

Interferon-induced transmembrane protein-3 rs12252-CC is associated with low differentiation and progression of hepatocellular carcinoma

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Abstract

Interferon-induced transmembrane protein 3 (*IFITM3*) is a component of ISG (Interferon-Stimulated Gene) family. The association between *IFITM3* and hepatocellular carcinoma (HCC) has been reported. While the relationship between this genetic variation and the progress of HCC remains unclear. To address this issue, we explore the relationship between the *IFITM3*-rs12252 genetic variants and the progression of HCC in this study.

A total of 336 candidates were enrolled in the study, including 156 patients with HBV related HCC and 180 patients with chronic Hepatitis B infections or liver cirrhosis. Liver cirrhosis or chronic hepatitis B were diagnosed with clinical characteristics and staging, laboratory testing, and imaging results of viral infection and hepatic damage. Polymerase chain reaction (PCR) was employed to determine the gene polymorphism of *IFITM3*, and analyzed with the GraphPad Prism v 5.

The patients with HCC had a significantly higher proportion of *IFITM3* rs12252-CC as compared with the patients with chronic HBV infection or liver cirrhosis. Moreover, the distribution of CC genotype in HCC patients with low differentiation was significantly higher than that in those with high differentiation. Furthermore, the patients with CC genotype were found with bigger tumor size, higher percentage of vascular thrombosis, higher distribution of low differentiation and higher 5-year relapse rate than those with CT/TT genotypes.

This study indicates a correlation between the *IFITM3*-rs12252 CC genotype and the progression of HCC.

Abbreviations: AFP = alpha-fetoprotein, AFU = α -L-fucosidase, ALB = albumin, ALT = alanine transaminase, AST = aspartate aminotransferase, BCLC = Barcelona clinic liver cancer, DBIL = direct bilirubin, EASL = European association for the study of the liver, GLB = globulin, HBV = hepatitis B virus, HCC = Hepatocellular carcinoma, *IFITM3* = Interferon-induced transmembrane protein 3, PCR = polymerase chain reaction, PTA = prothrombin activity, SD = standard deviation, TBIL = total bilirubin, TTR = time to relapse.

Keywords: Hepatocellular carcinoma, interferon-inducible transmembrane protein 3, liver cirrhosis, rs12252-CC genotype, tumor

1. Introduction

Hepatocellular carcinoma (HCC) is a major public health problem worldwide, and the incidence of HCC has been steadily increasing since the late 1990s.^[1] An estimated 782,500 new

cases of liver cancer and 745,500 deaths occurred worldwide during 2012, with China alone accounting for about 50% of the total number of cases and deaths.^[2] HCC represents the country's second most leading cause of cancer death in major cities and in rural areas.^[3] The high incidence rate of hepatocellular

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All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

The authors declared that they had no conflicts of interest.

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carcinoma in parts of China largely reflect the elevated prevalence of chronic hepatitis B virus (HBV) infection, with over 5% of the populations in these regions chronically infected with the virus.^[4] This trend is likely to continue in the future due to the long life expectancy of patients with viral hepatitis.^[5] The prognosis of HBV-related HCC patients is still unsatisfactory, despite recent advancements in diagnosis and treatment. Therefore, it is important to delineate the genetic mechanism underlying the development and progression of HBV-related HCC, and to find new biomarkers for the diagnosis and treatment of HCC patients.

Various genetic and environmental mediators are involved in the progression of liver diseases and may explain the observed variations in individuals' susceptibility for HCC development.^[6] Identification of genetic risk factors such as genetic polymorphism can serve as an important indication for early detection and therapy. Recent studies have successfully identified many potential genetic variations associated with the susceptibility to liver cancer.^[7–11]

Interferon-induced transmembrane protein 3 (*IFITM3*), a 10 to 15 kDa protein known as 1-8U, is a member of the IFN-inducible transmembrane protein family.^[12] The *IFITM3* gene was initially isolated from a genetic screen performed to identify the genes involved in the acquisition of germ-cell competence.^[13] The human homologues including *IFITM1*, -2, -3, and -5 are clustered on chromosome 11.^[14] *IFITM3* plays an important role in different cell processes, including cell adhesion, immune-cell regulation, germ-cell homing and maturation, and bone mineralization.^[15] *IFITM3* gene was reported to be positively correlated with the risk of colon cancer, breast cancer, gastric cancer and HCC.^[16–20] The *IFITM3* polymorphism rs12252-C, which encodes an *IFITM3* isoform ($\Delta 21$ *IFITM3*) lacking 21 amino acids at the amino terminus,^[21] has been positively associated with susceptibility and severity of influenza virus and hemorrhagic fever with renal syndrome in the Chinese patients.^[22–24] Furthermore, a study demonstrated that the *IFITM3*-rs12252-C genetic variant is associated with more rapid progression of HIV-1 infection.^[25]

While, there is no report about the relationship between the *IFITM3*-rs12252 genomic variants and the progression of cancer, especially for hepatocellular carcinoma. The single-nucleotide polymorphism (SNP-rs-12252-C) in *IFITM3* locus is more commonly reported in the Chinese population, and has been found in much higher homozygous frequency in patients with HCC. Until now, the effect of this association of *IFITM3*-rs12252 genetic polymorphism and the risk of HCC has not been investigated successfully. In order to address this issue, we performed this study to investigate whether the *IFITM3* rs12252 genetic polymorphism influences the risk of HCC.

2. Materials and methods

2.1. Patients

Patients with HBV related HCC were enrolled in the study, and patients with hepatitis B or HBV related cirrhosis were included as control group. The patients were from Beijing You'an Hospital, Capital Medical University between January 2009 and June 2014, and all of them were from the Han population in the northern area of China and most of them come from Beijing. HCC patients met the diagnostic criteria of HCC based on the European Association for the Study of the liver (EASL) guideline.^[26] Liver cirrhosis or chronic hepatitis B were diagnosed according to clinical characteristics, laboratory

testing, and imaging results of viral infection and hepatic damage. All cirrhosis patients were confirmed by imaging techniques without a vascular enhancing mass. Considering the comparability of the research (patients with decompensated cirrhosis and HCC patients do not receive interferon treatment and interferon can impact the expression of *IFITM3*), all patients were recruited without the use of interferon. The HCC patients and hepatitis B or cirrhosis patients were age (± 10 years) and gender matched. Patients were excluded if they had other cancers, or history of chemotherapy/radiotherapy for other cancers.

The degree of pathological differentiation of tumors and the immunohistochemistry staining of tumor tissues were defined by 2 pathologists. The tumor size was identified by CT scan and clinical staging of HCC was done according to the Barcelona Clinic Liver Cancer (BCLC) system. Among the HBV related HCC patients, we then stratified HCC patients into high, medium and low differentiation based on pathology. All patients were treated according to the EASL guideline.

The study was approved by the Beijing You'an Hospital Research Ethics Committee (Beijing, China). All the participants provided written informed consent. The methods were carried out in accordance with the approved guidelines and regulations. Consents to publish data in the form of document, images, videos, voice recordings etc. were obtained from the participants or legal representatives.

2.2. Follow-up

The HCC patients were followed up every month during the first 3 months after treatment, every 3 months at the first 2 years, every 6 months in the third year, and once a year subsequently. The patients underwent CT scan and ultrasound during the follow-up visits. The time to relapse (TTR) was defined as the time from the end of treatment to the development of new tumor diagnosed by CT scan.

2.3. DNA extraction and genotyping assays

Peripheral blood samples were collected in EDTA tube and stored at -80°C . DNA was purified from whole blood using the Pure Gene DNA Isolation kit (Gentra Systems, Detroit, MI, USA) according to the manufacturer's protocol. The region encompassing the human *IFITM3* rs12252 sequences were amplified by polymerase chain reaction (PCR). The amplification was performed using the following forward and reverse primers: 5'-GGAAACTGTTGAGAAACCGAA-3' and 5'-CATA-CGCACCTTCACGGAGT-3' (Beijing Institute of Genomics (BGI), Shenzhen, China). The PCR products were purified and sequenced on an Applied Biosystems 3730xl DNA analyzer (GATC Biotech). Single-nucleotide polymorphism was identified using Chromas (Technelysium Pty Ltd.) at the BGI, Shenzhen, China.

2.4. Laboratory examinations

Approximately 10 ml blood sample was collected from each participant during follow-up examinations. Fractionated aliquots of serum samples were frozen at -80°C before being tested. Serum samples were tested for a battery of 10 serum-markers including aspartate aminotransferase (AST), alanine transaminase (ALT), α -L-fucosidase (AFU), alpha-fetoprotein (AFP), total bilirubin (TBIL), direct bilirubin (DBIL), albumin (ALB), globulin (GLB), serum HBV DNA level and the prothrombin activity

(PTA). AST, ALT, TBIL, DBIL, ALB, GLB were tested by auto analyzer (Olympus AU5400, Japan) with commercial reagents. AFU and AFP was measured by automatic electrochemiluminescence immune assay analyzer (Roche E601, Roche, Switzerland). PTA was measured by automatic blood coagulation instrument (Stago, French). Serum HBV DNA level was quantified using a PCR (ABI7500, Life Technologies, USA).

2.5. Statistical analysis

Categorical variables were expressed as frequency and percentage. Statistical analysis of the genetic distribution data was performed using the chi-square test. For association testing under different genetic models, Fisher exact test was used since the chi-square approximation might not hold true for small sample size. Continuous variables were expressed as mean±standard deviation (SD) and Student *t* test was used to compare values between IFITM3-CT/TT and CC groups in which case data were normally distributed (evaluated with Kolmogorov-Smirnov test). Continuous variables were expressed as median (quartile), and Mann-Whitney *U* test was used when data were not normally distributed. Genetic model was analyzed using the Cochran-Armitage trend

test. For survival analysis, log-rank (Mantel-Cox) test was employed to identify the rate of relapse between patients with CT/TT and CC genotypes. Statistical test differences were considered significant if $P < .05$. Analyses were performed with the Graph Pad Prism version 5 (Graph Pad Software, LaJolla, CA, USA).

3. Results

3.1. Characteristics and laboratory information in patients with HBV related HCC

In the present study, the 336 base pairs of the *IFTM3* locus encompassing SNP rs 12252 was sequenced in the HBV related HCC group ($n=156$) and chronic hepatitis B or HBV related cirrhosis group ($n=180$). There were no differences in both groups in baseline characteristics. Table 1 showed the characteristics and laboratory information of the patients with HBV related HCC. Family history of cancer was more prevalent in HCC patients with CC genotype than those with CT/TT genotypes (25.0% vs 7.1%, $P < .05$). The tumors family history of CC genotype include 1 case of lung cancer, 1 case of gastric

Table 1
Characteristics and laboratory information in patients with HBV related HCC.

Variable	IFTM3-rs12252 genotype		P
	CT/TT (n=112)	CC (n=44)	
Gender			.460 [‡]
Male	97 (86.6)	40 (90.9)	
Female	15 (13.4)	4 (9.1)	
Age (years)	50.84±9.8	50.93±8.45	.957 [*]
Tumor family history	8 (7.1%)	11 (25.0%)	<.05 [‡]
Log DNA (IU/ml)	2.47±0.51	3.01±0.31	.374 [*]
ALT (U/L)	41.4 (27.5–77.5)	42.2 (26.2–51.4)	.775 [‡]
AST (U/L)	40.8 (30.5–68.1)	36.9 (28.7–56.4)	.429 [‡]
TBIL (μmol/L)	24.33±17.5	24.96±2.9	.871 [*]
DBIL (μmol/L)	4.1 (2.5–6.5)	3.3 (2.4–5.7)	.755 [‡]
ALB (g/L)	39.55±5.04	41.59±5.15	.058 [*]
GLB (g/L)	27.63±0.549	26.95±3.75	.414 [*]
PTA (%)	84.31±15.3	88.51±10.9	.065 [*]
AFU (U/L)	42.24±15.1	42.76±15.2	.864 [*]
AFP (ng/ml)	167.3 (28.44–480.0)	339 (32.94–818.25)	.993 [‡]
BCLC			.233 [‡]
O-A	74 (66.1)	23 (52.3)	
B	21 (18.8)	10 (22.7)	
C-D	17 (15.2)	11 (25.0)	
Diameter of tumor (cm)	3.89±0.24	4.91±0.50	<.05 [*]
Tumor number			.907 [‡]
Single	85 (75.9)	33 (75.0)	
Multiple	27 (24.1)	11 (25.0)	
Distal metastasis	18 (17.3)	11 (25.0)	.281 [‡]
Histopathological immunohistochemistry staining			
Ki67	20.62±2.244	21.32±3.184	.862 [*]
HBcAb (positive rate)	22.4%	26.8%	.663 [‡]
CD34 (positive rate)	87.9%	88.4%	.934 [‡]
Hepa (positive rate)	82.2%	88.4%	.353 [‡]
Treatment			.274 [‡]
Intervention	32 (28.6)	17 (40.5)	
Resection	54 (48.2)	19 (45.2)	
Transplantation	26 (23.2)	6 (14.3)	

AFP = alpha fetoprotein, AFU = α-L-Fucosidase, ALB = albumin, ALT = alanine aminotransferase, AST = aspartate aminotransferase, BCLC = Barcelona Clinic Liver Cancer, DBIL = direct bilirubin, GLB = globulin, HBV = hepatitis B virus, HCC = Hepatocellular carcinoma, PTA = Prothrombin Activity, TBIL = total bilirubin.

* Variables were expressed as mean±standard deviation, and *P* were calculated by *t* test.

[‡] Variables were expressed as median (quartile), and *P* were calculated by Mann-Whitney *U* test.

[‡] Variables were expressed as n (%), and *P* were calculated by chi-square test.

Table 2**Allele and genotype frequencies of IFITM3 rs12252 in hepatitis B or HBV related cirrhosis group and HBV related HCC group.**

Variable	Hepatitis B or Cirrhosis (n=180)	Total (n=156)	Tumor differentiation		
			High (n=39)	Medium (n=68)	Low (n=49)
Genotype, n (%)					
TT	36 (20.0)	28 (18.0)	11 (28.2)	12 (17.6)	5 (10.2)
CT	109 (60.6)	84 (53.8)	24 (61.5)	35 (51.5)	25 (51.0)
CC	35 (19.4)	44 (28.2)	4 (10.3)	21 (30.9)	19 (38.8)
Allele T	181 (50.3)	140 (44.9)	46 (59.0)	60 (43.5)	34 (35.4)
Allele C	179 (49.7)	172 (55.1)	32 (41.0)	78 (56.5)	62 (64.6)
Statistical analysis [#]					
Genotype [*]	P	.168	.282	.178	<.05
Allele [*]	P	.162	.163	.174	<.05

HBV=hepatitis B virus, HCC=Hepatocellular carcinoma.

[#] Compared with hepatitis B or cirrhosis group.^{*} P value were calculated by chi-square test.

cancer, and 9 cases of HCC. The tumors family history of TT/CT genotypes include 1 case of lung cancer and 7 cases of HCC). The tumor diameter of HCC patients with *IFITM3* rs12252-CC was larger than that in CT/TT patients (4.91 ± 0.5 cm vs 3.89 ± 0.24 cm, $P < .05$).

3.2. The CC genotype at single-nucleotide polymorphism 12252 was associated with HCC low differentiation

We further analyzed the relationship between the distribution of *IFITM3* rs12252 genetic variants and the differentiation of HCC. Totally, 39 high, 68 medium and 49 low differentiation HCC patients were identified. The HCC patients with high and medium differentiation had similar proportion of rs12252 genotype and allele compared with the patients with hepatitis B and cirrhosis, while, the HCC patients with low differentiation had a significantly different genotype and allele frequency compared with the patients with hepatitis B or cirrhosis ($P < .05$). A significantly higher frequency of the homozygous CC genotype was found in patients who developed low differentiation than hepatitis B or cirrhosis patients (38.8% vs 19.4%, $P < .05$). Moreover, a significantly higher frequency of the C allele was found in patients with low differentiation than hepatitis B or cirrhosis patients (64.6% vs 49.7%, $P < .05$, Table 2). The distribution of CC genotype in HCC patients with low differentiation was significantly higher than that in those with high differentiation (38.8% vs 10.3%, $P < .01$, Table 2). Furthermore, with the development of tumor differentiation, the rate of CC

genotype increased whereas TT genotype decreased ($P < .05$, Fig. 1A–B).

3.3. The recessive model has the largest odds ratio

The association of rs12252 with tumor low and medium differentiation was analyzed by comparing against the high differentiation through different genetic models (Table 3). We could explicitly evaluate, whether 1 (dominant) or 2 copies (recessive) of the CC genotype increase the risk of HCC low differentiation. All models apart from the dominant model showed significant association to tumor low differentiation. Under the recessive model, the HCC patients of CC genotype showed a threefold increase in risk for medium differentiation compared with carriers of the TT/CT [$P < .05$, OR (95%CI)= 3.910 (1.231–12.413)], and a 5-fold increase in risk for low differentiation compared with HCC patients of TT/CT [$P < .01$, OR (95%CI)= 5.542(1.697–18.095)].

3.4. More rapid tumor progression in patients with CC genotype

Typically, the course of disease runs through chronic hepatitis and cirrhosis before the development of HBV related HCC. However in few cases, it was evident that, HCC developed directly from chronic hepatitis without any history of liver cirrhosis. The frequency of development of HCC from chronic hepatitis was higher in patients carrying CC genotype than the patients with CT/TT genotypes (47.7% vs 23.2%, $P < .01$,

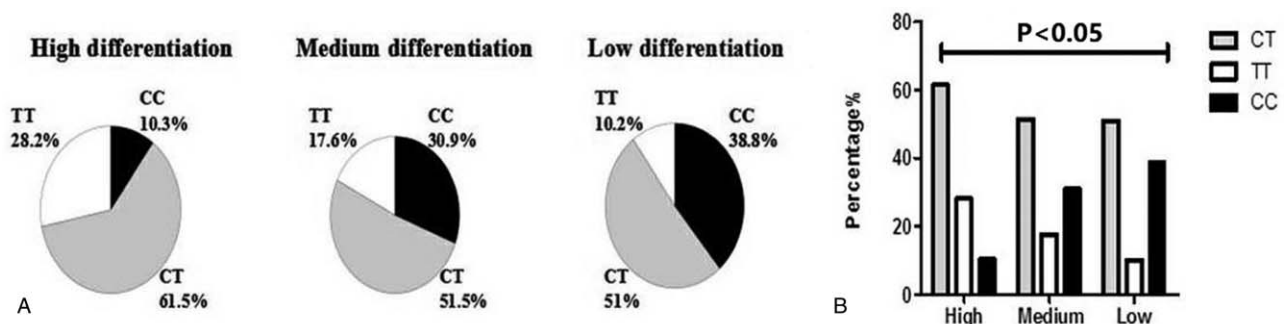


Figure 1. CC genotype of IFITM3-12252 was associated with tumor differentiation in HCC patients. (A) Genotype frequencies obtained from various degrees of HCC differentiation. (B) Proportion of HCC patients carrying CC, CT and TT genotype in various degrees of tumor differentiation.

Table 3**Association of rs12252 to HCC tissue differentiation under different genetic models.**

Genetic model	Tumor differentiation	P (χ^2 approximately)			OR (95%CI)
General/genotypic		TT	CT	CC	
	High (%)	11 (28.2)	24 (61.5)	4 (10.3)	
	Medium (%)	12 (17.6)	35 (51.5)	21 (30.9)	
Additive	Low (%)	5 (10.2)	25 (51.0)	19 (38.8)	
		T	C		
	High (%)	46 (59)	32 (41)		
Allelic/multiplicative	Medium (%)	59 (43.4)	77 (56.6)		
	Low (%)	35 (35.7)	63 (64.3)		
		CT/TT	CC		
Recessive	High (%)	35 (89.7)	4 (10.3)		
	Medium (%)	47 (69.1)	21 (30.9)		
	Low (%)	30 (61.2)	19 (38.8)		
Dominant		TT	CC/CT		
	High (%)	11 (28.2)	28 (71.8)		
	Medium (%)	12 (17.6)	56 (82.4)		
	Low (%)	5 (10.2)	44 (89.8)		

HCC = Hepatocellular carcinoma.

* Medium differentiation compared with high differentiation.

Low differentiation compared with high differentiation.

Fig. 2A). Moreover, the patients with CC genotype also showed bigger tumor size than CT/TT genotypes ($14.70 \pm 2.57 \text{ cm}^3$ vs $8.646 \pm 1.05 \text{ cm}^3$, $P < .05$, Fig. 2B). The percentage of tumors with vascular thrombosis was higher in CC genotype than CT/TT

genotypes (27.9% vs 7.1%, $P < .01$, Fig. 2C). Similarly, the distribution of low differentiation was higher in HCC patients with CC genotype than those with CT/TT genotypes ($P < .05$, Fig. 2D).

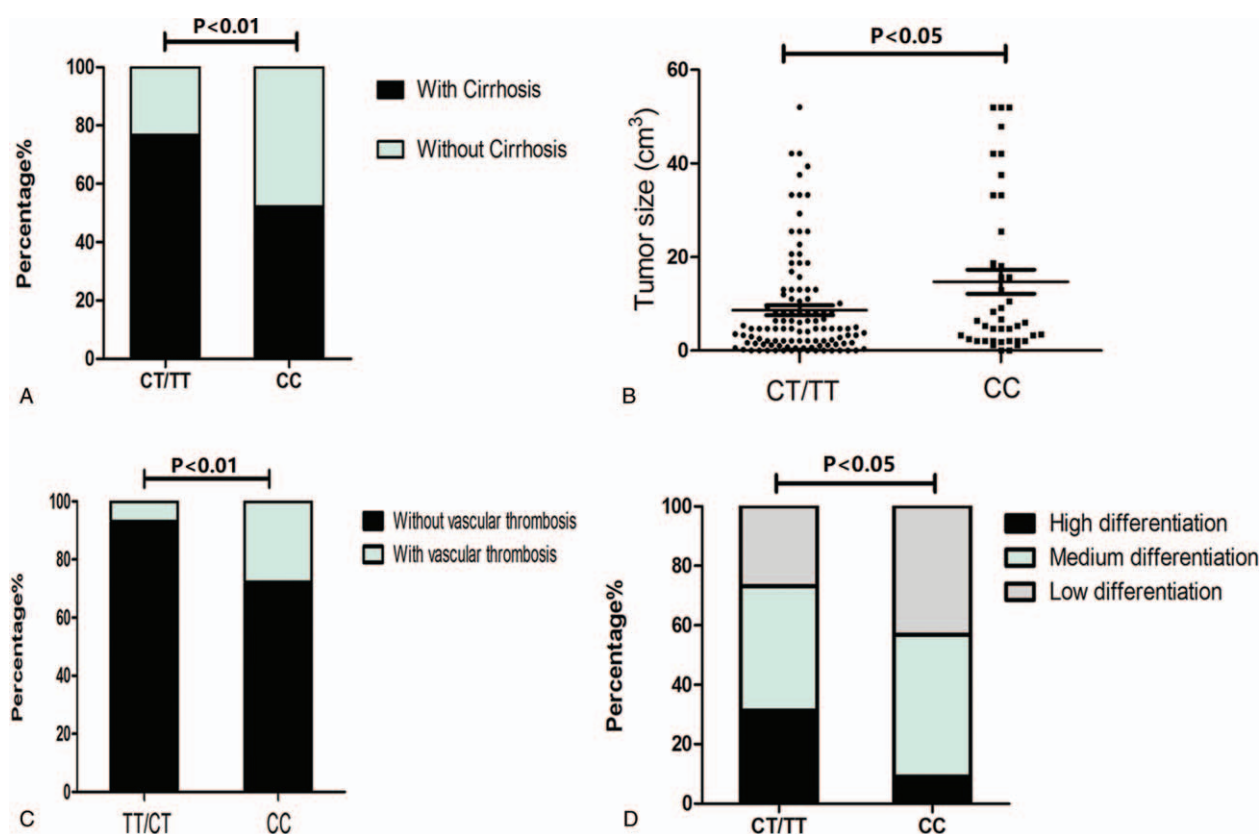


Figure 2. More rapid tumor progression in patients with CC genotype. (A) The rate of HCC developing without cirrhosis comparison between the HCC patients with CT/TT genotypes and with CC genotype. (B) The tumor size comparison between the HCC patients with CT/TT genotypes and with CC genotype. (C) The percentage with vascular thrombosis comparison between the HCC patients with CT/TT genotypes and with CC genotype. (D) The distribution of tumor differentiation comparison between the HCC patients with CT/TT genotypes and with CC genotype.

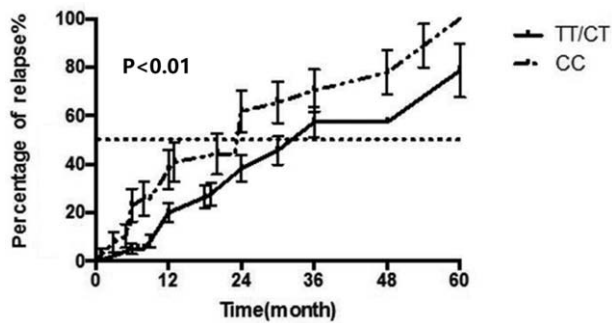


Figure 3. The relapse rate comparison between the HCC patients with CT/TT genotypes and with CC genotype (The bar means the average \pm SE of the relapse rate).

3.5. The higher relapse rate in HCC patients with CC genotype

The tumor relapse risk was significantly increased in patients carrying the CC genotype after treatment. The time of tumor relapse after receiving treatment was calculated and the tumor relapse rate was compared between the different genotypes (Fig. 3). Overall, 12 and 24 months after treatment the relapse rate were 24.67% and 44.5%, respectively. The median TTR of HCC with CT/TT genotypes and with CC genotype was 36 months (95%CI: 28.35–43.66), 24 months (95%CI: 19.90–28.11), respectively. The difference in relapse time of HCC after receiving treatment was significant ($P < .01$). Five years after treatment, the risk of relapse in CC genotype HCC patients was 1.5 times higher than that with CT/TT genotypes (95%CI: 0.93–2.43). There was a higher percentage of relapse in patients with CC genotype when compared with CT/TT genotypes in 5 years ($P < .01$).

4. Discussion

In this current study, we successfully showed that IFITM3-rs12252-C genetic polymorphism is associated with progression of chronic hepatitis to HCC. Initially, by comparing the HCC pathological differentiation, tumor size and recurrence between IFITM3 variants, provided novel information about the effects of genetic polymorphism in IFITM3-rs12252 on the susceptibility and clinic pathological development of HCC in Chinese patients. IFITM3-rs12252 CC genotype was strongly associated with a low tumor differentiation state and bigger tumor mass. It is the first time to investigate the relationship between the genetic polymorphism of IFITM3-rs12252 and progression of HCC.

Recent studies have demonstrated that members of IFITM family may be crucial factors in carcinogenesis. IFITM1 and IFITM3 expression levels were much higher in astrocytoma cells than in normal astrocytes.^[27] Furthermore, up-regulation of IFITM1 affected the proliferation and invasion of glioma.^[28] IFITM2 also played a critical role in regulating cell death through a p53-independent pro-apoptotic signaling pathway.^[29] Higher levels of IFITM3 could be detected in colon tumors than in normal colon tissue, indicating a potential role of IFITM3 in carcinogenesis. Another analysis studying IFITM3 gene polymorphisms, showed that C>T polymorphisms in the IFITM3 gene could be associated with susceptibility to UC (ulcerative colitis).^[30]

In the present study, a significantly higher frequency of the CC genotype was observed in low differentiation HCC patients

compared to hepatitis and liver cirrhosis. Furthermore, a significantly positive tumor history was observed in HCC patients with CC genotype than CT/TT genotypes, which can be contributed to the genetic susceptibility of HCC. We further noticed that the higher frequency of CC genotype was greatly associated with this verity and intensity of HCC, further indicating a strong association between CC genotype and HCC progression. Collectively, those patients with the CC genotype had a significantly higher rate of low differentiation, faster tumor progression including bigger tumor size, higher rate of vascular thrombosis and higher rate of tumor relapse than CT/TT patients.

The incidence of HCC shows a clear geographical variation with higher annual incidence in Asia than in Western countries.^[31] It is intriguing that the CC genotype is rare in Northern Europeans and common in Asian populations.^[22] Our results might indicate possible explanations on, why there are more HCC patients in China than Western countries.

It was well documented that, IFITM3 can function as a cancer-promoting gene, and could exert profound influences on cell proliferation, migration and invasion. Through modulation of the Wnt/ β -catenin signaling pathway, IFITM3 is implicated in the G0/G1 checkpoint to control the cell cycle in tumor tissue development.^[16] It is still not clear how SNP rs12252 affects the expression of IFITM3. It has been predicted that a mutation may result in expression of truncated IFITM3, which lacks the first 21 amino acids at the N-terminus.^[32] Previous mechanistic studies have all been based on this assumption, and the conclusions have been rather controversial.^[21] In order to study the mechanism of IFITM3 in HCC, we also conducted related cell research (under submission).

However, this study has some limitations. First, the sample size was small. Second, we could not follow the survival rate of patients with HCC, and hence could not analyze the IFITM3 rs12252 SNP on the survival rate of patients with HCC. Third, the patients with CC genotype have higher HCC tumor family history, they may be congenital HBV carriers through mother-to-child transmission, and the duration of HBV infection is longer, which may interfere with the study of CC genotype susceptibility to HCC. An extensive future study with a large sample size, prolonged period of follow up and with an elaborate analysis of the survival rate may help to derive more convincing conclusions.

5. Conclusion

Our data shows a clear correlation between the variants of IFITM3-rs12252 and the progression of HCC in Chinese population. IFITM3-rs12252 CC genotype is found strongly associated with the malignant degree of HCC, including tumor differentiation and tumor size. Additionally, patients with CC genotype seem to progress faster and easier to recrudescence. Based on our report, patients of the CC genotype with liver disease should undergo early prevention of HCC and early treatment of HCC to prevent the disease from worsening.

Author contributions

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