Cytokine 85 (2016) 26–29

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Serum progranulin levels are elevated in patients with chronic hepatitis B virus infection, reflecting viral load

Yi Gong, Tingxi Zhan, Qing Li, Guozhen Zhang, Bing Tan, Xiaoliang Yang, Yan Wu, Wenjuan Que, Yan Xing, Hui Liu, Xue Hu, Zebo Yu *

Department of Blood Transfusion, The First Affiliated Hospital of Chongqing Medical University, Chongqing, China

ARTICLE INFO

Article history:
Received 20 April 2016
Received in revised form 26 May 2016
Accepted 30 May 2016

Keywords:
Progranulin
HBV
Growth factor
Infection
Patients

ABSTRACT

Progranulin (PGRN) is implicated in infection, immunity and host defense, but its role in the pathogenesis of HBV infection remains unknown. Here we investigated whether there is dysregulated production and the clinical significance of circulating PGRN in patients with chronic HBV infection. Serum concentrations of PGRN were analyzed by enzyme-linked immunosorbent assay. Serum PGRN levels were significantly higher in patients with chronic HBV infection than healthy subjects. PGRN levels were significantly associated with HBV-DNA levels, but did not correlate with the concentrations of alanine aminotransferase and aspartate aminotransferase. This study demonstrates increased circulating PGRN production and association between PGRN levels and viral loads in patients with chronic HBV infection, suggesting a functional role of PGRN in the pathogenesis of HBV infection.

1. Introduction

Hepatitis B virus (HBV) is a noncytopathic, hepatotropic, double-stranded DNA virus that affect more than 350 million individuals [1]. HBV can cause acute and chronic hepatitis, which may progress to liver cirrhosis or hepatocellular carcinoma after infection [2]. The outcome of HBV infection was influenced by the interaction between the virus and host immune response [3]. Thus, elucidating the expression or biological function of immunoregulatory factors in the patients with chronic HBV infection is required for the successful development of novel immune modulatory therapies against HBV infection.

Progranulin (PGRN) is a 593-aa growth factor, which is widely expressed in epithelia, bone marrow, immune cells, solid organs, and the nervous system [4]. PGRN plays a regulatory role in tissue development, proliferation, regeneration, infection and inflammatory response. PGRN could activate many of the typical cell proliferation signaling pathways, including extracellular signal-regulated kinase (ERK), phosphoinositide-3 kinase (PI3K/Akt) pathways, not only in tumors but also in neurons [5]. PGRN has also been considered as a multifunctional immunoregulatory molecule by regulating the activation of signaling pathways involved in infection and immunity, especially TNF/TNFR signaling [6,7]. PGRN has a classic N-terminal signal peptide and several N-glycosylation sites, and it could be detected in sera or cerebrospinal fluids [5]. Detecting protein levels of PGRN may be helpful in early diagnosis of some diseases, such as frontotemporal lobar degeneration (FTLD) [8]. However, data about the expression of PGRN during virus infection is limited. In this study, we investigated the production and clinical significance of PGRN in patients with chronic HBV infection, which may provide new evidence for the possible role of PGRN in the immune response during HBV infection, as well as new drug targets for immunotherapy against chronic HBV infection.

2. Materials and methods

2.1. Human subjects

This study examined 38 Chinese patients with chronic hepatitis B virus infection including 21 HBe Ag positive and 17 HBe negative ones. All patients were HBsAg positive, and concurrence of hepatitis C virus, hepatitis D virus, hepatitis G virus, human immunodeficiency virus, metabolic liver disease, liver cancer, and other possible causes for chronic liver injuries, such as alcohol, drug, congestive heart failure and autoimmune diseases was excluded. Patients received any anti-HBV treatments or steroids 6 months

* Corresponding author at: Department of Blood Transfusion, The First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing 400016, China.
E-mail address: 53293936@qq.com (Z. Yu).

http://dx.doi.org/10.1016/j.cyto.2016.05.026
1043-4666/

before blood sampling were also excluded. 36 sex- and age-matched healthy Chinese individuals were enrolled in this study as normal controls. This study protocol was approved by the Clinical Research Ethics Committee of The First Affiliated Hospital of Chongqing Medical University (No. 201603006), and informed consent was obtained from each subject according to the Declaration of Helsink.

2.2. Laboratory studies

The levels of HBsAg, HBeAg, anti-HBs, anti-HBc, and anti-HBe were determined by commercial enzyme immunoassay kits (ARCHITECT i2000, Abbott, Wiesbaden, Germany). Intra-assay coefficient of variation (CV) value was 3.1% for HBsAg, 4.7% for HBeAg, 4.6% for anti-HBs, 5.5% for anti-HBc, and 4.8% for anti-HBe, while inter-assay CV value was 4.3% for HBsAg, 5.1% for HBeAg, 5.7% for anti-HBs, 6.5% for anti-HBc, and 5.3% for anti-HBe. The HBV DNA level was assayed by real-time PCR on the Roche Cobas Z480 real-time detection system (Roche systems) with a determination sensitivity of 1×10^3 copies/ml. Intra-assay CV value was 3.3% and inter-assay CV value was 4.1% for HBV DNA. Serum anine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TB), direct bilirubin (DB), total cholesterol (TC), and triglyceride (TG) were measured by autoanalyzer, Beckman Coulter synchron LX 20 (Beckman Coulter Inc., USA). Intra-assay CV value was 3.1% for ALT, 3.5% for AST, 1.8% for TB, 2.5% for DB, 2.8% for TC, and 5.3% for TG, while inter-assay CV value was 4.5% for ALT, 5.0% for AST, 2.6% for TB, 3.4% for DB, 3.2% for TC, and 6.1% for TG. C-reactive protein (CRP) analysis was performed on a Beckman Coulter AU 2700 analyzer. Intra-assay CV value was 3.1% and inter-assay CV value was 4.8% for CRP.

2.3. Quantification of PGRN

Serum concentrations of PGRN in all chronic HBV patients and control subjects were determined by ELISA (R&D Systems Minneapolis, MN, USA) according to the manual. The detection limit was 0.54 ng/mL. Intra-assay CV value was 5.2% and inter-assay CV value was 6.5% for PGRN.

2.4. Statistical analysis

Mann–Whitney rank sum test was used to analyze the difference in PGRN concentrations between patient and healthy control groups. Spearman's rank correlation test was used to test the relationship among the variables in human data. All analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical software for Windows, version 9.0 (SPSS, IL, USA). $P < 0.05$ was considered statistically different.

3. Results

3.1. Characteristics of chronic HBV patients and healthy control subjects

This study investigated 38 Chinese patients with chronic HBV infection and 36 healthy control subjects. Their age, sex, body mass index (BMI), albumin, virological characteristics, ALT, AST, TB, DB, and CRP were described in Table 1. No participant had any autoimmune diseases. The serum concentrations of ALT, AST, TB and DB were significantly increased in chronic HBV patients compared to those of healthy controls, suggesting the presence of liver injury caused by chronic HBV infection in these patients.

Table 1
Clinical and virological characteristics of the subjects enrolled in the study.

	Healthy controls (n = 36)	Chronic hepatitis B (n = 38)
Age, years	43 (33–56)	41 (25–58)
Female/male (%)	20/16	21/17
BMI	21 (18–24)	22 (18–26)
Albumin, g/dL [median (IQ range)]	4.2 (3.6–4.9)	3.0 (1.8–4.1)
TC, mmlol/L [median (IQ range)]	3.6 (2.8–5.2)	4.0 (2.8–5.2)
TG, mmlol/L [median (IQ range)]	1.1 (0.35–1.7)	1.2 (0.35–1.7)
CRP, mg/L	<5	<5
HBsAg positive	0/36	38/38
HBeAg positive	0/36	21/38
HBcAb positive	0/36	38/38
ALT, U/ml [median (IQ range)]	<30	161 (30–522)***
AST, U/ml [median (IQ range)]	<20	131 (20–460)***
TB, μmol/L [median (IQ range)]	<20	52 (21–178)***
DB, μmol/L [median (IQ range)]	<10	43 (16–111)***
HBV DNA log10 (copies/ml)	0	5.5 (3.1–8.0)
Autoimmune diseases	None	None

Note: Data are expressed as median (interquartile range) unless otherwise indicated. IQ: interquartile, BMI: body mass index, ALT: alkanine aminotransferase, AST: aspartate aminotransferase, TB: total bilirubin, DB: direct bilirubin, TC: total cholesterol, TG; triglyceride, CRP: C-reactive protein, n: number of individuals.
*** $p < 0.001$ when compared to healthy controls.

3.2. The serum PGRN levels were elevated in patients with chronic HBV infection

To investigate the role of PGRN in the pathogenesis of chronic HBV infection, we first compared serum PGRN levels between 38 patients with chronic HBV infection and 36 healthy controls by using ELISA (Fig. 1). Serum PGRN concentrations in healthy controls were always within the range of 16–83 ng/ml (Fig. 1A). Serum PGRN levels in patients with chronic HBV infection (median: 65.4 ng/ml) were significantly and markedly higher than those in healthy controls (median: 45.8 ng/ml). Furthermore, serum PGRN concentrations were not statistically different between HBeAg-positive and HBeAg-negative patients (Fig. 1B).

3.3. Correlation analysis of serum PGRN with ALT and AST levels in chronic HBV patients

By analyzing the relationship between serum PGRN and ALT or AST concentrations in the patients with chronic HBV infection, we found that serum PGRN concentrations did not significantly correlate with ALT or AST in chronic HBV patients (Fig. 2).

3.4. Correlation analysis between serum PGRN and HBV-DNA levels

We next tested whether serum PGRN levels correlate with viral loads in the patients with chronic HBV infection. Serum PGRN levels showed a significantly positive correlation with serum HBV-DNA levels in these patients ($r = 0.37$; $p = 0.02$; Fig. 3A). When the data of the healthy control group was combined with that of patient group in correlation analysis, there was still a significant and positive correlation between serum PGRN levels and HBV loads in all participants ($r = 0.58$; $p < 0.001$; Fig. 3B).

4. Discussions

HBV is the prototypical member of hepadnaviruses, which naturally infect only humans and great apes, and HBV infection has gained increasing public attention [9]. A large body of studies have demonstrated that a variety of regulatory molecules participate in immune response, contributing to chronic HBV infection, dysregulated liver inflammation and disease progression [10,11]. A better understanding of the role of immune factors in the