Clinical significance of decoy receptor 3 upregulation in patients with hepatitis B and liver fibrosis

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Received September 28, 2017; Accepted April 16, 2018

DOI: 10.3892/ol.2018.8762

Abstract. Decoy receptor 3 (DcR3) is a tumor necrosis factor receptor, which may inhibit apoptosis. The aim of the present study was to investigate the clinical significance of DcR3 upregulation in patients with chronic hepatitis B (CHB) and hepatic fibrosis. A total of 128 patients with a clinical diagnosis of CHB who underwent liver biopsy were included in the present study. The expression levels of DcR3, hyaluronic acid (HA), type III procollagen, type IV collagen (IV-C) and laminin protein were assessed. The diagnostic value of DcR3 in patients with CHB with hepatic fibrosis was determined using receiver operating characteristic (ROC) curve analysis. DcR3 was significantly upregulated in patients with CHB, particularly in patients with active CHB. The expression of DcR3 was significantly increased in patients with CHB with liver fibrosis and liver cirrhosis, compared with patients with CHB without liver fibrosis. The area under the ROC curve for the diagnosis of CHB liver fibrosis based on DcR3 or DcR3 combined with IV-C/HA was 0.807 or 0.869, with a sensitivity and specificity of 76.9 and 77.8% or 84.6 and 81.2%, respectively. DcR3 is a marker for liver fibrosis in patients with hepatitis B infection. The use of DcR3 in combination with IV-C and HA may further increase its diagnostic value for liver fibrosis.

Introduction

Chronic viral hepatitis B (CHB) poses a serious threat to human health. An epidemiological study demonstrated that the seroprevalence rate of hepatitis B virus surface antigen in the Chinese population is 7.18% (1). Hepatitis B may lead to hyperplasia of hepatic fibrous connective tissues, resulting in liver fibrosis and cirrhosis (2,3). Detection of hepatitis B virus (HBV) DNA is the most common laboratory method for the evaluation of virus replication activity. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and \( \gamma \)-glutamyl transferase (\( \gamma \)-GGT) are serum indicators of liver function in patients with CHB. Hyaluronic acid (HA), type III procollagen (PCIII), type IV collagen (IV-C) and laminin protein (LN) are commonly used as indicators of liver fibrosis (4-6). However, these indicators do not completely meet the clinical requirements due to their limited sensitivity and specificity (7). Pathological examination by liver biopsy, which carries a risk of complications, is required for the diagnosis of liver fibrosis (8-10). Thus, it is required to identify noninvasive markers of liver fibrosis with clinical diagnostic or therapeutic significance. Decoy receptor 3 (DcR3), a novel member of the tumor necrosis factor receptor (TNFR) superfamily, is a surface receptor that competitively binds Fas Ligand (FasL), lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells (LIGHT) and TNF-like ligand 1A (TL1A) ligands to inhibit apoptosis. It has been indicated that the expression of DcR3 is increased in the inflammatory response to bacterial infection, rheumatoid arthritis, acute ulcerative colitis and appendicitis, and in tumors (11). DcR3 is closely associated with cancer and inflammation (12). The aim of the present study was to examine the expression of DcR3 in patients with hepatitis B and hepatic fibrosis to explore its clinical diagnostic value.

Materials and methods

Study subjects. A total of 128 patients (male, n=72; female, n=56; median age, 38 years; range, 20-65 years) diagnosed with CHB were recruited in The Liver Department of Songjiang Hospital Affiliated to First People's Hospital (Shanghai, China) between December 2014 and December 2015. All patients with CHB met the criteria for the diagnosis of CHB published in ‘Chronic HBV Hepatitis Treatment and Prevention Guide’ (13). Patients with a history of fatty liver, alcoholic liver disease, obesity, liver cancer, hepatitis C, hepatitis D, autoimmune liver disease and other liver diseases, as well as those with a recent history of infection and allergy were excluded. All patients provided written informed consent. All procedures were performed in compliance with the relevant provisions of the Ethics Committee of Songjiang District Center Hospital.

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Key words: decoy receptor 3, chronic hepatitis B, liver fibrosis, receiver operating curve
The correlations between DcR3 and HBV DNA, ALT, AST, GGT, HA, IV-C, PCIII, LN, or NF-κB were analyzed using Pearson's correlation test. The gender difference was analyzed using a χ² test. P<0.05 was considered to indicate a statistically significant difference. All the data are presented as median and ranges or mean ± standard error.

**Results**

**DcR3 expression is significantly increased in patients with CHB.** Serum DcR3 levels (196.88±26.67 ng/ml) were significantly higher in the CHB group, compared with the healthy control group (0.047±0.006 ng/ml; P<0.05; Table I). In addition, γ-GGT, AST and ALT levels were significantly higher in the CHB group, compared with the healthy control group (P<0.05; Table I). However, there was no significant difference in procalcitonin and interleukin 6 between the two groups, which demonstrated similar responses to acute inflammation. The serum levels of HA, PCIII, IV-C and LN were significantly higher in the CHB group, compared with the control group (P<0.05; Table I).

**DcR3 expression is significantly increased in patients with active hepatitis B.** Patients with CHB with ALT>40 IU/L, HBV DNA>10⁷ copies/ml were classified as active hepatitis B group (active CHB). The remaining patients who did not meet any of these criteria were classified as hepatitis B carriers (CHB carrier). Serum DcR3 levels were significantly higher in the active CHB group (253.82±32.12 ng/ml), compared with the CHB carrier group (151.35±12.03 ng/ml; P<0.05; Fig. 1).

**DcR3 levels are increased in patients with hepatitis B and liver fibrosis.** Patients with CHB were divided into three groups according to pathology results: S₀, CHB group without fibrosis group; S₁, CHB group complicated with fibrosis group; and S₂, group with cirrhosis. NC was the healthy control group. The results demonstrated that the DcR3 levels were significantly higher in the S₁, group (227.37±18.71 ng/ml) and the S₂, group (355.26±5.054 ng/ml), compared with the S₀, group (109.66±16.08 ng/ml). DcR3 levels were significantly higher in the S₁, group (227.37±18.71 ng/ml; Fig. 2). The levels of HA, PCIII, IV-C and LN were significantly higher in the S₁, and S₂, groups, compared with the S₀, group (P<0.05; Table II).

**Significant association between DcR3 and fibrosis indicators.** Results demonstrated that DcR3 levels were significantly increased in the hepatic fibrosis group (S₁, and S₂) and significantly associated with the four indicators of hepatic fibrosis: HA (r=0.51, P<0.001); IV-C (r=0.34, P=0.0013); PCIII (r=0.49, P<0.001); and LN (r=0.40, P<0.001). In addition, serum DcR3 levels in patients with CHB were positively associated with HBV DNA, ALT, AST and GGT levels (r=0.27, 0.53, 0.54 and 0.48, respectively; P<0.001; Table III). However, DcR3 levels had no association with sex and age in patients with CHB.

**Diagnostic value of DcR3 in CHB and hepatic fibrosis.** The diagnostic ability of DcR3 and liver fibrosis indicators (IV-C, HA, PCIII and LN) for CHB with hepatic fibrosis was evaluated using ROC curve analysis (Fig. 3), with area under the curve (AUC) values of 0.807, 0.770, 0.688, 0.626 and 0.584,
respectively. The 95% confidence interval, cut-off value, sensitivity and specificity are displayed in Table IV. When the cutoff value for DcR3 was set at 168.67 ng/ml, its diagnostic sensitivity and specificity were 76.9 and 77.8%, respectively (Table IV). When liver fibrosis indicators with improved diagnostic ability, namely IV-C and HA, were used in combination with DcR3 for diagnosis, the AUC for DcR3 combined with IV-C and HA was 0.869, with sensitivity and specificity of 84.6 and 81.2%, respectively (Table IV). This indicated that DcR3 may significantly improve the combined diagnostic effect of IV-C and HA (AUC of 0.798, with sensitivity and specificity of 71.8 and 75.0%, respectively) and demonstrated the value of DcR3 as a clinical diagnostic index of liver fibrosis.

Nuclear factor (NF)-κB levels are increased in patients with hepatitis B and liver fibrosis. The serum levels of NF-κB in patients with hepatitis B and liver fibrosis were detected, which demonstrated that NF-κB levels were elevated in S0 (2,128.01±552.67 pg/ml), S1 (2,199.50±460.40 pg/ml) and S4 (2,179.56±384.11 pg/ml) groups, compared with in S0 (P<0.001) or NC groups (P=0.001). DcR3 levels were significantly higher in the S4 group, compared with the S1 group (P=0.013). Patients with CHB were divided into three groups according to the liver biopsy diagnosis as follows: S0 (n=45), CHB without fibrosis; S1 (n=74), CHB complicated with fibrosis; and S4 (n=9), cirrhosis. DcR3, decoy receptor 3; CHB, chronic hepatitis B.
tissues, compared with normal liver tissues (P=0.021). DcR3 levels increased with the grade of hepatic cell carcinoma (P=0.033), and DcR3 mRNA was significantly higher in patients with HCC who died within 5 years, compared with those who survived >5 years (P=0.008; Fig. 5). Assessment of DcR3 levels in patients with CHB associated with liver fibrosis indicated that DcR3 serves an important role in the CHB-liver fibrosis-liver cirrhosis-liver cancer progression, although the specific underlying mechanism of action requires further examination.

Table II. Clinical data of patients with CHB liver fibrosis (mean ± standard deviation).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>S₀ (n=45)</th>
<th>S₁-3 (n=74)</th>
<th>S₄ (n=9)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female (n)</td>
<td>20/15</td>
<td>41/33</td>
<td>6/3</td>
<td>a₁&lt;0.001, a₂&lt;0.001, b=0.031</td>
</tr>
<tr>
<td>DcR3 (ng/ml)</td>
<td>109.66±16.08</td>
<td>227.37±18.71</td>
<td>355.26±50.54</td>
<td>a₁=0.003, a₂&lt;0.001, b=0.014</td>
</tr>
<tr>
<td>HA (ng/ml)</td>
<td>0.74±0.060</td>
<td>1.03±0.05</td>
<td>1.49±0.39</td>
<td>a₁&lt;0.001, a₂&lt;0.001, b=0.031</td>
</tr>
<tr>
<td>PCIII (ng/ml)</td>
<td>22.88±0.95</td>
<td>35.18±1.28</td>
<td>49.41±2.81</td>
<td>a₁=0.049, b=0.037</td>
</tr>
<tr>
<td>IV-C (pg/ml)</td>
<td>548.51±39.33</td>
<td>910.52±37.40</td>
<td>1,266.32±255.32</td>
<td>a₁&lt;0.001, a₂&lt;0.001, b=0.005</td>
</tr>
<tr>
<td>LN (ng/ml)</td>
<td>0.05±0.01</td>
<td>0.10±0.003</td>
<td>0.15±0.032</td>
<td>a₁=0.006, b=0.002</td>
</tr>
<tr>
<td>γ-GGT (IU/l)</td>
<td>23.47±4.00</td>
<td>51.35±7.89</td>
<td>46.89±11.69</td>
<td>a₁=0.008</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>35.31±4.50</td>
<td>95.78±16.09</td>
<td>176.80±115.45</td>
<td>a₁&lt;0.001, a₂=0.006</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>45.26±5.62</td>
<td>198.72±35.09</td>
<td>186.93±123.06</td>
<td>a₁=0.008</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>15.33±1.81</td>
<td>18.01±2.03</td>
<td>19.91±5.97</td>
<td>a₁=0.008</td>
</tr>
<tr>
<td>PCT (ng/ml)</td>
<td>0.11±0.071</td>
<td>0.16±0.02</td>
<td>0.12±0.091</td>
<td>a₁=0.008</td>
</tr>
</tbody>
</table>

γ-GGT, γ-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HA, hyaluronidase; PCIII, type III procollagen; IV-C, type IV collagen; LN, laminin; DcR3, decoy receptor 3; CHB, chronic hepatitis B; PCT, procalcitonin.

Table III. Association between DcR3 and other clinical biomarkers.

<table>
<thead>
<tr>
<th>Index</th>
<th>Sex</th>
<th>Age</th>
<th>HBV-DNA</th>
<th>γ-GGT</th>
<th>AST</th>
<th>ALT</th>
<th>HA</th>
<th>PCIII</th>
<th>IV-C</th>
<th>LN</th>
<th>NF-κB</th>
</tr>
</thead>
<tbody>
<tr>
<td>DcR3</td>
<td>-0.122</td>
<td>-0.355</td>
<td>-0.27</td>
<td>-0.53</td>
<td>-0.54</td>
<td>-0.48</td>
<td>-0.51</td>
<td>-0.49</td>
<td>-0.34</td>
<td>-0.40</td>
<td>-0.06</td>
</tr>
<tr>
<td>P=0.434</td>
<td>P=0.020</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P=0.001</td>
<td>P&lt;0.001</td>
<td>P&lt;0.001</td>
<td>P=0.0013</td>
<td>P&lt;0.001</td>
<td>P=0.726</td>
<td></td>
</tr>
</tbody>
</table>

γ-GGT, γ-glutamyl transferase; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HA, hyaluronidase; PCIII, type III procollagen; IV-C, type IV collagen; LN, laminin protein; DcR3, decoy receptor 3; NF-κB, nuclear factor-κB; HBV, hepatitis B virus.
Discussion

Accurate assessment of the degree of viral hepatic fibrosis is important for patient treatment, prognosis and surveillance. Liver biopsy, which is considered the gold standard for the diagnosis of liver fibrosis, has certain limitations. Such invasive procedures are associated with certain complications. The majority of patients refuse to undergo liver biopsy, and they are even more reluctant to accept a second liver biopsy, making it difficult to assess the efficacy of the procedure (14). Additionally, sample errors, and intraobserver or interobserver differences may affect the accuracy of the diagnostic results (15-17).

Within the TNFR family, DcR3 is the only member capable of competitively binding the three ligands TL1A, LIGHT and FasL (12). In humans, DcR3 is upregulated in pathological conditions including cancer, and autoimmune and inflammatory diseases (18,19). The data from the present study demonstrated that DcR3 expression was significantly associated with ALT, AST and γ-GGT. Assessment of DcR3 protein levels may provide an important diagnostic biomarker, for example for inflammatory diseases or high-grade carcinoma. The present study compared the performance of DcR3 with that of direct serological markers (HA, CIV, PCIII and LN) for the diagnosis of liver fibrosis and its stages.

In the present study, it was indicated that serum DcR3 levels were significantly increased in patients with hepatic fibrosis and hepatic cirrhosis (P<0.01) and significantly associated with the four indicators of hepatic fibrosis, namely HA (r=0.51, P<0.0001), IV-C (r=0.34, P<0.0013), PCIII (r=0.49, P<0.0001) and LN (r=0.40, P<0.0001), indicating the clinical value of DcR3 for the diagnosis of hepatic fibrosis. A study by Kim et al (20) on the different stages of chronic hepatitis and fibrosis determined that DcR3 expression levels were elevated in bile duct epithelial cells and infiltrating lymphocytes, in regenerated and poorly developed bile ducts, as well as in cultured hepatoma cells, indicating that DcR3 serves an important role in the progression of chronic hepatitis to hepatic fibrosis, leading to the occurrence of liver cirrhosis and HCC.

To determine the diagnostic value of DcR3 for liver fibrosis, ROC analysis was performed. The AUC values for the diagnosis of hepatitis B and hepatic fibrosis based on DcR3 and the four indicators of liver fibrosis, including IV-C, HA,
PCIII and LN, were 0.807, 0.770, 0.688, 0.626 and 0.584, respectively. When IV-C and HA, which have demonstrated superior diagnostic accuracy among the four indicators, were used in combination with DcR3 as diagnostic markers, the AUC was 0.869, with sensitivity and specificity of 84.6 and 81.2%, respectively, indicating that DcR3 significantly improves the combined diagnostic value of IV-C and HA. These results indicated that the diagnostic value of DcR3 for CHB may be superior to that of the four conventional liver fibrosis indicators.

The role of DcR3 in HBV infection remains unclear. A previous study indicated that DcR3 expression is closely associated with the degree of inflammation during the pathogenetic process of acute ulcerative colitis, as increased DcR3 expression levels are observed in the peripheral blood of patients with a high incidence of inflammation, and these are significantly reduced following effective treatment (21). A previous study indicated that DcR3 is upregulated in patients with hepatitis B e antigen-negative CHB (22). However, the present study determined that the DcR3 levels are increased in patients with active hepatitis and in those with liver fibrosis. Yang et al (23) indicated that DcR3 levels were significantly increased in the sera of patients with HCC and associated with liver cirrhosis, tumor metastasis and recurrence, which indicated that the highly expressed and distributed DcR3 may serve as an important role in occurrence and development of primary HCC.

Bioinformatics analysis of a human HCC DNA microarray database indicated that DcR3 expression was significantly higher in HCC tissues compared with that in normal liver tissues. DcR3 levels increased with the grade of HCC, and the copy number of DcR3 DNA of patients with HCC who died within 5 years was significantly higher compared with that of patients with HCC who survived >5 years. These results indicated that DcR3 may serve as an important role in the occurrence and development of CHB, liver fibrosis, liver cirrhosis and liver cancer, although the underlying molecular mechanism requires further exploration.

The expression of DcR3 is high in patients with CHB, liver fibrosis and liver cirrhosis. However, the molecular mechanisms underlying liver cancer progression has not yet been investigated. DcR3 levels are increased in patients with inflammatory bowel disease, primary Sjogren's syndrome, rheumatoid arthritis and primary biliary cirrhosis. DcR3 is also upregulated in Kaposi's sarcoma-associated herpes virus-infected human umbilical vein endothelial cells (24) and skin lesions of psoriasis patients (25). In human keratinocytes, DcR3 is transcriptionally regulated by epidermal growth factor via the NF-κB signaling pathway (26). In nasopharyngeal carcinoma (NPC) cases with Epstein Barr Virus (EBV) infection, EBV binds to the promoter of the transcriptional activation factor RTA and upregulates DcR3 expression (27). Furthermore, its latent membrane protein-1 activates the NF-κB signaling pathway to promote DcR3 expression, and the upregulated DcR3 enhances the metastatic and invasive abilities of the NPC cell line HONE-1 (28). HBV protein HBx upregulates gene expression by binding to pattern recognition receptor at key sectors of host genes, and indirectly activates host gene promoters by interacting with the transcription factor NF-κB and regulating gene transcription (29). In the
The present study demonstrated that DcR3 is a novel indicator for the diagnosis of hepatitis B and liver fibrosis. Further in-depth study would provide indications to elucidate the underlying molecular mechanism and assist in designing strategies for the clinical treatment of liver fibrosis, liver cirrhosis and liver cancer induced by hepatitis B.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of China (grant no. 81702729), the Scientific Foundation of Shanghai Municipal Commission of Health and Family Planning (grant nos. 201540119 and 201640Y0273) and the Science and Technology Research Project of Songjiang of Shanghai (grant nos. 15SJGG25 and 15SJGG47).

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

YH conceived the idea and designed the study. YH and XL contributed to the data analysis and writing of the manuscript. HC helped to conduct the experiments and data analysis. JZ collected clinical blood specimens and clinical data. FZ performed indicator assays. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The human data reported in this manuscript were collected according to procedures approved by the Institutional Ethical Review Board of Songjiang District Center Hospital. All patients provided written informed consent for the publication of their data.

Consent for publication

All patients provided written informed consent for the publication of their data.

Competing interests

The authors declare that they have no competing interests.

References


