Role of ErbB3 binding protein 1 (EBP1) as a predictor in hepatocellular carcinoma.

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Abstract

Background: The role of ErbB3 Binding Protein 1 (EBP1) in Hepatocellular Carcinoma (HCC) prognosis was inconsistent. Therefore, we performed this study to determine the effect of EBP1 in HCC prognosis.

Methods: 146 patients underwent resection of HCC were collected between 2010 and 2015. Overall Survival (OS) analysis was performed with Kaplan-Meier with log rank test. The prognostic value of EBP1 was evaluated by cox regression analysis.

Results: The expression of EBP1 was lower compared with adjacent normal tissues (p=0.01). EBP1 expression was significantly correlated with larger tumour size (p=0.03), metastasis (p=0.04), and advanced clinical stage (p=0.01). Univariate analysis of OS revealed that the relative level of EBP1 expression (p=0.002), tumor size (p=0.05), metastasis (p=0.04), and clinical stage (p=0.001) were prognostic indicators. Furthermore, multivariate analysis revealed that clinical stage (p=0.01) and EBP1 expression (p=0.03) were independent prognostic indicator for OS in HCC.

Conclusions: In conclusion, this study suggested that EBP1 expression was an independent prognostic indicator for OS in HCC.

Keywords: Hepatocellular carcinoma, ErbB3 binding protein 1, Overall survival.

Introduction

Hepatocellular Carcinoma (HCC) is one of the most common cancers and ranks fifth among causes of cancer mortality worldwide [1]. Among the major risk factors for HCC, chronic infection with Hepatitis B Virus (HBV) is of particular interest for its coherent distribution with the HCC prevalence [2]. In Western countries and Japan, infection with HCV is the more common cause of HCC, while in Asia and developing countries, HBV is more common [3].

ErbB3 Binding Protein 1 (EBP1) is the human homologue of the mouse protein p38-2AG4, which regulates cell proliferation [4]. EBP1 also associates with mature ribosomes and suppresses the phosphorylation of the eukaryotic initiation factor 2 alpha under stress condition, and thus is possibly involved in the control of protein translation [5]. Ko et al. proposed that p42 Ebp1 functions as a potent tumor suppressor of non-small cell lung cancer through interruption of Akt signaling [6]. Awasthi et al. suggest that the ability of EBP1 to activate ErbB2 signaling pathways results in increased lapatinib sensitivity [7]. Ghosh et al. suggested that phosphorylation of EBP1 may be one mechanism of PAK1-induced hormone resistance and that PAK1 inhibitors may be useful in cells in which EBP1 is overexpressed [8]. Hu et al. indicated that EBP1 may be a valuable prognostic marker and promising therapeutic target of HCC [9]. However, the role of EBP1 in HCC prognosis was inconsistent. Therefore, we performed this study to determine the effect of EBP1 in HCC prognosis.

Materials and Methods

Patients and tissue samples

146 patients underwent resection of HCC were collected between 2010 and 2015. None of the patients received preoperative chemotherapy or radiotherapy. All tissue samples were immediately snap frozen in liquid nitrogen after surgery and stored at -80°C. All patients’ slides were reviewed to confirm the diagnosis and to classify the tumour according to the sixth edition of the Tumour Node Metastases (TNM) classification of the International Union against Cancer (UICC). The clinicopathologic features of the patients with
HCC are shown in Table 1. Patients with evidence of other diseases were excluded from this study. Approval for the study was obtained from the Ethics Committee of Hospital.

**Real-time quantitative RT-PCR for EBP1**

EBP1 expression in tissues and cells was performed by real-time quantitative RT-PCR. Briefly, total RNAs were isolated from the tissues and cells by TRIzol (Invitrogen, Carlsbad, CA, USA) following the manufacturer's protocol. Real-time (RT) and quantitative Polymerase Chain Reaction (qPCR) kits were used to evaluate the EBP1 expression from tissue samples. Primers for EBP1: 5'-TCAAAGCTGTAAGCTTATGTCGG-3' and 5'-CATAGAAATTCATACAAGGT-3'; β-actin: forward 5'-CCACTGGCATCGTGA TGGA-3', reverse 5'-CGCTCGTGAGGATCTTCAT-3'. Relative gene expression was calculated using the comparative Cycle Threshold (CT) (2^\(\Delta\Delta\text{CT}\)) method, with β-actin served as an endogenous control for normalization.

**Statistical analysis**

All statistical analyses were performed using SPSS 20.0 software (SPSS Inc., Chicago, IL, USA). The significance of between-group differences was estimated using Student’s t-test and \(\chi^2\) test, as appropriate. Overall Survival (OS) was calculated as the time interval from the date of surgery to either the day of the last follow-up or cancer-related death. OS rates were calculated using the Kaplan-Meier method, with the log-rank test applied for comparison. Variables with a value of \(p<0.05\) in univariate analysis were used in a subsequent multivariate analysis, based on the Cox proportional hazards model. Two-sided \(p\)-values were calculated, and \(P<0.05\) indicated statistical significance.

**Results**

**EBP1 expression and clinicopathologic factors of HCC**

As shown in Figure 1, the expression of EBP1 was lower compared with adjacent normal tissues (\(p=0.01\)). As shown in Table 1, EBP1 expression was significantly correlated with larger tumor size (\(p=0.03\)), metastasis (\(p=0.04\)), and advanced clinical stage (\(p=0.01\)). No significant association was found between EBP1 expression and age (\(p=0.33\)), gender (\(p=0.27\)), tumor number (\(p=0.11\)).

**EBP1 expression and prognosis of HCC**

Kaplan-Meier method and log-rank test were used to investigate the prognostic value of EBP1 expression in HCC. As shown in Figure 2, HCC patients with low EBP1 expression were correlated with shorter OS (\(p=0.001\)) compared with those with high EBP1 expression. As shown in Table 2, univariate analysis of OS revealed that the relative level of EBP1 expression (\(p=0.002\)), tumor size (\(p=0.05\)), metastasis (\(p=0.04\)) and clinical stage (\(p=0.001\)) were prognostic indicators. Furthermore, multivariate analysis revealed that clinical stage (\(p=0.01\)) and EBP1 expression (\(p=0.03\)) were independent prognostic indicator for OS in HCC (Table 2).

**Table 1. The relationship between EBP1 expression and clinicopathologic parameters.**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Low (n=71)</th>
<th>High (n=75)</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>36</td>
<td>32</td>
<td>0.33</td>
</tr>
<tr>
<td>(\leq 55)</td>
<td>35</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>(&gt;55)</td>
<td>35</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td>0.27</td>
</tr>
<tr>
<td>Male</td>
<td>44</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>27</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Tumor size (cm)</td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>(\leq 5)</td>
<td>28</td>
<td>43</td>
<td></td>
</tr>
<tr>
<td>(&gt;5)</td>
<td>43</td>
<td>32</td>
<td></td>
</tr>
<tr>
<td>Tumor number</td>
<td></td>
<td></td>
<td>0.11</td>
</tr>
<tr>
<td>Solitary</td>
<td>35</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Multiple</td>
<td>36</td>
<td>38</td>
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</table>
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Discussion
In this study, we found that the expression of EBP1 was lower compared with adjacent normal tissues. EBP1 expression was significantly correlated with larger tumour size, metastasis, and advanced clinical stage. In addition, multivariate analysis revealed that clinical stage and EBP1 expression was independent prognostic indicator for OS in HCC.

Miao et al. suggested that EBP1 participates in the regulation of intestinal inflammation via mediating Akt signaling pathway [10]. Liu et al. suggested that Ebp1 suppressed thyroid cancer cell lines by upregulating RASRAL expression [11]. Gong et al. suggested that EBP1 is a novel prognostic indicator and potential therapeutic target of PDAC, shedding new insights into the important role of EBP1 in cancer development [12]. Nguyen et al. demonstrate an important role of Ebp1 in promoting cell proliferation in AML cells through the regulation of both rRNA synthesis and PCNA expression [13]. Wang et al. establish distinct physical and functional interactions between FBXW7 and EBP1 isoforms, which yield their mechanistically unique isoform-specific functions of EBP1 in cancer [14].

Several limitations were showed in the present study. Firstly, the sample size was relatively small. Secondly, the specific mechanisms for EBP1 regulating HCC were not investigated in the study.

In conclusion, this study suggested that EBP1 expression was an independent prognostic indicator for OS in HCC.

Table 2. Univariate and multivariate analysis for overall survival.

<table>
<thead>
<tr>
<th>Prognostic factors</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
<th>HR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (≤ 55/&gt;55)</td>
<td>1.15</td>
<td>0.63-1.92</td>
<td>0.62</td>
<td></td>
<td></td>
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<tr>
<td>Gender (male/female)</td>
<td>0.95</td>
<td>0.58-1.55</td>
<td>0.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor size (≤ 5 cm/&gt;5 cm)</td>
<td>1.58</td>
<td>1.01-2.48</td>
<td>0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tumor number (solitary/multiple)</td>
<td>1.26</td>
<td>0.96-1.64</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metastasis (yes/no)</td>
<td>2.67</td>
<td>1.07-6.67</td>
<td>0.04</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Clinical grade (I and II/III)</td>
<td>2.66</td>
<td>1.48-4.80</td>
<td>0.001</td>
<td>2.11</td>
<td>1.16-3.94</td>
<td>0.01</td>
</tr>
<tr>
<td>EBP1 (high/low)</td>
<td>2.51</td>
<td>1.42-4.44</td>
<td>0.002</td>
<td>1.97</td>
<td>1.08-3.53</td>
<td>0.03</td>
</tr>
</tbody>
</table>

HR: Hazard Ratio; CI: Confidence Interval.

Disclosure of Conflict of Interest
The authors have declared that no competing interests exist.

References


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