor Allele Frequencies of the FAT10 Gene Among the Study Subjects

Name	Chromosome Position	Observed Heterozygocity	Predicted Heterozygocity	Hardy-Weinberg P-value	Major allele	Minor allele	Minor Frequency	Genotyping rate (100%)
-143A/G	29,527,621	0.14	0.13	0.14	A	G	0.07	100
-121A/G	29,527,599	0.18	0.19	0.27	A	G	0.11	100
+3446C/T	29,524,033	0.02	0.02	1.00	C	T	0.01	100
+3476T/C	29,524,003	0.18	0.19	0.27	T	C	0.11	100
+3527T/C	29,523,952	0.25	0.27	0.13	T	C	0.16	100
+3607T/C	29,523,872	0.18	0.19	0.27	T	C	0.11	100
+3620C/G	29,523,859	0.18	0.19	0.27	C	G	0.11	100
+3803C/G	29,523,676	0.06	0.06	1.00	C	G	0.03	100
+3809G/T	29,523,670	0.18	0.19	0.27	G	T	0.11	100
+3833G/C	29,523,646	0.39	0.32	0.00	G	C	0.20	100

Table 3. Frequency of FAT10 Selected SNPs Alleles and Genotypes Among Patients and Controls and the Associations with the Risk of HCC

Variables	control(%) (N = 268)	HCC(%) (N = 254)	P -value	OR[a](95%CI)
-143				
A	485 (90.5)	487 (95.9)		1 (reference)
G	51 (9.51)	21 (4.13)	0.00	0.41 (0.24-0.69)
A/A	217 (81.0)	233 (91.7)		1 (reference)
A/G	51 (19.0)	21 (8.27)	0.00	0.38 (0.22-0.66)
G/G	0 (0)	0 (0)		
-121				
A	456 (85.1)	475 (93.5)		1 (reference)
G	80 (14.9)	33 (6.50)	0.00	0.40 (0.26-0.61)
A/A	194 (72.4)	224 (88.2)		1 (reference)
A/G	68 (25.4)	27 (10.6)	0.00	0.34 (0.21-0.56)
G/G	6 (2.24)	3 (1.18)	0.32	0.43 (0.11-1.76)
+3476				
T	456 (85.1)	475 (93.5)		1 (reference)
C	80 (14.9)	33 (6.50)	0.00	0.40 (0.26-0.61)
T/T	194 (72.4)	224 (88.2)		1 (reference)
T/C	68 (25.4)	27 (10.6)	0.00	0.34 (0.21-0.56)
C/C	6 (2.24)	3 (1.18)	0.32	0.43 (0.11-1.76)
+3527				
T	449(83.77)	431 (84.8)		1 (reference)
C	87(16.23)	77 (15.2)	0.63	0.92 (0.66-1.29)
T/T	190 (70.90)	186 (73.2)		1 (reference)
T/C	69 (25.74)	59 (23.2)	0.51	0.87 (0.58-1.31)
C/C	9 (3.36)	9 (3.55)	0.97	1.02 (0.40-2.63)
+3607				
T	456 (85.1)	475 (93.5)		1 (reference)
C	80 (14.9)	33 (6.50)	0.00	0.40 (0.26-0.61)
T/T	194 (72.4)	224 (88.2)		1 (reference)
T/C	68 (25.4)	27 (10.6)	0.00	0.34 (0.21-0.56)
C/C	6 (2.24)	3 (1.18)	0.32	0.43 (0.11-1.76)
+3620				
C	456 (85.1)	475 (93.5)		1 (reference)
G	80 (14.9)	33 (6.50)	0.00	0.40(0.26-0.61)
C/C	194 (72.4)	224 (88.2)		1 (reference)
C/G	68 (25.4)	27 (10.6)	0.00	0.34 (0.21-0.56)
G/G	6 (2.24)	3 (1.18)	0.32	0.43 (0.11-1.76)
+3809				
G	456 (85.1)	475 (93.5)		1 (reference)
T	80 (14.9)	33 (6.50)	0.00	0.40(0.26-0.61)
G/G	194 (72.4)	224 (88.2)		1 (reference)
G/T	68 (25.4)	27 (10.6)	0.00	0.34 (0.21-0.56)
T/T	6 (2.24)	3 (1.18)	0.32	0.43 (0.11-1.76)

[a]Adjusted by age, gender, smoking and drinking status

are show in Table 3. There was a significant association among the SNPs -143 A/G, -121 A/G, +3476 T/C, +3607 T/C, +3620 C/G, +3809 G/T with a decreased risk of HCC. There was no significant association between FAT10 polymorphisms and HCC clinical pathological characteristics such as the tumor size, number of nodes, liver cirrhosis, HBsAg, TNM stage, metastasis (lymph node metastasis and distant metastasis) (all P-values > 0.05).

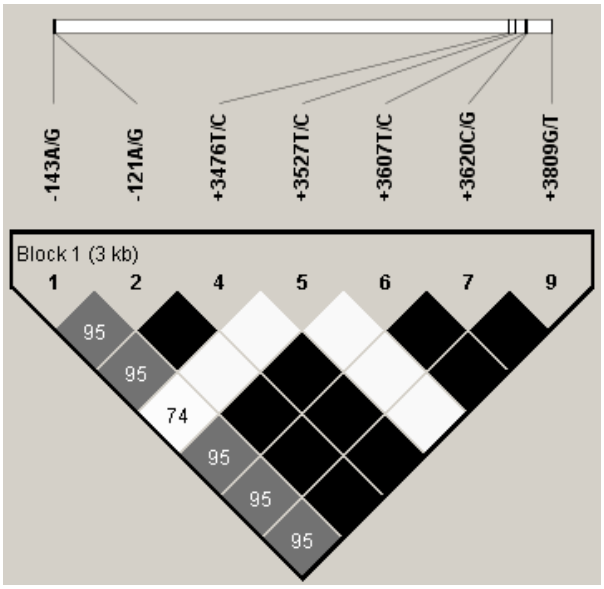


Figure 3. Linkage Disequilibrium (LD) Maps of the Seven Selected SNPs in the HCC Cases and Controls. Pair-wise LD analysis across the seven FAT10 polymorphisms. Black indicates strong LD, dark and light gray indicate moderate to low LD, and white indicates no significant LD between the polymorphisms.

Haplotype block structure and analysis

The pairwise linkage disequilibrium (LD) (D') values for the selected seven SNPs and LD structure of FAT10 are shown in Figure 3. There was pronounced differences in the LD map (Figure 3). The SNPs -143 A/G, -121 A/G, +3476 T/C, +3527 T/C, +3607 T/C, +3620 C/G, +3809 G/T polymorphisms clustered in a block. A strong LD was observed among the seven SNPs (D'>0.70), and complete linkage disequilibrium (D'=1) was also detected among the seven polymorphisms.

Four potential haplotypes were identified in our population (all haplotypes frequency > 1%), namely AATTTCG, AATCTCG, GGCTCGT, AGCTCGT (Table 4). The most frequent haplotype in both patients and controls was AATTTCG (78.35% in patients and 69.36% in controls) that contains the -143 A: -121 A: +3476 T: +3527 T: +3607 T: +3620 C: +3809 G SNPs. The haplotypes GGCTCGT, containing the SNPs -143 G: -121 G: +3476 C: +3527 T: +3607 C: +3620 G: +3809 T: and AGCTCGT that contains the SNPs -143 A: -121 G: +3476 C: +3527 T: +3607 C: +3620 G: +3809 T were associated with protective effect against HCC (OR =0.41,

Table 4. Frequencies of the Haplotypes of the FAT10 Gene in the HCC Patients and Controls and the Associations with the Risk of HCC

Haplotype[a]	Control(N = 536) (%)	HCC (N = 508) (%)	P-value	OR (95%CI)	Permutated P* (n=10000000)
AATTTCG	369 (69.4)	398 (78.4)	0.00	1.64 (1.24-2.17)	0.01
AATCTCG	86 (16.0)	77 (15.2)	0.69	0.93 (0.67-1.31)	1
GGCTCGT	50 (9.32)	20 (3.94)	0.00	0.41 (0.24-0.70)	0.01
AGCTCGT	31 (5.78)	13 (2.56)	0.01	0.43 (0.22-0.83)	0.05

[a]seven SNP alleles from left to right -143 A/G , -121 A/G, +3476 T/C, +3527 T/C, +3607 T/C, +3620 C/G, +3809 G/T were used for construction of the haplotypes; *Calculated with the Haploview software

95% CI:0.24-0.70 and OR =0.43, 95% CI:0.22-0.83, respectively). The most common haplotype AATTTCG was associated with an increased risk of HCC (OR= 1.64, 95% CI=1.24-2.17). Using 10-million permutation testing, the GGCTCGT and AATTTCG haplotypes were significantly associated with HCC ($P < 0.05$). Analysis of hapatocellular carcinoma clinical pathological features revealed that AATTTCG haplotype was associated with higher tumor and Clinical stage($P < 0.05$). We also found GGCTCGT haplotype was associated with lower tumor and clinical stage ($P < 0.05$).

Discussion

Specific genes and areas of genomic instability have been identified as being associated with the risk of HCC (Luo et al., 2006). Increasing evidence has revealed that genomic changes may alter cellular phenotype so that they evolve from preneoplastic stage into HCC Thorgeirsson (Thorgeirsson and Grisham, 2002), indicating that individual genetic factors play an important role in the course of hepatocarcinogenesis. Multiple gene alterations, such as allelic insertion, deletion, mutation, polymorphism, and methylation are strongly linked to HCC (Buendia, 2000). Comparing HCC patients and healthy control subjects, genetic information is particularly valuable to mark a target gene for predicting risk and pathological development of HCC.

The mRNA of FAT10 has been reported to be over expressed in HCC (Lee et al., 2003; Lukasiak et al., 2008), but its pathogenesic role in cancer is not understood. The present study provides new insight into the effects of FAT10 polymorphisms on the susceptibility and clinicopathological statuses of HCC. To our knowledge, this is the first report to identify FAT10 gene DNA sequences that differ between HCC patients and healthy controls. We also identified a novel SNP, +3446 C/T.

We identified 6 polymorphisms that may influence the risk of HCC. It is possible that the SNPs at positions -143 and -121 which are located in the promoter region of FAT10 may affect the transcriptional activity. SNPs in exon 2 (+3476 T/C, +3607 T/C, +3620C/G, +3809G/T) result in amino acid changes in the FAT10 protein (Leu51Ser, Ser95Pro, Ala99Gly, Cys162Ala) and may alter the protein activity. We speculate that the effect of the six genetic polymorphisms on the functional activity of FAT10 protein maybe is more apparent than other identified SNPs, finally, the susceptibility of hepatocellular carcinoma is changed. Further studies are required to test these hypotheses.

In recent years, the association between disease gene polymorphisms and diversity of clinical phenotypes has been actively studied. The phenotype of polygenic disease is often varied, including tumor characteristics. Links between the genotype and phenotype has increased the understanding of the mechanism of disease and disease outcomes. The FAT10 protein expression level has been associated with p53 mutation, lymph node metastasis, and TNM stage (Ji et al., 2009). Our analysis of tumor size, number of nodes, liver cirrhosis, HBsAg, TNM stage, metastasis in the HCC group, found no significant association between the individual polymorphism we identified in FAT10 and these clinical pathological characteristics. Recent studies have suggest that haplotypes versus SNPs have greater power for detecting association of a genetic marker with disease (Akey et al., 2001). We found a significantly decreased risk for HCC for the individuals with the haplotype GGCTCGT (OR =0.41, 95% CI:0.24-0.70) and AGCTCGT (OR =0.43, 95% CI:0.22-0.83), The haplotype AATTTCG was associated with an increased risk of HCC (OR=1.64, 95%CI:1.24-2.17). The GGCTCGT and AATTTCG haplotypes remained significant after the permutation test (both P-values = 0.01). We also found that the AATTTCG haplotype was associated with higher tumor and Clinical stage. In addition, the GGCTCGT haplotype was significantly associated with lower tumor and Clinical stage.

The major difference between the two haplotypes was the presence or absence of the minor allele of -143 A/G, -121 A/G, +3476 T/C, +3607 T/C, +3620 C/G, +3809 G/T. The minor allele of -143 A/G, -121 A/G, +3476 T/C, +3607 T/C, +3620 C/G, +3809 G/T also demonstrated a decreased risk of HCC. These findings indicated that the -143 A/G, -121 A/G, +3476 T/C, +3607 T/C, +3620 C/G, +3809 G/T SNPs, or closely linked genetic variants, may be a biologically active genetic variants. The seven studied SNPs in FAT10 were in strong LD (Figure 3). Our analysis suggests that this region of the FAT10 may help modulate HCC susceptibility and clinicopathological development.

In conclusion, in this case-controlled study, we report for the first time that genetic variants located in exonic and flanking sequences of FAT10 may be associated with susceptibility and clinicopathological development of HCC in a Chinese Han population. Considering the small sample size, nonrandom sampling, and pitfalls of unknown confounders, further research is reuired to further test this hypothesis. In addition, more studies should be carried out both in vivo and in vitro to clarify the genetic and molecular epidemiology of HCC.

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