Gene 569 (2015) 218–224

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Research paper

Genetic polymorphisms in *Toll-like receptor 3* gene are associated with the risk of hepatitis B virus-related liver diseases in a Chinese population

Xiamei Huang *, Haiwei Li, Jian Wang, Chunni Huang, Yu Lu, Xue Qin *, Shan Li *

Department of Clinical Laboratory, First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi, China

ARTICLE INFO

Article history:
Received 15 February 2015
Received in revised form 20 May 2015
Accepted 22 May 2015
Available online 27 May 2015

Keywords:
Chronic hepatitis B
Liver cirrhosis
Hepatocellular carcinoma
Toll-like receptor 3
Polymorphism

ABSTRACT

Toll-like receptors (TLRs) are well known to play a critical role in antiviral and antitumor response. Available evidence has demonstrated that TLRs are implicated in the immunopathogenesis of hepatitis B virus (HBV) infection and tumor carcinogenesis. Therefore, we performed the present study to investigate the relationship with *TLR3* gene polymorphisms and the risk of HBV-related liver disease in a Chinese population. A total of 623 individuals were included: 172 chronic hepatitis B (CHB) patients, 91 HBV-related liver cirrhosis (LC) patients, 174 HBV-related hepatocellular carcinoma (HCC) patients, and 186 healthy controls. Allelic and genotypic frequencies of *TLR3* rs1879026 and rs3775290 polymorphisms were detected by PCR-RFLP and DNA sequencing analysis. Association analysis showed that the TT genotype of *TLR3* rs3775290 was associated with a decreased risk for CHB, HBV-related LC, and HCC (OR = 0.52, 95% CI: 0.27–0.99, $P = 0.048$; OR = 0.32, 95% CI: 0.14–0.76, $P = 0.010$; OR = 0.49, 95% CI: 0.26–0.92, $P = 0.027$). Nevertheless, a lack of association was found between *TLR3* rs1879026 and HBV-related liver diseases. In addition, haplotype analysis revealed that individuals who carried the GT haplotype might have a decreased risk of HBV-related liver diseases. The results indicated that genetic variant in *TLR3* gene rs3775290 polymorphisms may be a protective factor for CHB, HBV-related LC, and HCC in the Chinese population.

1. Introduction

Hepatitis B virus (HBV) infection is a severe public health problem worldwide. It is estimated that more than 2 billion people around the world are infected with HBV, with 75% of these distributed within the Asia-Pacific region (Liaw and Chu, 2009). In China, over 8% of the population is chronically infected with HBV, being the major cause of chronic hepatitis (CHB), liver cirrhosis (LC), and hepatocellular carcinoma (HCC) in Chinese patients (Liu et al., 2011). The clinical course of HBV infection varies from spontaneous to chronic persistent infection, and persistent HBV infection will finally lead to severe liver injury. Most HCC cases in China are derived from initial HBV infection, followed by LC, and finally carcinogenesis (Chemin and Zoulim, 2009; Balmasova et al., 2014). Despite the poor prognosis and high relapse rates of HCC, targeted drugs and surgical treatment are still unsatisfactory. Although the molecular and cellular mechanisms of HCC pathogenesis have been well investigated in previous studies, the occurrence and development mechanisms of HCC are still not completely elucidated. It is widely accepted that HCC pathogenesis involves a complex multifactorial process including genetic factors (Farazi and DePinho, 2006). Further, many studies have indicated the association of genetic variants with HCC susceptibility (Li et al., 2011; Minmin et al., 2011; Baldissera et al., 2012).

Toll-like receptors (TLRs) belong to the pattern-recognition receptor family and play a crucial role in the innate and adaptive immune responses through recognition of microbial pathogens, which are essential in host defense mechanisms (Werling and Jungi, 2003). HBV infection and clearance depends on host immune responses (Balmasova et al., 2014). Additionally, it has been reported that TLR signaling contributes to immunopathogenesis of HBV infection (Wu et al., 2009).

Toll-like receptor 3 (TLR3), as a nucleic acid receptor, recognizes poly(I:C) and double-strand RNA (dsRNA) in internalized bacteria and viruses (Akira, 2009). Activated TLR3 signaling leads to the activation of the transcription factors interferon-regulatory factor-3(IRF3) and nuclear factor (NF)-κB, and induces the production of interferon-β (IFN-β) and inflammatory cytokines, mediating antiviral and antitumor immunity responses. TLR3 is expressed in dendritic cells (DCs), natural killer (NK) cells, Kupffer cells, and hepatocytes (Testro and Visvanathan, 2009). Evidence has shown that expression of TLR3 and IFN-β was

Abbreviations: TLR3, Toll-like receptor 3; HBV, hepatitis B virus; CHB, chronic hepatitis B; LC, liver cirrhosis; HCC, hepatocellular carcinoma; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; HWE, Hardy–Weinberg equilibrium; ORs, odds ratios; CIs, confidence intervals; SNPs, single-nucleotide polymorphisms; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; AFP, alpha fetoprotein.

* Corresponding authors.
E-mail addresses: huang_xiamei@163.com (X. Huang), qinxue919@126.com (X. Qin), lis8858@126.com (S. Li).

http://dx.doi.org/10.1016/j.gene.2015.05.054
0378-1119/

significantly decreased in monocyte-DCs from patients with CHB, compared with healthy individuals (Li et al., 2009). Furthermore, a previous study revealed that TLR3 ligand signaling induces antiviral cytokines to inhibit HBV replication in a mouse model (Isogawa et al., 2005). TLR3 is expressed not only in immune cells but also in cancer cells (Sato et al., 2009). A recent study found HCC patients with high levels of TLR3 expression had a 17-fold longer median survival than patients with low TLR3 expression levels (Chew and Abastado, 2013). In addition, TLR3 agonists have an influence on the tumor microenvironment by suppressing angiogenesis, induction of tumor cell apoptosis, and inhibition of tumor cell migration in HCC (Guo et al., 2012). These studies indicate that TLR3 is closely correlated with HBV infection and may play critical roles in the development and prognosis of HBV-related HCC.

Genetic variations in *TLR3* have been correlated with susceptibility to various diseases, including viral hepatitis and HCC (He et al., 2007; Ishizaki et al., 2008; Etem et al., 2011; Al-Qahtani et al., 2012; Sironi et al., 2012; Lee et al., 2013; Li and Zheng, 2013; Rong et al., 2013). However, little is known concerning *TLR3* gene polymorphisms and HBV-related CHB, LC, and HCC. Therefore, this study has investigated the role of *TLR3* rs1879026 and rs3775290 gene polymorphisms in HBV-related liver disease (HBV-related CHB, LC, and HCC) susceptibility in a Chinese population.

2. Materials and methods

2.1. Study population

The study was approved by the ethics and investigation committee of the First Affiliated Hospital of Guangxi Medical University, and all the participants gave their written informed consent at the time of inclusion.

A total of 437 consecutive patients with HBV-related liver diseases and 186 healthy controls participated in this case–control study. All participants were from the Chinese Han population enrolled at the First Affiliated Hospital of Guangxi Medical University between July 2011 and October 2012. Patients with HBV-related liver diseases included 172 CHB patients, 91 LC patients with HBV infection, and 174 patients with HBV-related HCC.

Control subjects were matched to cases on the basis of age, sex, and smoking and drinking status, undergoing a routine medical check-up in the hospital's outpatient clinic. The selection criteria for the controls included no HBV history or family history of HCC and free of any cancer or other serious illness.

As described in our previous article (Li et al., 2011), CHB was diagnosed based on HBsAg seropositivity, positivity for serum HBV-DNA levels, and continuously elevated alanine aminotransferase (ALT) over a period of 6 months. LC was diagnosed on the basis of pathologic exams, laboratory features, and the findings of computed tomography (CT) or ultrasonography. HCC was diagnosed by the elevation of alpha-fetoprotein (AFP) (>400 ng/mL) combined with at least one positive iconography examination result, including CT and magnetic resonance imaging, or positive findings on cytological or pathological examination. All the patients were newly diagnosed and treatment-naive at the time of recruitment. In addition, patients positive for hepatitis C virus antibody and hepatitis D virus antibody, or who had any other type of liver disease (for example, autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis, or Budd–Chiari syndrome), or a second primary cancer, were excluded.

The clinical characteristics of patients and controls, including age, gender, and smoking and drinking consumption status were investigated at the time of blood collection. The following laboratory parameters were obtained for each participant: serum aspartate aminotransferase (AST), ALT, gamma-glutamyltransferase (GGT), AFP, and HBV-DNA.

2.2. DNA isolation and genotyping

Peripheral blood samples were obtained from all the participants. Genomic DNA was isolated from whole blood samples by the standard phenol-chloroform method. Genomic DNA samples were stored at −20 °C prior to amplification. We detected genotypes of *TLR3* rs1879026 and rs3775290 by the polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method. Specifically, primers for *TLR3* rs1879026 were forward (5′-GCTCTC AGGAGGATGACACGA-3′) and reverse (5′-TGTCACATACACAGGAAT GTAATGC-3′). Primers for *TLR3* rs3775290 were forward (5′-ATGT TGGCTATGTTGTTGTTGC-3′) and reverse (5′-CAAAGTATTTCCCTTG CCTCAC-3′). PCRs were run at 95 °C for 5 min, followed by 32 cycles at 95 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 5 min. PCR products were then digested with the restriction endonucleases BsmI (GAATGCN^) and BstBI (TT^CGAA), respectively, at 37 °C overnight, and subjected to 3% agarose gel electrophoresis to identify the *TLR3* alleles on the basis of the respective allele size. The polymorphism genotypes of *TLR3* rs1879026 and rs3775290 were characterized as GG/TG/TT and CC/TC/TT, respectively (Fig. 1).

The genotypes were assigned by an investigator who was blinded to the patients' clinical status. To control the quality of genotyping, 10% of the PCR-amplified DNA samples were randomly selected and sequenced to validate the results obtained with PCR-RFLP by other investigators. Results between PCR-RFLP and DNA sequencing analysis were completely concordant (Figs. 2 and 3).

2.3. Statistical analysis

Statistical analysis was carried out using SPSS statistical software package ver.16.0 (SPSS Inc., Chicago, USA). Continuous variables were tested using the one-way ANOVA test. And the categorical variables were evaluated by χ^2 test. The direct counting method was used to estimate the genotype and allele frequencies. The genotype distribution was analyzed for deviations from the Hardy–Weinberg equilibrium (HWE) using the goodness-of-fit χ^2 test, performed by using a professional web-based program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Differences in the allele or genotype frequencies between the cases and control subjects were evaluated by means of χ^2 analysis with a 2×2 or 2×3 contingency table. Associations between genotypes and HBV-related liver diseases were estimated using a binary logistic regression

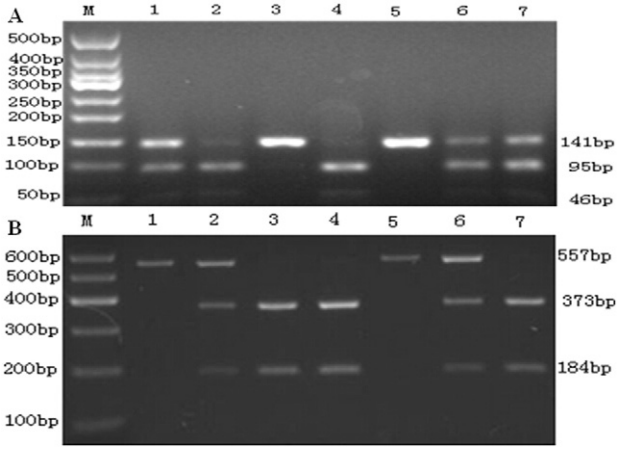

Fig. 1. PCR-RFLP assay for analyzing the rs1879026 and rs3775290 of the *TLR3* gene. (A) TLR 3 rs1879026. Lane M: DNA marker; Lanes 1, 6 and 7: TG genotypes; Lanes 2 and 4: GG genotypes; Lanes 3 and 5: TT genotypes. (B) TLR3 rs3775290. Lane M: DNA marker; Lanes 1 and 5: TT genotypes; Lanes 2 and 6: TC genotypes; Lanes 3, 4 and 7: CC genotypes.