Cancer Immunol Immunother (2009) 58:1433–1440
DOI 10.1007/s00262-009-0660-4

ORIGINAL ARTICLE

−509C>T polymorphism in the TGF-β1 gene promoter, impact on the hepatocellular carcinoma risk in Chinese patients with chronic hepatitis B virus infection

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Received: 18 November 2008 / Accepted: 7 January 2009 / Published online: 25 January 2009

Abstract Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. The risk for developing HCC increases with severity of inflammation and fibrosis. Transforming growth factor-β1 (TGF-β1) is most frequently upregulated in tumor cells. The most studied −509C>T polymorphism of TGF-β1 gene has been associated with colorectal, gynecologic, and lung cancers. To assess whether this polymorphism in TGF-β1 gene is associated with susceptibility to and/or clinicopathologic characteristics of HBV-related HCC, a total of 575 patients with chronic HBV infection and 299 healthy volunteers with no evidence of recent or remote HBV infection were prospectively enrolled. The patients were divided into two groups: those without ($n = 196$) and those with HCC ($n = 379$). These 379 HCC patients with chronic HBV infection were designated as cases, the remaining 196 patients without HCC and 299 healthy volunteers served as disease and healthy controls, respectively. −509C>T polymorphism in the TGF-β1 gene promoter was studied using restriction fragment-length polymorphism. In addition, tumor tissues of liver ($n = 60$) were obtained from the studied HCC patients for measurement of TGF-β1 mRNA expression levels. We also assessed the plasma TGF-β1 levels of HBV patients without ($n = 94$) or with HCC ($n = 136$) and healthy subjects ($n = 120$). In our study group, the risk of HCC in Chinese patients with HBV infection was significantly lower with the TT genotypes than in those with the CC genotypes at position −509 of TGF-β1 gene ($P = 0.01$). In addition, in the case group, patients with the CC genotype had a statistically significant higher median plasma TGF-β1 or liver tumor tissue TGF-β1 mRNA level compared with the individuals with the TT genotype. However, in a subsequent analysis of the association between this polymorphism and clinicopathological characteristics including tumor number, size, grade, stage, and invasiveness, there was no significant difference in both the distribution of genotype or allelic frequency within HCC patients, indicating that −509C>T exchange in TGF-β1 gene may play an important role in the occurrence, not the progression of HBV-related HCC through influencing plasma concentrations of TGF-β1 or TGF-β1 mRNA expression of liver tumor tissue.

Keywords Chronic hepatitis · HBV · Hepatocellular carcinoma · Transforming growth factor-β1 · Single-nucleotide polymorphism

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide [12]. Major etiologic

factors associated with HCC include infection with hepatitis C (HCV) and hepatitis B (HBV) viruses, excess alcohol intake and aflatoxin B1 exposure [2, 8]. It ranks the second among all malignancies in China [13, 18], where HBV infection is highly endemic and where chronic infection is usually acquired in early infancy [21]. The risk for developing HCC increases with severity of inflammation and fibrosis [17]. However, the host genetic factors that affect the HCC association remain unclear.

It is becoming increasingly apparent that most population-attributable cancer heritability is related not to the rare deleterious gene defects but to polymorphic variations in the DNA sequence [14]. The most common sequence variation in the human genome is the stable substitution of a single base; the single-nucleotide polymorphism (SNP). Cytokines, as the product of host responses to inflammation, play an important role in the defense against viral infections and carcinogenesis [11]. Transforming growth factor-β (TGF-β) is encoded by three different genes—TGF-β1, TGF-β2, and TGF-β3. Of these, TGF-β1 is most frequently upregulated in tumor cells. The human TGF-β1 gene is located on chromosome 19q13.1 and the variation among individuals for TGF-β1 production has been considered to be under genetic control [7]. Up to date, several TGF-β1 polymorphisms have been identified: three upstream of exon 1, an insertion/deletion of a cytosine residue within the 5′ untranslated region, and three in the coding region of the gene, which result in amino acid substitutions. The most studied −509C>T polymorphism is located within an YY1 consensus binding site [15] and has been associated with colorectal, gynecologic, and lung cancers [1, 4, 9]. Our previous study demonstrated that although there was no significant difference in genotyping distribution between liver cirrhosis patients and control subjects at −509 in Chinese, the C allele at −509 could play important roles in progression of liver cirrhosis. Moreover, the −509C allele has been associated with increased TGF-β1 plasma levels [20]. To assess whether this polymorphism in TGF-β1 gene is associated with susceptibility to and/or clinicopathologic characteristics of HBV-related HCC, we performed a case-control association study in Chinese samples.

Materials and methods

Patients

A total of 575 patients with chronic HBV infection and 299 healthy volunteers with no evidence of recent or remote HBV infection were periodically enrolled between December 2005 and October 2007 at the Eastern Hepatobiliary Hospital. They were regularly followed with measurements of serum ALT, AST, γ-GT, and HBV markers such as HBsAg, HBeAg, and anti-HBeAb using commercially available radioimmunoassay kits every month, and ultrasonography or computed tomography of the liver every 3 months. All patients were positive for HBsAg and did not have any other types of liver diseases such as chronic hepatitis C, alcoholic liver diseases, autoimmune liver diseases, or metabolic liver diseases. The patients were divided into two groups: those without (n = 196) and those with HCC (n = 379). These 379 HCC patients with chronic HBV infection were designated as cases, the remaining 196 patients without HCC and 299 healthy volunteers served as disease and healthy controls, respectively. The diagnosis of HCC was confirmed by several imaging techniques, including abdominal US, computed tomography (CT), magnetic resonance imaging and histologically on liver biopsy obtained by guided US. The following clinicopathologic characteristics of HCC patients were obtained at the time of whole blood collection: tumor number, size, growth phase, stage, and invasiveness. Age, sex, and laboratory values used in the Child classification—total bilirubin, albumin, prothrombin time—and additional levels of platelets, alpha fetoprotein, CEA, CA 19-9 were also obtained from all studied subjects. In addition, tumor tissues of liver (n = 60) were obtained from the studied HCC patients for measurement of TGF-β1 mRNA expression level.

Analysis of TGF-β1 gene polymorphisms

Genomic DNA was extracted from peripheral blood with the Qiagen QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). TGF-β1 gene polymorphism genotyping at positions −509 in the promoter region, was carried out according to the restriction fragment-length polymorphism (RFLP) method described by Carturan [3] without knowing the subjects' case or control status. The PCR reactions were performed in a total volume of 20 μL containing 10 ng of genomic DNA, 1 × PCR buffer, 1.5 mM $MgCl_2$, 5% DMSO, 5 pmol of each primer, 50 mM dNTP, and 0.4 unit of Taq polymerase (Takara, Japan). This PCR reaction mixture was initially denatured at 95°C for 10 min, followed by 35 cycles of 94°C for 30 s, 58°C for 30 s, 72°C for 45 s, and a final extension cycle at 72°C for 10 min. The used primers were listed in Table 1. The 808 bp PCR products were digested with the Eco81I (SauI) (Takara, Japan) and separated by polyacrylamide gel electrophoresis: the C allele was cut into 617 and 191 bp fragments (the T allele was not digested). The results of the RFLP analysis were confirmed by sequencing 18 study subjects for the C to T substitution at position −509 in the promoter region of the TGF-β1 gene, with perfect correspondence.

Table 1 Primer sequences for RFLP and real-time PCR

	Primer and probe sequence
RFLP	Sense: 5′-CCCGGCTCCATTTCCAGGTG-3′
	Antisense: 5′-GGTCACCAGAGAAAGAGGAC-3′
Quantification of TGF-β1 mRNA	Sense: 5′-GCGTCTGCTGAGGCTCAAG-3′
	Antisense: 5′- CAAAAGATAACCACTCTGGCGA-3′
	Probe: 5′-FAM-ACCAGAAATACAGCAACAATTCCTGGCGA-TAMRA-3′
Quantification of β-actin mRNA	Sense: 5′-CCTGGCACCCAGCACAAT-3′
	Antisense: 5′-GCTGATCCACATCTGCTGGAA-3′
	Probe: 5′-FAM-ATCAAGATCATTGCTCCTCCTGAGCGC-TAMRA-3′

Quantification of TGF-β1 mRNA expression by real-time PCR

Total RNA was isolated from tumor tissues of liver with the TRIzol Reagent (Invitrogen, USA) and subsequently treated with DNase I (Takara, Japan). Two hundred nanograms of total RNA from each sample was used to synthesize complementary DNA (cDNA). The obtained cDNA was amplified by a regular PCR and cloned into pMD18-T vector to create a quantitative standard control as described previously [16]. The level of TGF-β1 mRNA present is expressed as the ratio of TGF-β1 PCR product to β-actin PCR product. All reactions were performed in duplicate and experiments were repeated to ensure accuracy. The primers and probes used in real-time PCR for quantification of TGF-β1 and β-actin mRNA expression were listed in Table 1.

Measurement of plasma TGF-β1 concentration

The concentration of TGF-β1 in plasma was determined for 120 individuals selected from healthy subjects, 94 individuals from HBV patients without HCC, and 136 individuals from HBV patients with HCC, in a double antibody sandwich ELISA (Jing Mei, Shanghai, China) according to the manufacturer's instructions.

Statistical analysis

Deviation of Hardy–Weinberg equilibrium was tested by using the χ^2 test for goodness of fit. The significance of the differences between the genotype and allelic frequencies in the case and control groups were determined using 2×2 tables and a standard χ^2 test. Association was expressed as odds ratios (OR) as risk estimates with 95% confidence intervals (95% CI). χ^2 test was used to perform for the association of clinicopathologic characteristics and TGF-β1 −509 genotypes and allelic frequencies of HCC patients with chronic HBV infection. Power calculations were performed using the software program Power And Precision (Biostat, Inc., USA). *t* test was applied for the statistical analysis of association between plasma TGF-β1 concentration or TGF-β1 mRNA expression level and genotype. All statistical tests were two-sided, and a probability level of $P < 0.05$ was considered to be statistically significant. Data analysis was done using SPSS 11.0 software (SPSS, Inc.).

Results

General characteristics of the subjects

A total of 575 local ethnic Han Chinese subjects with chronic HBV infection and 299 healthy volunteers were enrolled in our study. General characteristics of the subjects are summarized in Table 2. There were no significant differences in terms of distributions on age and gender within the groups. Patients with HCC had poor status of liver function such as higher level of TBIL, ALT, AST, γ-GT, CEA, CA 19-9, and lower level of albumin, platelets.

With regard to clinicopathologic characteristics of the HCC patients, single-tumor was found in 250 patients (66.0%), 15 patients (4.1%) had lymph node metastasis. Tumor diameter was <5 cm in 143 patients (37.7%). The histologic grade of HCC was grade I–II in 41 cases, grade III–IV in 336 cases. Tumor stage was obtained according to the TNM criteria, 188, 53, and 120 patients was in stage I, II, III, and only 6 patients was in stage IV. According to the Child classification, 267, 97, and 5 patients was with mild (grade A), moderate (grade B), and severe (grade C) liver damage, respectively.

TGF-β1 −509 polymorphisms of the subjects

Genotype and allelic frequencies of TGF-β1 gene polymorphism (rs1800469) of the patients and healthy volunteers are presented in Table 3. The genotype distributions were in Hardy–Weinberg equilibrium in each group studied. Of 575 patients with chronic HBV infection, 379 also had HCC. As shown in Table 3, the CT genotype at position −509 of TGF-β1 gene prevailed in all three groups, the CC and TT genotype distribution of the TGF-β1 −509 polymorphism was significantly different in those with HCC and without HCC or healthy controls; the risk of HCC was

Table 2 General characteristics of the subjects

	Healthy control (n = 299)	HBV patients without HCC (n = 196)	HBV patients with HCC (n = 379)	P value (case vs. healthy control)	P value (case vs. disease control)
Age, median, y	55	56	58	0.56	0.38
Men, n (%)	215 (72)	143 (73)	284 (75)	0.38	0.62
Laboratory values mean (SD)					
Total bilirubin, μmol/L	10.1 (2.9)	13.8 (5.8)	19.6 (11.7)		
Albumin, g/L	49.4 (3.3)	46.7 (2.9)	40.3 (4.6)		
Prothrombin time, s	10.2 (1.1)	10.8.3 (1.1)	11.2 (1.2)		
Platelets $\times 10^9$/L	205.0 (43.1)	182.6 (52.0)	136.3 (68.4)		
Alanine aminotransferase, U/L	18.9 (9.2)	26.2 (11.0)	128.9 (268.1)		
Aspartate aminotransferase, U/L	20.2 (4.8)	23.2 (6.8)	130.2 (270.9)		
γ-Glutamyltranspeptidase, U/L	27.2 (18.9)	29.8 (28.2)	114.2 (138.2)		
Carcinoembryonic antigen, μg/L	1.6 (0.9)	2.8 (1.2)	5.1 (9.2)		
Carbohydrate antigen 19-9, U/mL	11.6 (8.6)	9.1 (3.9)	30.8 (39.6)		
n (%)					
Total bilirubin > 20 μmol/L	3 (1.0)	29 (14.8)	136 (35.9)		
Alpha fetoprotein > 200 μg/L	0 (0)	0 (0)	222 (58.6)		
Platelets < 100 $\times 10^9$/L	3 (1.0)	9 (4.6)	120 (31.7)		
HBeAg	0 (0)	56 (28.6)	287 (75.7)		
Anti HBeAg	0 (0)	155 (79.1)	130 (34.3)		
Tumor number (n = 379) n (%)					
Single			250 (66.0)		
Multiple			129 (34.0)		
Tumor size (n = 379) n (%)					
<5 cm			143 (37.7)		
≥5 cm			236 (62.3)		
Tumor grade (n = 377) n (%)					
I and II			41 (10.9)		
III and IV			336 (89.1)		
Tumor stage (n = 367) n (%)					
I			188 (51.2)		
II			53 (14.5)		
III			120 (32.7)		
IV			6 (1.6)		
Tumor invasiveness (n = 368) n (%)					
Without lymph node			353 (95.9)		
With lymph node			15 (4.1)		
Child-pugh grade (n = 369) n (%)					
A			267 (72.4)		
B			97 (26.3)		
C			5 (1.3)		

Tumor stage was obtained according to the TNM criteria

significantly lower among patients with the TT genotype or carrying at least one T allele at position −509 than among the patients with the CC genotype ($P = 0.01$ and 0.04, respectively). The power based on sample size and allelic distribution exceeds 80% when the effect size is set at 10% (assuming that alpha, two-tailed, is set at 0.05), indicating that the sample size was large enough to detect association.

TGF-β1 mRNA expression in HCC patients with chronic HBV infection

In the real-time PCR, fluorescence was detected in all tissue specimens from HCC patients with chronic HBV infection (Table 4). The mean value of TGF-β1 mRNA expression was 0.88 (±0.60, ranged from 0.48 to 2.98) for patients

Table 3 Genotype and allelic frequencies of TGF-β1 gene polymorphism in cases and controls

TGF-β1 polymorphism	Healthy control (n = 299)	HBV patients without HCC (n = 196)	HBV patients with HCC (n = 379)	Odds ratio (95% CI) (case vs. healthy control)	Odds ratio (95% CI) (case vs. disease control)	P value (case vs. healthy control)	P value (case vs. disease control)
Genotypes							
CC	50 (16.7)	31 (15.8)	89 (23.5)	1 (Reference)	1 (Reference)	–	–
CT	156 (52.2)	101 (51.5)	198 (52.2)	0.71 (0.48–1.07)	0.68 (0.43–1.10)	0.11	0.13
TT	93 (31.1)	64 (32.7)	92 (24.3)	0.56 (0.35–0.87)	0.50 (0.30–0.84)	0.01	0.01
CT + TT	249 (83.3)	165 (84.2)	290 (76.5)	0.65 (0.45–0.96)	0.61 (0.39–0.96)	0.04	0.04
Alleles							
C	256 (42.8)	163 (41.6)	376 (49.6)	1 (Reference)	1 (Reference)	–	–
T	342 (57.2)	229 (58.4)	382 (50.4)	0.76 (0.61–0.94)	0.72 (0.57–0.93)	0.01	0.01

Data are presented as n (%)

Table 4 TGF-β1 mRNA expression of liver tumor tissue in HCC patients with chronic HBV infection

TGF-β1 polymorphism	Value		P value
	Mean ± SD	Range	
Genotypes			
CC (n = 21)	0.88 ± 0.60	0.48–2.98	–
CT (n = 23)	0.55 ± 0.42	0.46–1.28	0.10
TT (n = 16)*	0.28 ± 0.16	0.04–0.25	0.0002
CT + TT (n = 39)*	0.44 ± 0.29	0.04–1.28	0.006

* Compared with genotype CC at position −509, $P < 0.05$

with CC genotype, 0.55 (±0.42, ranged from 0.46 to 1.28) for patients with CT genotype, and 0.28 (±0.16, ranged from 0.04 to 0.25) for patients with TT genotype. TGF-β1 mRNA expression levels were significantly higher in CC patients than TT patients or patients carrying at least one T allele (0.88 vs. 0.28 or 0.44; P = 0.0002 or 0.006).

TGF-β1 gene polymorphisms and plasma TGF-β1 concentration

We assessed the plasma TGF-β1 levels of HBV patients without (n = 94) or with HCC (n = 136) and healthy subjects (n = 120). As shown in Table 5, in HBV patients without or with HCC, the plasma concentration of TGF-β1 was 10.25 ± 6.72 and 10.14 ± 6.82 ng/ml, respectively, slightly higher than that in healthy subjects (9.97 ± 4.59 ng/ml). In addition, the TGF-β1 concentration on plasma among disease (P = 0.26) or healthy controls (P = 0.80) did not show any significant difference between CC and TT genotypes at position −509, however, the concentration of plasma TGF-β1 was statistically higher in patients with CC genotype (12.83 ± 8.72 ng/ml) than in those with TT genotype (8.98 ± 5.80 ng/ml) in the case group (P = 0.04).

TGF-β1 gene polymorphisms and clinicopathological characteristics

Genetic polymorphisms in the TGF-β1 gene promoter were shown to interfere with the transcriptional activity of this gene [10], and TGF-β1 seems to affect the development of HCC. Analysis of the association between a more aggressive disease behavior (malignant or moderate/severe disease) and the TGF-β1 gene polymorphism is described in Table 6. There was no significant difference between the distribution of genotype or allelic frequency and tumor grade (well or moderately vs. poorly differentiation). However, we observed that high tumor grade was more frequent

Table 5 TGF-β1 gene polymorphisms and plasma TGF-β1 concentrations in patients and healthy controls

TGF-β1 polymorphism	Healthy control (n = 120)			P value	HBV patients without HCC (n = 94)			P value	HBV patients with HCC (n = 136)			P value
	Value				Value				Value			
	n	Mean ± SD	Range		n	Mean ± SD	Range		n	Mean ± SD	Range	
Genotypes												
CC	24	10.43 ± 5.54	1.86–28.65	–	22	11.86 ± 7.81	2.34–29.38	–	26	12.83 ± 8.72	2.10–36.20	–
CT	54	9.64 ± 4.51	2.02–23.18	0.51	37	10.04 ± 4.89	2.28–27.87	0.33	74	9.75 ± 6.36	1.58–31.64	0.06
TT	42	10.13 ± 4.19	2.01–28.91	0.80	35	9.46 ± 7.62	1.43–36.46	0.26	36	8.98 ± 5.80	2.02–24.22	0.04

Table 6 Clinicopathological characteristics and TGF-β1 −509C>T genotype and allelic frequencies of HCC patients with chronic HBV infection

	Genotype			*P* value		Allele		*P* value
	CC	CT	TT	CT versus CC	TT versus CC	C	T	
Tumor number								
Single	59 (23.6)	135 (54.0)	56 (22.4)	0.79	0.54	253 (50.6)	247 (49.4)	0.49
Multiple	30 (23.3)	63 (48.8)	36 (27.9)			123 (47.7)	135 (52.3)	
Tumor size								
<5 cm	36 (25.2)	77 (53.8)	30 (21.0)	0.90	0.28	149 (52.1)	137 (47.9)	0.82
≥5 cm	53 (22.4)	121 (51.3)	62 (26.3)			227 (53.0)	201 (47.0)	
Tumor grade								
I and II	14 (34.2)	21 (51.2)	6 (14.6)	0.24	0.06	49 (59.8)	33 (40.2)	0.06
III and IV	74 (22.0)	176 (52.4)	86 (25.6)			324 (48.2)	348 (51.8)	
Tumor stage								
I and II	54 (22.4)	129 (53.5)	58 (24.1)	0.34	0.76	237 (49.2)	245 (50.8)	0.76
III and IV	33 (26.2)	61 (48.4)	32 (25.4)			127 (50.4)	125 (49.6)	
I	44 (23.4)	97 (51.6)	47 (25.0)	1.00	0.89	185 (49.2)	191 (50.8)	0.88
II and III and IV	43 (24.0)	93 (52.0)	43 (24.0)			179 (50.0)	179 (50.0)	
Tumor invasiveness								
Without lymph node	82 (23.2)	182 (51.6)	89 (25.2)	0.55	0.27	346 (49.0)	360 (51.0)	0.27
With lymph node	5 (33.3)	8 (53.3)	2 (13.3)			18 (60.0)	12 (40.0)	
Child-pugh grade								
A	60 (22.5)	152 (56.9)	55 (20.6)	0.07	0.27	272 (50.9)	262 (49.1)	0.22
B + C	27 (26.5)	39 (38.2)	36 (35.3)			93 (45.6)	111 (54.4)	

in HCC patients with TT genotype or T allele compared with CC genotype or C allele.

Since several previous reports addressed that the TGF-β1 polymorphism is associated with tumor invasiveness and prognosis, we analyzed the possible association between lymph node metastasis, representing tumor invasiveness, and TGF-β1 genotype or allelic frequency. Among 379 HCC patients with chronic HBV infection, lymph node status could be evaluated in 368 (97.1%) patients. Frequencies of lymph node metastasis were 5 of 15 (33.3%) in HCC patients with CC genotype, 8 of 15 (53.3%) in HCC patients with CT genotype and 2 of 15 (13.3%) in patients with TT genotype. There was still no significant difference in both the distribution of genotype or allelic frequency between HCC patients without and with lymph node metastasis. Since lymphatic metastasis can be largely influenced by tumor stage, we analyzed the association between TGF-β1 genotype and tumor stage according to the TNM criteria (Table 6). However, both the distribution of genotype or allelic frequency were similar between TNM I and II group versus TNM III and IV group ($P = 0.76$ for genotype and $P = 0.76$ for allele) or TNM I group versus II, III and IV group ($P = 0.89$ for genotype and $P = 0.88$ for allele).

Finally, we stratified patients according to the Child classification. In 369 HCC patients, the severity of liver damage was very similar in patients with C and T allele, without statistical significance ($P = 0.22$). However, we found a significant association between TGF-β1 genotype and liver damage (Table 6). We also found that the TGF-β1 −509T allele is in non-significantly higher numbers in multiple-tumor HCC patients compared with single-tumor population.

Discussion

HCC is the major primary liver cancer. It is the fifth most common cancer and the third cause of cancer-related death worldwide. The estimated annual number of cases exceeds 500,000. The main risk factors for HCC are cirrhosis of any etiology, but mainly due to HBV, HCV, and chronic alcohol consumption. In Eastern Asia, Middle, and Western Africa where HBV infection is hyperendemic, HCC is a public health problem and the incidence is more than 20 per 100,000 per year. The incidence of HCC depends on several factors, including age over 50 years, male sex, etiology, and severity stage of the cirrhosis [5]. Anyway, genetic factors are likely to modify the risk of HCC; however, the genetic factors that determine progression to HCC remain mostly to be investigated.

TGF-β1 regulates cell growth, differentiation, and function. It is a multifunctional cytokine known to induce the expression of collagen genes and to provoke extracellular

matrix fibrosis [10]. In the past decades, with the identification of SNPs within the TGF-β1 gene, much effort had been made to determine the correlation between host genetic background and liver disease, including liver fibrosis and cirrhosis [6, 19, 20]. Our previous study show that no polymorphism at codon25, 263 or positions −800, −988 in Chinese, and a complete linkage disequilibrium was shown between −509 and codon10, therefore, we focused on whether −509C>T exchange in TGF-β1 gene is associated with susceptibility to and/or clinicopathologic characteristics of HBV-related HCC in Chinese samples.

Results of this study strongly suggest that TGF-β1 gene single-nucleotide polymorphism analysis and plasma TGF-β1 or TGF-β1 mRNA of liver tumor tissue measurements may serve as novel markers for the occurrence of HCC in patients with chronic HBV infection in Chinese. However, in a subsequent analysis of the association between the polymorphism and clinicopathological characteristics including tumor number, size, grade, stage, and invasiveness, there was no significant difference in both the distribution of genotype or allelic frequency within HCC patients, indicating that although −509C>T exchange in TGF-β1 gene was associated with susceptibility to HBV-related HCC, it had little influence on the progression of HCC. This fact is not unexpected since HCC is a multifactorial disease whose development is dependent on several genetic and environmental factors.

The control mechanisms of the TGF-β1 concentration in plasma are poorly understood. Evidences show that the concentration of active TGF-β1 may be predominantly under genetic control. Grainger et al. [7] have observed that the −509C>T polymorphism is significantly associated with the TGF-β1 plasma concentration. In their study, the presence of the T allele at −509 is associated with higher concentrations of TGF-β1 and this increase in concentration is higher among individuals homozygous for T than in heterozygotes, suggesting a dose-response effect of the T allele on circulating concentrations of TGF-β1. In our study, the exact opposite was found suggesting that the presence of a C rather than a T residue at position –509 of the TGF-β1 gene may lead to higher plasma TGF-β1 in HCC patients with chronic HBV infection in Chinese samples. This might be the reason to explain why the TGF-β1 −509C>T TT genotype may confer protection from HBV-related HCC. However, the reason for the apparent discrepancy on the genetic control of plasma TGF-β1 remains unclear. It might be attributable to the difference in genetic backgrounds and environmental factors of the study subjects.

In conclusion, our data support the hypothesis that TGF-β1 polymorphisms contribute to the occurrence of HCC in patients with chronic HBV infection, and this effect may carry out through the modification of plasma TGF-β1 or liver tumor tissue TGF-β1 mRNA levels. However, this polymorphism may not contribute to the progression of HBV-related HCC in Chinese population.

Acknowledgments The research in the author's laboratory was supported by National Natural Science Foundation of China, No. 30770994.

References

1. Berndt SI, Huang WY, Chatterjee N, Yeager M, Welch R, Chanock SJ, Weissfeld JL, Schoen RE, Hayes RB (2007) Transforming growth factor beta 1 (TGFB1) gene polymorphisms and risk of advanced colorectal adenoma. Carcinogenesis 28:1965–1970
2. Brunetto MR, Oliver F, Colombatto P, Bonino F (1995) Hepatocellular carcinoma and infections with multiple hepatitis viruses. Princess Takamatsu Symp 25:61–66
3. Carturan S, Roccatello D, Menegatti E, Di Simone D, Davit A, Piazza A, Sena LM, Matullo G (2004) Association between transforming growth factor beta1 gene polymorphisms and IgA nephropathy. J Nephrol 17:786–793
4. De Ruyck K, Van Eijkeren M, Claes K, Bacher K, Vral A, De Neve W, Thierens H (2006) TGFbeta1 polymorphisms and late clinical radiosensitivity in patients treated for gynecologic tumors. Int J Radiat Oncol Biol Phys 65:1240–1248
5. Ganne-Carrié N, Chastang C, Chapel F, Munz C, Pateron D, Sibony M, Dény P, Trinchet JC, Callard P, Guettier C, Beaugrand M (1996) Predictive score for the development of hepatocellular carcinoma and additional value of liver large cell dysplasia in Western patients with cirrhosis. Hepatology 23:1112–1118
6. Gewaltig J, Mangasser-Stephan K, Gartung C, Biesterfeld S, Gressner AM (2002) Association of polymorphisms of the transforming growth factor-b1 gene with the rate of progression of HCV-induced liver fibrosis. Clin Chim Acta 316:83–94
7. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD (1999) Genetic control of the circulating concentration of transforming growth factor type beta1. Hum Mol Genet 8:93–97
8. Hifnawy MS, Mangoud AM, Eissa MH, Nor Edin E, Mostafa Y, Abouel-Magd Y, Sabee EI, Amin I, Ismail A, Morsy TA, Mahrous S, Afefy AF, el-Shorbagy E, el-Sadawy M, Ragab H, Hassan MI, el-Hady G, Saber M (2004) The role of aflatoxin-contaminated food materials and HCV in developing hepatocellular carcinoma in Al-Sharkia Governorate, Egypt. J Egypt Soc Parasitol 34:479–488
9. Kang HG, Chae MH, Park JM, Kim EJ, Park JH, Kam S, Cha SI, Kim CH, Park RW, Park SH, Kim YL, Kim IS, Jung TH, Park JY (2006) Polymorphisms in TGF-beta1 gene and the risk of lung cancer. Lung Cancer 52:1–7
10. Kim SJ, Glick A, Sporn MB, Roberts AB (1989) Characterization of the promoter region of the human transforming growth factor-beta 1 gene. J Biol Chem 264:402–408
11. Koziel MJ (1999) Cytokines in viral hepatitis. Semin Liver Dis 19:157–169
12. Nguyen MH, Keeffe EB (2004) Epidemiology and treatment outcomes of patients with chronic hepatitis C and genotypes 4 to 9. Rev Gastroenterol Disord 4:S14–S21
13. Peto J (2001) Cancer epidemiology in the last century and the next decade. Nature 411:390–395
14. Ponder BA (2001) Cancer genetics. Nature 411:336–341
15. Pulleyn LJ, Newton R, Adcock IM, Barnes PJ (2001) TGFbeta1 allele association with asthma severity. Hum Genet 109:623
16. Qi P, Gao CF, Fang M, Ji Q, Zhao YP, Liu Y, Sun XJ (2008) Establishment of a real-time PCR for quantifying transforming

growth factor beta1 in blood of hepatocellular carcinoma patients. J Med Coll PLA 23:243–250
17. Sherlock S (1994) Viruses and hepatocellular carcinoma. Gut 35:828–832
18. Srivatanakul P, Sriplung H, Deerasamee S (2004) Epidemiology of liver cancer: an overview. Asian Pac J Cancer Prev 5:118–125
19. Wang H, Mengsteab S, Tag CG, Gao CF, Hellerbrand C, Lammert F, Gressner AM, Weiskirchen R (2005) Transforming growth factor-b1 gene polymorphisms are associated with severity of liver fibrosis in Caucasians with chronic hepatitis C infection. World J Gastroenterol 11:1929–1936
20. Wang H, Zhao YP, Gao CF, Ji Q, Gressner AM, Yang ZX, Weiskirchen R (2008) Transforming growth factor beta 1 gene variants increase transcription and are associated with liver cirrhosis in Chinese. Cytokine 43:20–25
21. Yu MC, Yuan JM, Govindarajan S, Ross RK (2000) Epidemiology of hepatocellular carcinoma. Can J Gastroenterol 14:703–709