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Original Article

Serum DHCR24 Auto-antibody as a new Biomarker for Progression of Hepatitis C

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ABSTRACT

Background: New biomarkers are needed to identify the stage of hepatitis C virus (HCV)-infected diseases in order to reduce the mortality rates. Herein, we investigated whether serum 3β-hydroxysterol Δ24-reductase antibody (DHCR24 Ab) may serve as a prognostic marker for hepatitis C infection progression to hepatocellular carcinoma (HCC).

Methods: Serum DHCR24 Abs from 395 HCV-positive patients, including 133 chronic hepatitis (CHC), 85 liver cirrhosis (LCC), and 177 HCC (HCC-C) patients; 232 hepatitis B virus (HBV)-positive patients, including 103 chronic hepatitis (CHB), 56 liver cirrhosis (LCB), and 73 HCC (HCC-B) patients; and 24 healthy controls, were measured using enzyme-linked immunosorbent assay.

Results: The serum DHCR24 Ab levels were significantly higher in patients with CHC than in healthy controls, in LCC than in CHC, and in LCC than in HCC-C ($P < 0.0001$ for all). The concentration of serum DHCR24 Ab in HCC-B patients showed no significant difference compared to CHB and LCB patients ($P = 0.1247$). The DHCR24 Ab levels were significantly higher in early HCC-C than CHC or LCC patients and in late HCC-C compared to early HCC-C patients. The sensitivity of the DHCR24 Ab for HCC-C detection (70.6%) was higher than that of alpha-fetoprotein (AFP; 54.8%) and protein induced by vitamin K absence or antagonist-II (PIVKA-II; 42 · 5%). Moreover, DHCR24 was up-regulated in HCV-positive, but not HBV-positive tissues or HBV-negative, HCV-negative HCC specimens.

Conclusions: DHCR24 auto-antibody represents a potential noninvasive biomarker for HCV-related liver disease and may facilitate the diagnosis of PIVKA-II and AFP-negative HCC.

1. Introduction

Hepatitis C virus (HCV) infection has a high prevalence, incidence, and pathogenicity, and may evolve into cirrhosis and hepatocellular carcinoma (HCC) (Castello et al., 2010). HCV infection is considered a chronic liver disease, likely resulting from immune evasion by HCV quasi-species generated from high rates of replication errors (Farazi and DePinho, 2006). Since HCV does not integrate into the host genome, HCV-related HCC (HCC-C) is mainly induced through indirect pathways, including chronic inflammation, cirrhosis, cell death, and proliferation (Lorusso and Ruegg, 2008). A hallmark of HCV infection is perturbed lipid metabolism; HCV exploits host lipids for replication and further infection. The 3β-hydroxysterol Δ24-reductase (DHCR24) protein catalyzes the conversion of desmosterol to cholesterol; Bae and Paik, 1997 this reaction appears to be remarkable in HCV infection (Schaefer and Chung, 2013). Previously, we found that HCV up-regulated DHCR24 expression in human hepatoblastoma-derived RzM6-LC cells and that DHCR24 overexpression impaired p53 activity by suppressing acetylation and increasing interactions with the MDM2 proto-oncogene. In turn, this suppressed the hydrogen peroxide-induced apoptotic response in

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hepatocytes (Nishimura et al., 2009; Tsukiyama-Kohara, 2012; Saito et al., 2012).

The development of novel biomarkers and surveillance strategies in high-risk populations will permit earlier discovery of HCC and improve the survival outcomes. Currently, the most commonly used biomarker for early-stage HCC is alpha-fetoprotein (AFP) (Zhang et al., 2004). However, the AFP levels remain normal in 15–20% of advanced-stage HCC patients, and only 10–20% of early-stage HCC patients show abnormal AFP values (Monsour et al., 2013). Therefore, additional HCC biomarkers, including protein induced by vitamin K absence or antagonist-II (PIVKA-II; des-gamma-carboxy-prothrombin), have been developed (Makuuchi et al., 2008). Nonetheless, the need for novel biomarkers for early detection of HCC remains an important issue. Moreover, no biomarker corresponding to the disease progression of HCV-infected diseases, such as chronic hepatitis, cirrhosis, and HCC, has been established. Accordingly, this study aimed to evaluate the levels of serum DHCR24 auto-antibody (DHCR24 Ab) in a broad spectrum of chronic hepatitis diseases.

2. Materials and Methods

2.1. Subjects

The subjects were prospectively enrolled at the Tokyo Metropolitan Komagome Hospital, Showa University Fujigaoka Hospital, and Kanazawa University Hospital, Japan. This study was approved by the ethics committee of each hospital and conducted in accordance with the Helsinki Declaration. All patients provided informed consent.

Frozen liver samples (cancerous and noncancerous) were obtained from five HCC-HCV, five HCC-HBV and five HCC-nonBnonC (NBNC) patients that were obtained from 15 HCC patients at the Liver Unit of the Tokyo Metropolitan Komagome Hospital, Japan and used for Western blotting and immunohistochemistry.

Six hundred fifty-one serum samples, collected from September 2007 to November 2014, were obtained from 395 HCV-positive patients, including 133 moderate chronic hepatitis C (CHC), 85 liver cirrhosis (LCC), and 177 HCC (HCC-C) patients; 232 HBV patients, including 103 chronic HBV (CHB), 56 liver cirrhosis (LCB), and 73 HCC (HCC-B) patients; and 24 healthy controls (Table 1).

Chronic HCV infection was defined as detectable serum anti-HCV antibodies and HCV RNA. Liver cirrhosis was by presence of ascites and/or gastroesophageal varices and defined by the aspartate transaminase (AST) to platelet ratio index (APRI) and Fibrosis-4 index. APRI (cutoff value ≧1.00, sensitivity 89%, specificity 74%, PPV38%, NPV 98%) was calculated as follows: (AST [IU / L] / upper normal limit) × 100/platelets (10^9/L) (Wai et al., 2003). The Fibrosis-4 index (cutoff value ≧1.45, sensitivity 70%, specificity 74%, PPV42%, NPV 90%) was calculated by: age (years) × AST (IU / L) / (platelets [10^9/L] × [alanine aminotransferase (ALT; IU/L)]^{½}) (Sterling et al., 2006). HCC was diagnosed by ultrasonography and computed tomography and confirmed by liver biopsy, and classified into early and late HCCs according to the pathological criteria of the international consensus group for hepatocellular neoplasia. Early-stage HCC was defined as a single lesion between 2 and 5 cm, or less than or equal to three lesions each ≤3 cm. Late-stage HCC was defined by a single lesion >5 cm, or greater than three lesions (International Consensus Group for Hepatocellular Neoplasia and The International Consensus Group for Hepatocellular N, 2009).

The diagnosis of chronic HBV infection was confirmed by the presence of HBV surface antigen in the blood for greater than six months, HBV core antibodies, and HBV-DNA detection by real-time polymerase chain reaction. The healthy controls had no medical history of liver disease, were negative for viral hepatitis markers, and had normal ALT and AST levels. Table 1 summarizes the main characteristics of the study patients.

2.2. Western Blotting

Fifteen frozen liver tissue specimens were homogenized on ice using a type-A glass homogenizer (Wheaton Science Products, Millville, NJ, USA) in RIPA buffer (1% sodium dodecyl sulfate, 0.5% NP40, 0.15 M NaCl, 10 mM Tris pH 7 · 4, 5 mM ethylenediaminetetraacetic acid [EDTA], and 1 mM dithiothreitol). Proteins were transferred to Immobilon P polyvinylidene fluoride membranes (Merck Millipore, Jaffrey, NH, USA). After blocking with 5% Block Ace™ powder (Dainippon Pharmaceutical Co., Osaka, Japan) in TBST (0.1% Tween 20, 20 mM Tris-Cl pH 7.6, and 137 mM NaCl) at room temperature for 2 h, the membranes were incubated for 2 h with 1-μg/mL of primary antibody in TBST containing 1% Block Ace. After washing, the membranes were incubated with 1:2000 horseradish peroxidase-conjugated rabbit anti-mouse antibody (Dako, Glostrup, Denmark) in TBST containing 1% Block Ace. Western blotting was performed using DHCR24 monoclonal antibody (MoAb) 2-152a[6] as the primary antibody. Actin (polyclonal antibody; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used for normalization.

2.3. Immunohistochemistry of Liver Tissues

Frozen sections of cancerous and noncancerous liver tissues from HCC patients were prepared in optimal cutting temperature compound (Ted Pella Inc., Redding, CA, USA), placed on glass slides, thawed, washed with phosphate-buffered saline (PBS), and fixed with 1% paraformaldehyde for 10 min in PBS. After blocking for 1 h with PBS containing 1% bovine serum albumin and 1 mM EDTA, the slides were washed with PBS and incubated overnight at 4 °C with the primary monoclonal Abs (5 μg/mL). Next, the slides were washed with PBS thrice and incubated for 30 min with 1:1000 Alexa 488-conjugated goat anti-mouse immunoglobulin G (IgG) $(Fab')_2$ fragment or Alexa 568-conjugated goat anti-rabbit IgG $(Fab')_2$ fragment (Molecular Probes, Eugene, OR, USA) in PBS containing 0.05% Tween-20. Subsequently, the slides were washed thrice with PBS and cover-slipped using Vector-shield (Vector

Table 1
Baseline demographic and disease characteristics.

	Healthy controls (N = 24)	Hepatitis C virus group (N = 395)	Hepatitis B virus group (N = 232)
Mean age ± SD, years	45.08 ± 16.84	68.32 ± 11.40	58.31 ± 12.90
Gender (male/female)	9/15	185/210	151/81
ALT (IU/L), mean ± SD	21.50 ± 10.10	54.55 ± 44.80	63.01 ± 159
AST (IU/L), mean ± SD	21.92 ± 6.47	58.32 ± 36.70	79.25 ± 450
AFP (ng/mL), mean ± SD	4.50 ± 3.23	233.31 ± 18823	1850 ± 180900
PIVKA-II (mIU/mL), mean ± SD	–	5991.35 ± 75342.65	471.60 ± 3736.78
Clinical status			
Moderate chronic hepatitis (n)	–	133	103
Liver cirrhosis (n)	–	85	56
HCC (n)	–	177	73

Abbreviations: SD, standard deviation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; AFP, alpha-fetoprotein; PIVKA-II, protein induced by vitamin K absence or antagonist-II; HCC, hepatocellular carcinoma.