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Original Article

Associations of toll-like receptor 4, 5 and 9 genetic variants with hepatitis B virus-related hepatocellular carcinoma and viral clearance in a Guangxi male population

Jia Zhu[1*], Aihua Tan[1,2*], Ziting Yao[1], Yong Gao[1], Weimin Xie[2], Yongkui Lu[2], Zengnan Mo[1]

[1]*Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning 530021, Guangxi, China;* [2]*Department of Chemotherapy, The Affiliated Tumor Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China.* [*]*Co-first authors.*

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Abstract: Toll-like receptors 4, 5 and 9 (TLR4, 5 and 9) are involved in the induction of innate immune response against hepatitis B virus (HBV) infection. This study aimed to explore the potential role of TLR4, 5 and 9 polymorphisms in the susceptibility to HBV clearance and HBV-related hepatocellular carcinoma (HCC). In this study, five single nucleotide polymorphisms (SNPs) (rs1927911, rs11536889, rs10759930, rs10983755 and rs2149356) at the *TLR4* gene, one SNP (rs5744174) at the *TLR5* gene and two SNPs at the *TLR9* gene (rs187084 and rs352140) were genotyped in 395 patients with HBV-positive HCC, 293 with persistent HBV carriers and 686 with HBV natural clearance subjects. In the case of *TLR5* rs5744174, the GA genotype (heterozygote model: adjusted OR=0.67; 95% CI, 0.50-0.90; P=0.008) and GA+GG genotype (dominant model: adjusted OR=0.67; 95% CI, 0.51-0.89; P=0.006) carriers were associated with HBV clearance as compared to the AA genotype carriers. In the case of *TLR4* rs10983755, the AA genotype carriers were associated with HBV clearance as compared to the AG+GG genotype (recessive model: adjusted OR=0.39; 95% CI, 0.16-0.94; P=0.036) and GG genotype (homozygote model: adjusted OR=0.39; 95% CI, 0.16-0.93; P=0.034) carriers. All other SNPs showed no significant associations with HBV-positive HCC and HBV natural clearance. These results demonstrated that SNP rs10983755 in *TLR4* and SNP rs5744174 in *TLR5* were associated with HBV natural clearance, and may be risk factors for HBV clearance.

Keywords: *TLR4*, *TLR5*, *TLR9*, genetic variants, HBV clearance, HCC

Introduction

HCC is the fifth most common cancer and the third highest cause of cancer-related deaths. The major risk factor for HCC is persistent infection with HBV [1]. The main cause of HBV persistence is weak antiviral immune response to its antigens [2]. The persistent and weak activation of the immune system within the chronically inflamed liver are key determinants in the development of HCC [3-5]. TLRs are a group of pattern recognition receptors (PRRs) that can recognize pathogen-associated molecular patterns (PAMPs), including virus, bacteria and other pathogens, and trigger innate immune responses against them [6]. HBV infection triggers an innate immune response, and the activation of TLR signaling inhibits HBV replication [7, 8]. Previous studies have shown that TLR4, 5 and 9 play crucial roles in controlling HBV replication [9, 10]. Many *TLR* SNPs were identified to be associated with the susceptibility to infections and a spectrum of inflammatory diseases [11]. A recent study showed that *TLR5* rs5744174 was associated with HBV natural clearance in chronic HBV-infected patients [12]. Shi et al. [13] found that the SNP of *TLR4* decreased the risk of development of HBV-related HCC in a Chinese population. Meanwhile, Xie et al. [14] reported a lack of association of *TLR9* rs352140 and rs352139 with susceptibility to HCC. Associations between *TLR5* variants and HCC risk, and between *TLR4*, *TLR9* and HBV clearance have not been reported.

Table 1. SNPs and PCR primer for TLR4, 5, 9 allele genotyping

SNPs	Chromosome position	PCR primer
rs187084	52261031	rs187084F: CGTCTTATTCCCCTGCTGGAATG
		rs187084R: CCTGCCATGATACCACCCAGAGT
rs352140	52256697	rs352140F: GGGACACTTGGCTGTGGATGTT
		rs352140R: GCTGGACCTCTACCACGAGCAC
rs5744174	223284528	rs5744174F: AGAAGCCCCGGAACTTTGTGAC
		rs5744174R: TATAGCTGGGCCTCCTGCAGAC
rs10983755	120464670	rs10983755F: AATGGTCCCTCACAGCTTGGTTTT
		rs10983755R: TGGGATTAAATGAACTGGCATTTG
rs1927911	120470054	rs1927911F: GCTGGCTTCTGCAAGGAATTTTG
		rs1927911R: TGGCCCAGATTTTGACAACTGC
rs11536889	120478131	rs11536889F: CTGGGATCCCTCCCCTGTACC
		rs11536889R: TTTCTGAGGAGGCTGGATGAACA
rs10759930	120461621	rs10759930F: GTCTGGGGAGGAGATGGCACT
		rs10759930R: ATGTGCCATGGACCAATGCTCT
rs2149356	120474199	rs2149356F: CCACAAAACTCGCTCCTATCACCT
		rs2149356R: TGGATCAAGTTTAGCCATTTTCTGTCA

SNPs: Single nucleotide polymorphisms; PCR: polymerase chain reaction; F: forward; R: reverse.

To understand the roles of *TLR4*, *5*, and *9* variants in HBV clearance and HCC development, we investigated five SNPs (rs1927911, rs11536889, rs10759930, rs10983755 and rs2149356) at the *TLR4* gene, one SNP (rs5744174) at the *TLR5* gene and two SNPs (rs187084 and rs352140) at the *TLR9* gene in a Chinese Guangxi male population.

Methods

Subjects

The study was approved by the ethics committee of Affiliated Tumor Hospital of Guangxi Medical University. All of the participants were male. The HCC patients were enrolled from January 2012 to December 2014, diagnosed by histopathological examination in combination with computerized tomography (CT) and/or magnetic resonance imaging (MRI), and ascertained to be infected with HBV for more than six months. In the end, 395 HBV-positive HCC patients consented to participate in the study.

There were two groups of controls were designed in this study: the HBV persistent carrier group and the HBV natural clearance group. All the controls were cancer-free, and selected from the Fangchenggang Area Male Health and Examination Survey [15] in Guangxi. The HBV persistent carriers were positive for HBsAg and antibodies against hepatitis B core antigen (anti-HBc). The HBV natural clearances subjects were negative for HBsAg but positive for antibodies against hepatitis B virus surface antigen (anti-HBs) and anti-HBc. As a result, 293 HBV persistent carriers and 686 HBV natural clearance subjects were enrolled.

SNP genotyping

Whole DNA was isolated from blood sampl-es using the BloodGen mini kit (CoWin Biotech) according to the manufacturer's instructions. SNP genotyping was performed using the improved multiplex ligase detection reaction (iMLDR) method (Shanghai Genesky Bio-Tech Co., Ltd. http://biotech.geneskies.com/index.html). The SNPs and PCR primers for *TLR* 4, 5 and 9 allele genotyping are listed in **Table 1**.

Statistical analysis

Differences of demographic and clinical data between the cases and controls were evaluated by one-way ANOVA (for continuous variables) and chi-square test (for categorical variables). Each SNP frequency in the controls was assessed for departure from Hardy-Weinberg Equilibrium using the chi-square test. The genotype distribution of cases and controls were analyzed by the Chi-square test. Odds ratio (OR) with 95% confidence intervals (CIs) were determined using binary logistic regression to estimate the associations of SNPs with HBV clearance and HBV-related HCC risks. Haplotype and linkage disequilibrium (LD) analyses were performed by SHEsis software. The statistical analyses were conducted using spss 16.0 software package. All *P* values were two-sided, and $P<0.05$ was considered to be statistically significant.

Results

General characteristics of the HBV-positive HCC patients and controls are summarized

Table 2. Demographic and selected variables in HCC patients, HBV persistent carriers and HBV natural clearance subjects

Variables	HCC patients	HBV persistent carriers	HBV natural clearance subjects	P
N	395	293	686	
Age, year (mean ± SD)	48.0 ± 10.1	37.1 ± 10.0	37.0 ± 10.8	<0.001
Smoking status n (%)				0.002
Ever	170 (43.0%)	157 (53.6%)	367 (53.5%)	
Never	225 (57.0%)	136 (46.4%)	319 (46.5%)	
Drinking status n (%)				0.333
Ever	140 (35.5%)	106 (36.2%)	272 (39.7%)	
Never	254 (64.5%)	187 (63.8%)	414 (60.3%)	

in **Table 2**. Significant differences were not found between the groups in drinking status (P=0.333), but were found in age and smoking status (P<0.001, P=0.002, respectively). The distribution of eight SNPs in the controls were in accordance with the Hardy-Weinberg equilibrium (all P>0.05). In the case of TLR5 rs5744174, the GA genotype (heterozygote model: adjusted OR=0.67; 95% CI, 0.50-0.90; P=0.008) and GA+GG genotype (dominant model: adjusted OR=0.67; 95% CI, 0.51-0.89; P=0.006) carriers were associated with HBV clearance as compared to the AA genotype carriers. In the case of TLR4 rs10983755, the AA genotype carriers were associated with HBV clearance as compared to the AG+GG genotype (recessive model: adjusted OR=0.39; 95% CI, 0.16-0.94; P=0.036) and GG genotype (homozygote model: adjusted OR=0.39; 95% CI, 0.16-0.93; P=0.034) carriers (**Table 3**).

All other SNPs showed no significant associations with the risk of HBV-positive HCC and HBV natural clearance. Linkage disequilibrium (LD) information of TLR4 variations is shown in **Table 4**. Furthermore, the haplotype frequencies of the five SNPs of TLR4 were evaluated. The most frequent haplotype in the three groups was TGGGG. None of the haplotypes were significantly associated with HBV-positive HCC and HBV natural clearance (all P>0.05) (**Table 5**).

Discussion

This study revealed the association of five SNPs (rs1927911, rs11536889, rs10759930, rs10983755 and rs2149356) at the *TLR4* gene, one SNP (rs5744174) at the *TLR5* gene, and two SNPs at the *TLR9* gene (rs187084 and rs352140) with HBV-related HCC and HBV clearance. *TLR5* rs5744174 and *TLR4* rs10983755 were significantly associated with HBV clearance. The other SNPs showed no significant associations with HBV-positive HCC and HBV natural clearance.

Host genetic factors are associated with the prognosis of HBV infection [16]. An in-depth study of host genetic factors and HBV could enhance the disease management [17]. TLRs are important components for the development of autoimmunity, and play crucial roles in the defense against different pathogens, including bacteria, fungi and viruses [18-21]. They also mediate innate immune responses to inhibit HBV replication [22-25], and might hinder HBV infection and contribute to viral clearance [26]. Visvanathan et al. [27] found that HBV replication was associated with up-regulation of the TLRs in chronic HBV infection. Recent studies found that *TLR4* rs10983755 polymorphism was related to inflammatory diseases and asymptomatic urinary tract infections in Swedish populations [28], and also played a protective role on asthma severity in Chinese populations [29]. Li et al. [30] reported that the *TLR4* rs10983755 polymorphism might play a protective role in the defense against H. pylori infection in a Chinese Han population. Additionally, a significant association between *TLR4* rs10983755 and preterm neonatal gram-negative bacterial infection was reported in Han Chinese neonates [31].

In this study, we found that *TLR4* rs10983755 was strongly associated with HBV natural clearance and played a role in HBV infection. This polymorphism might cause changes in immune-mediated inflammatory processes.

TLR5 rs5744174 c. T2487C variations, located in the promoter region, could lead to missense mutation, and replace phenylalanine to leucine [32]. A recent study suggested that *TLR5* rs5744174 was associated with HBV natural clearance and higher IFN-γ production. *TLR5* rs5744174 exerted its effects against HBV infection by probably changing the protein struc-

Table 3. Genotypes of TLR4, TLR5 and TLR9, HBV clearance, and HCC susceptibility

Genotype	HCC patients	HBV persistent carriers	HBV natural clearance subjects	OR (95% CI)[a]	*P*[a]	OR (95% CI)[b]	*P*[b]
rs2149356							
GG	206 (52.2%)	154 (53.5%)	338 (49.6%)	1.00		1.00	
TG	160 (40.5%)	112 (38.9%)	275 (40.3%)	1.08 (0.75-1.56)	0.673	0.89 (0.67-1.20)	0.447
TT	29 (7.3%)	22 (7.6%)	69 (10.1%)	1.22 (0.62-2.42)	0.567	0.70 (0.42-1.17)	0.171
Dominant				1.10 (0.78-1.57)	0.582	0.85 (0.65-1.13)	0.261
Recessive				1.18 (0.61-2.30)	0.625	0.73 (0.44-1.21)	0.223
rs10759930							
TT	202 (51.1%)	154 (52.9%)	338 (49.7%)	1.00		1.00	
CT	164 (41.5%)	114 (39.2%)	274 (40.3%)	1.11 (0.77-1.60)	0.591	0.91 (0.68-1.22)	0.539
CC	29 (7.3%)	23 (7.9%)	68 (10.0%)	1.14 (0.58-2.24)	0.712	0.74 (0.44-1.23)	0.245
Dominant				1.11 (0.78-1.58)	0.557	0.88 (0.67-1.16)	0.355
Recessive				1.09 (0.56-2.11)	0.802	0.77 (0.47-1.26)	0.297
rs11536889							
GG	177 (44.8%)	104 (48.4%)	274 (54.4%)	1.00		1.00	
CG	180(45.6%)	92 (42.8%)	200 (39.7%)	1.29 (0.86-1.93)	0.212	1.22 (0.87-1.70)	0.249
CC	38 (9.6%)	19 (8.8%)	30 (6.0%)	1.17 (0.59-2.31)	0.648	1.67 (0.90-3.09)	0.106
Dominant				1.27 (0.87-1.86)	0.222	1.28 (0.93-1.76)	0.135
Recessive				1.03 (0.54-1.98)	0.920	1.53 (0.84-2.78)	0.167
rs1927911							
GG	205 (51.9%)	156 (53.4%)	340 (49.6%)	1.00		1.00	
AG	161 (40.8%)	113 (38.7%)	276 (40.3%)	1.12 (0.78-1.62)	0.531	0.89 (0.67-1.19)	0.439
AA	29 (7.3%)	23 (7.9%)	69 (10.1%)	1.15 (0.59-2.26)	0.686	0.72 (0.44-1.20)	0.213
Dominant				1.13 (0.80-1.60)	0.498	0.86 (0.65-1.13)	0.274
Recessive				1.09 (0.57-2.11)	0.790	0.76 (0.46-1.25)	0.277
rs10983755							
GG	279 (70.6%)	205 (70.4%)	461 (67.3%)	1.00		1.00	
AG	104 (26.3%)	80 (27.5%)	189 (27.6%)	1.02 (0.69-1.52)	0.919	0.95 (0.70-1.30)	0.761
AA	12 (3.0%)	6 (2.1%)	35 (5.1%)	1.38 (0.45-4.25)	0.575	0.39 (0.16-0.93)	0.034
Dominant				1.05 (0.71-1.54)	0.809	0.86 (0.64-1.17)	0.337
Recessive				1.37 (0.45-4.21)	0.580	0.39 (0.16-0.94)	0.036
rs5744174							
AA	244 (61.8%)	187 (64.0%)	372 (54.4%)	1.00		1.00	
GA	128 (32.4%)	89 (30.5%)	267 (39.0%)	1.12 (0.77-1.64)	0.546	0.67 (0.50-0.90)	0.008
GG	23 (5.8%)	16 (5.5%)	45 (6.6%)	1.26 (0.58-2.74)	0.554	0.70 (0.39-1.27)	0.241
Dominant				1.14 (0.80-1.64)	0.466	0.67 (0.51-0.89)	0.006
Recessive				1.22 (0.57-2.61)	0.620	0.81 (0.45-1.46)	0.483
rs352140							
CC	183 (46.3%)	136 (46.7%)	322 (47.1%)	1.00		1.00	
TC	168 (42.5%)	121 (41.6%)	274 (40.1%)	1.24 (0.85-1.80)	0.263	1.03 (0.77-1.39)	0.822
TT	44 (11.1%)	34 (11.7%)	87 (12.7%)	1.09 (0.61-1.96)	0.774	0.92 (0.59-1.44)	0.712
Dominant				1.21 (0.85-1.72)	0.299	1.01 (0.76-1.33)	0.962
Recessive				0.98 (0.56-1.71)	0.947	0.91 (0.59-1.38)	0.645
rs187084							
AA	181 (45.8%)	134 (45.9%)	315 (46.0%)	1.00		1.00	
GA	169 (42.8%)	123 (42.1%)	278 (40.6%)	1.23 (0.85-1.79)	0.278	1.03 (0.77-1.38)	0.852
GG	45 (11.4%)	35 (12.0%)	92 (13.4%)	0.99 (0.56-1.76)	0.968	0.89 (0.57-1.38)	0.598
Dominant				1.17 (0.83-1.67)	0.372	0.99 (0.75-1.31)	0.963
Recessive				0.89 (0.52-1.54)	0.686	0.88 (0.58-1.33)	0.536

NOTE: Logistic regression analyses adjusted for age, smoking status and drinking status. [a]HCC patientsvs HBV persistent carriers. [b]HBV persistent carriers vs HBV natural clearance subjects.

Table 4. Linkage disequilibrium (LD) information of TLR4 variations

	HBV persistent carriers					HBV natural clearance				
	rs10983755	rs1927911	rs11536889	rs10759930	rs2149356	rs10983755	rs1927911	rs11536889	rs10759930	rs2149356
rs10983755	--	1.000[a]	0.999[a]	1.000[a]	0.993[a]	--	1.000[a]	0.999[a]	1.000[a]	1.000[a]
rs1927911	0.506[b]	--	0.970[a]	1.000[a]	0.993[a]	0.531[b]	--	1.000[a]	0.998[a]	1.000[a]
rs11536889	0.098[b]	0.183[b]	--	0.971[a]	0.970[a]	0.104[b]	0.193[b]	--	1.000[a]	1.000[a]
rs10759930	0.498[b]	0.986[b]	0.186[b]	--	1.000[a]	0.530[b]	0.993[b]	0.195[b]	--	1.000[a]
rs2149356	0.500[b]	0.982[b]	0.183[b]	0.982[b]	--	0.532[b]	1.000[b]	0.194[b]	0.995[b]	--

[a]D'; [b]R^2.

Table 5. Results of TLR4 haplotype association analysis

Haplotype	HCC patients, n (%)	HBV persistent carriers, n (%)	HBV natural clearance subjects, n (%)	OR (95% CI)[a]	P^a	OR (95% CI)[b]	P^b
CAATG	127 (16.1)	85 (20.0)	225 (22.5)	0.78 (0.57-1.05)	0.105	0.86 (0.65-1.14)	0.291
CGATG	85 (10.8)	56 (13.1)	121 (12.1)	0.81 (0.57-1.16)	0.248	1.10 (0.78-1.55)	0.577
TGGGC	249 (31.6)	129 (30.3)	260 (26.0)	1.08 (0.84-1.39)	0.563	1.24 (0.96-1.59)	0.094
TGGGG	319 (40.3)	155 (36.4)	393 (39.3)	1.20 (0.94-1.53)	0.140	0.89 (0.70-1.12)	0.309

NOTE: Haplotypes with a frequency less than 3% in all three groups were ignored in analysis. SNPs order: rs10759930, rs10983755, rs1927911, rs2149356, rs11536889. [a]HCC patients vs HBV persistent carriers. [b]HBV persistent carriers vs HBV natural clearance subjects.

ture and conformation [12]. Our results also demonstrated that *TLR5* rs5744174 was strongly associated with HBV natural clearance. However, we did not find any correlations between *TLR4* (rs1927911, rs11536889, rs10759930, rs10983755 and rs2149356), *TLR5* rs5744174, *TLR9* (rs187084 and rs352140) and HBV-positive HCC, or any associations of TLR4 (rs1927911, rs11536889, rs10759930 and rs2149356) and TLR9 (rs187084 and rs352140) with HBV natural clearance.

In conclusion, our study suggested that SNP rs10983755 in *TLR4* and SNP rs5744174 in *TLR5* were significantly associated with HBV natural clearance, and could be susceptibility factors for HBV clearance.

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Disclosure of conflict of interest

None.

Address correspondence to: Zengnan Mo, Center for Genomic and Personalized Medicine, Guangxi Medical University, Nanning 530021, Guangxi, China. Tel: +860771 5320761; Fax: +8607715320-761; E-mail: zengnanmo@hotmail.com

References

[1] Parkin DM, Bray F, Ferlay J and Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.

[2] Chisari FV and Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 1995; 13: 29-60.

[3] Chen L, Zhang Q, Chang W, Du Y, Zhang H and Cao G. Viral and host inflammation-related factors that can predict the prognosis of hepatocellular carcinoma. Eur J Cancer 2012; 48: 1977-1987.

[4] Chemin I and Zoulim F. Hepatitis B virus induced hepatocellular carcinoma. Cancer Lett 2009; 286: 52-59.

[5] Han YF, Zhao J, Ma LY, Yin JH, Chang WJ, Zhang HW and Cao GW. Factors predicting occurrence and prognosis of hepatitis-B-virus-related hepatocellular carcinoma. World J Gastroenterol 2011; 17: 4258-4270.

[6] Aderem A and Ulevitch RJ. Toll-like receptors in the induction of the innate immune response. Nature 2000; 406: 782-787.

[7] Pei RJ, Chen XW and Lu MJ. Control of hepatitis B virus replication by interferons and Toll-like receptor signaling pathways. World J Gastroenterol 2014; 20: 11618-11629.

[8] Zhang E and Lu M. Toll-like receptor (TLR)-mediated innate immune responses in the control of hepatitis B virus (HBV) infection. Med Microbiol Immunol 2015; 204: 11-20.

[9] Isogawa M, Robek MD, Furuichi Y and Chisari FV. Toll-like receptor signaling inhibits hepatitis B virus replication in vivo. J Virol 2005; 79: 7269-7272.

[10] Sajadi SM, Mirzaei V, Hassanshahi G, Khoramdelazad H, Daredor HY, Hosseini SM, Moogooi M, Ravary A, Arababadi MK and Kennedy D. Decreased expressions of Toll-like receptor 9 and its signaling molecules in chronic hepatitis B virus-infected patients. Arch Pathol Lab Med 2013; 137: 1674-1679.

[11] Misch EA and Hawn TR. Toll-like receptor polymorphisms and susceptibility to human disease. Clin Sci (Lond) 2008; 114: 347-360.

[12] Wu JF, Chen CH, Ni YH, Lin YT, Chen HL, Hsu HY and Chang MH. Toll-like receptor and hepatitis B virus clearance in chronic infected patients: a long-term prospective cohort study in Taiwan. J Infect Dis 2012; 206: 662-668.

[13] Minmin S, Xiaoqian X, Hao C, Baiyong S, Xiaxing D, Junjie X, Xi Z, Jianquan Z and Songyao J. Single nucleotide polymorphisms of Toll-like receptor 4 decrease the risk of development of hepatocellular carcinoma. PLoS One 2011; 6: e19466.

[14] Junjie X, Songyao J, Minmin S, Yanyan S, Baiyong S, Xiaxing D, Jiabin J, Xi Z and Hao C. The association between Toll-like receptor 2 single-nucleotide polymorphisms and hepatocellular carcinoma susceptibility. BMC Cancer 2012; 12: 57.

[15] Tan A, Gao Y, Yang X, Zhang H, Qin X, Mo L, Peng T, Xia N and Mo Z. Low serum osteocalcin level is a potential marker for metabolic syndrome: results from a Chinese male population survey. Metabolism 2011; 60: 1186-1192.

[16] Thursz M. Genetic susceptibility in chronic viral hepatitis. Antiviral Res 2001; 52: 113-116.

[17] Fattovich G, Bortolotti F and Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. J Hepatol 2008; 48: 335-352.

[18] Hoebe K, Du X, Georgel P, Janssen E, Tabeta K, Kim SO, Goode J, Lin P, Mann N, Mudd S, Crozat K, Sovath S, Han J and Beutler B. Identification of Lps2 as a key transducer of MyD88-independent TIR signalling. Nature 2003; 424: 743-748.

[19] Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, Walsh EE, Freeman MW, Golenbock DT, Anderson LJ and Finberg RW. Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. Nat Immunol 2000; 1: 398-401.

[20] Wang CM, Chang SW, Wu YJ, Lin JC, Ho HH, Chou TC, Yang B, Wu J and Chen JY. Genetic variations in Toll-like receptors (TLRs 3/7/8) are associated with systemic lupus erythematosus in a Taiwanese population. Sci Rep 2014; 4: 3792.

[21] Lee BL and Barton GM. Trafficking of endosomal Toll-like receptors. Trends Cell Biol 2014; 24: 360-369.

[22] Wu J, Lu M, Meng Z, Trippler M, Broering R, Szczeponek A, Krux F, Dittmer U, Roggendorf M, Gerken G and Schlaak JF. Toll-like receptor-mediated control of HBV replication by nonparenchymal liver cells in mice. Hepatology 2007; 46: 1769-1778.

[23] Nan XP, Zhang Y, Yu HT, Li Y, Sun RL, Wang JP and Bai XF. Circulating CD4+CD25high regulatory T cells and expression of PD-1 and BTLA on CD4+ T cells in patients with chronic hepatitis B virus infection. Viral Immunol 2010; 23: 63-70.

[24] Zhang Y, Lian JQ, Huang CX, Wang JP, Wei X, Nan XP, Yu HT, Jiang LL, Wang XQ, Zhuang Y, Li XH, Li Y, Wang PZ, Robek MD and Bai XF. Overexpression of toll-like receptor 2/4 on monocytes modulates the activities of CD4(+) CD25(+) regulatory T cells in chronic hepatitis B virus infection. Virology 2010; 397: 34-42.

[25] Lian JQ, Wang XQ, Zhang Y, Huang CX and Bai XF. Correlation of circulating TLR2/4 expression with CD3+/4+/8+ T cells and treg cells in HBV-related liver cirrhosis. Viral Immunol 2009; 22: 301-308.

[26] Ma Z, Zhang E, Yang D and Lu M. Contribution of toll-like receptors to the control of hepatitis B virus infection by initiating antiviral innate responses and promoting specific adaptive immune responses. Cell Mol Immunol 2015; 12: 273-282.

[27] Visvanathan K, Skinner NA, Thompson AJ, Riordan SM, Sozzi V, Edwards R, Rodgers S, Kurtovic J, Chang J, Lewin S, Desmond P and Locarnini S. Regulation of toll-like receptor-2 expression in chronic hepatitis B by the precore protein. Hepatology 2007; 45: 102-110.

[28] Ragnarsdottir B, Jonsson K, Urbano A, Gronberg-Hernandez J, Lutay N, Tammi M, Gustafsson M, Lundstedt AC, Leijonhufvud I, Karpman D, Wullt B, Truedsson L, Jodal U, Andersson B and Svanborg C. Toll-like receptor 4 promoter polymorphisms: common TLR4 variants may protect against severe urinary tract infection. PLoS One 2010; 5: e10734.

[29] Zhang Q, Qian FH, Zhou LF, Wei GZ, Jin GF, Bai JL and Yin KS. Polymorphisms in toll-like receptor 4 gene are associated with asthma severity but not susceptibility in a Chinese Han population. J Investig Allergol Clin Immunol 2011; 21: 370-377.

[30] Li P, He CY, Xu Q, Sun LP, Ha MW and Yuan Y. Effect of the -2081 G/A polymorphism of the TLR4 gene and its interaction with Helicobacter pylori infection on the risk of gastric cancer in Chinese individuals. Genet Test Mol Biomarkers 2014; 18: 610-615.

[31] Zhou JG, Zhang JP, Lee BH, Zhang SL, Zhu L and Chen C. Toll-like receptor 4 polymorphisms in gram-negative bacterial infections of Han Chinese neonates. Am J Perinatol 2015; 32: 363-370.

[32] Dhiman N, Ovsyannikova IG, Vierkant RA, Ryan JE, Pankratz VS, Jacobson RM and Poland GA. Associations between SNPs in toll-like receptors and related intracellular signaling molecules and immune responses to measles vaccine: preliminary results. Vaccine 2008; 26: 1731-1736.