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Original article

Expression of DBC1 is associated with poor prognosis in hepatitis virus-related hepatocellular carcinoma

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ABSTRACT

Purpose: Deleted in breast cancer 1 (DBC1) is a nuclear protein that was named by its deletion at a region 8p21 in some breast cancers and has been suggested as a poor prognostic indicator of various human cancers. However, the expression level of DBC1 protein and the prognostic role of DBC1 in hepatocellular carcinoma (HCC) have not been reported.
Methods: We investigated the effect of DBC1 protein expression in 199 hepatitis virus-related HCC patients. Immunohistochemical expression of DBC1 were evaluated by tissue microarray.
Results: High DBC1 immunoreactivity was observed in 177 (88.9%) of the 199HCC cases and was significantly associated with younger age ($P=0.001$), higher α-fetoprotein level ($P=0.008$), hepatitis B virus infection ($P=0.001$), and liver cirrhosis ($P=0.003$). High DBC1 expression showed an unfavorable effect on recurrence-free survival (RFS) ($P=0.036$) and tended to be an independent predictor of shorter RFS ($P=0.064$). High DBC1 expression did not show an unfavorable effect on overall survival ($P=0.575$). Five (45.5%) of 11 low grade dysplastic nodules (LGDNs), 8 (80%) of 10 high grade dysplastic nodules (HGDNs), and 10 (83.3%) of 12 early HCCs showed high DBC1 expression. The proportion of high DBC1 expression in LGDN, HGDN, early HCC, and HCC was significantly different, with a stepwise increase ($P=0.0002$).
Conclusion: DBC1 protein could be a prognostic marker of shorter RFS in HCC patients after hepatectomy and human hepatocarcinogenesis was a multistep process accompanied by a stepwise increase in high DBC1 expression from LGDN, through HGDN, to HCC. Patients with high DBC1 expression can be considered candidates for adjuvant treatment after hepatectomy.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common human malignancies and usually develops in chronic liver diseases. Globally, chronic hepatitis virus infection is the most frequent risk factor for the development of HCC. Even after resection, HCC is still related with high rates of mortality due to the high incidence of tumor recurrence and metastasis [1,2]. Examining molecular changes and clinical outcome is an effective method for search for prognostic factors for further therapies [3]. HCC associated with chronic liver disease develops through a multistep process of carcinogenesis [4]. Premalignant lesions of HCCs have been designated as dysplastic nodules by the International Working Party [5]. Dysplastic nodules, which usually occur in cirrhotic livers, are further divided into low grade dysplastic nodules (LGDNs) and high grade dysplastic nodules (HGDNs) depending on the degree of cytological or architectural atypia on histologic examination [5].

Deleted in breast cancer 1 (DBC1) is a nuclear protein that was named by its deletion at a region 8p21 in some breast cancers [6]. DBC1 has been shown to have many protein–protein interactions and appears to have multiple cellular functions including regulation of apoptosis and metabolism [7]. DBC1 regulates silent mating type information regulation 2 homolog 1 (SIRT1) activity and contributes to high-fat diet-induced liver steatosis in mice [8]. A recent

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study showed that DBC1 directly inhibited the deacetylase activity of SIRT1 and inhibited SIRT1-dependent cell survival through the p53 pathway [9]. Hamaguchi et al. reported that mRNA expression of DBC1 was down-regulated in some breast cancers [6]. However, some studies found that DBC1 was overexpressed and associated with poor prognosis in breast cancer, gastric carcinoma, esophageal squamous cell carcinoma, and colorectal cancer [10–13]. Another study showed that DBC1 protein was highly overexpressed in a subset of human HCC tissues compared with surrounding non-cancer tissues and DBC1 did not function as a negative regulator of SIRT1 activity in HCC cells [14]. However, the expression level of DBC1 protein and the prognostic role of DBC1 in HCC have not been reported.

We investigated the effect of DBC1 protein expression in 199 hepatitis virus-related HCC patients with long-term follow-up. Immunohistochemical expression of DBC1 were evaluated by tissue microarray.

2. Materials and methods

2.1. Patients and specimens

Hepatitis virus-related HCC tissues were obtained from 199 patients who underwent hepatectomy at Samsung Medical Center, Seoul, Korea between July 2000 and May 2006. Hepatitis virus-related 11 LGDNs, 10HGDNs, and 12 early HCC tissues were obtained from 33 patients with cirrhosis who underwent liver resection or liver transplantation. Freshly removed livers were serially sliced at 3- to 4-mm intervals and examined by a pathologist for the presence of nodular lesions. Any bulging nodules at least 5 mm in diameter or lesions macroscopically different in color from the surrounding liver were fixed in 10% neutral formalin and embedded in paraffin. HCC, early HCC, LGDN, and HGDN were confirmed histologically according to the guideline of the International Working Party (5) (Fig. 1). None of the patients received preoperative or postoperative chemotherapy. This study was approved by the Institutional Review Board of Samsung Medical Center. Tumors were staged according to both the American Joint Committee on Cancer (AJCC) staging system [15] and Barcelona Clinic Liver Cancer (BCLC) staging classification [16]. Intrahepatic metastasis and multicentric occurrence were defined according to the previously reported criteria [17]. Tumor tissue microarray blocks were constructed as described previously [18]. Two 2 mm cores from paraffin blocks of each specimen were taken.

The median follow-up period was 119.1 months (range, 24.0-151.4 months) for survivors. Patients after surgical resection were followed by monitoring serum α–fetoprotein levels and three phase dynamic computed tomography scans or magnetic resonance imaging every three months. Recurrence-free survival (RFS) was defined from the date of hepatectomy until the date of tumor recurrence, metastasis, or last follow-up. Overall survival (OS) was defined as the period from the date of hepatectomy until the date of death or last follow-up.

2.2. Immunohistochemical analysis

Immunohistochemical staining was performed as previously described [18]. Antigen retrieval was performed with 0.01 mol/L citrate buffer (pH 6.0) in a pressure cooker for 30 min. The sections were reacted with rabbit polyclonal antibody to DBC1 (IHC-00135, 1:100; Bethyl Lab. Inc., Montgomery, TX, USA) at room temperature for 60 min. To validate the concordance between tissue microarrays and whole tumor sections, DBC1 expression was also examined for 40 corresponding whole tumor sections randomly chosen from the 199 cases. All reactions were performed using appropriate negative and positive controls. For negative controls, sections were incubated with pre-immune rabbit serum instead of the primary antibody. Positive controls (human normal kidney) showed nuclear DBC1 expression in epithelial cells of convoluted tubules.

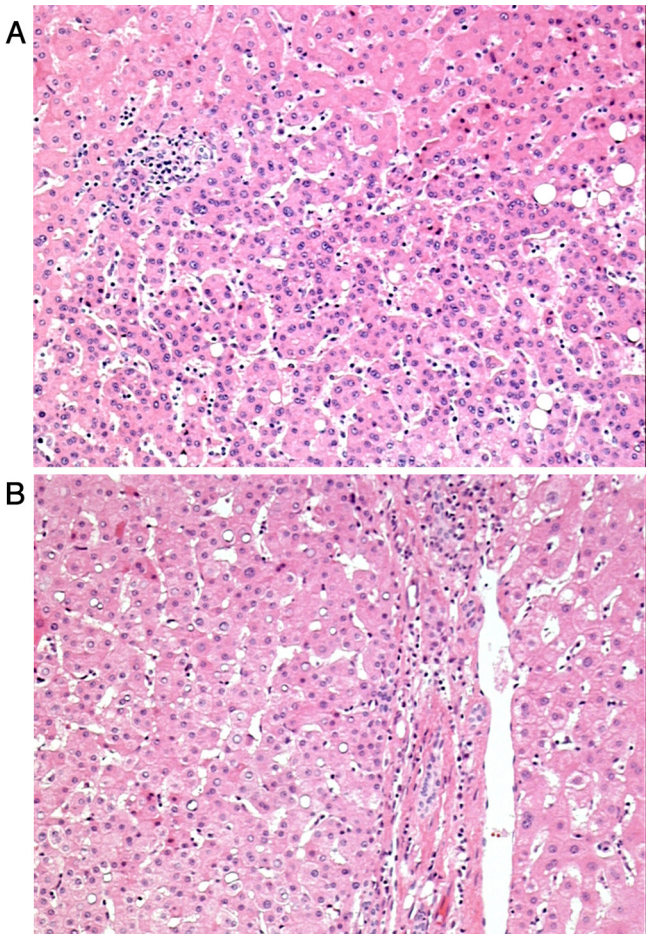

Fig. 1. Histological features of early hepatocellular carcinoma and the surrounding liver (left upper part) (A) and high grade dysplastic nodule and the surrounding liver (right one-third part) (B). Dysplastic nodules show diffuse small cell dysplasia. (H&E stain, ×100).

All sections were independently examined by two experienced pathologists (C.K. Park and S.Y. Ha) who were blinded to the clinical data. Nearly homogeneous nuclear immunostaining with moderate staining intensity was observed. The proportion of tumor cells with nuclear staining was determined semi-quantitatively. Duplicate tissue cores for each tumor showed high levels of homogeneity for the proportion of tumor cells with positive staining. DBC1 immunoreactivity was divided into a low- or high-expression group if the percentage of stained tumor cells regardless of staining intensity was 0–50% or >50%, respectively.

2.3. Statistical analysis

The SPSS software, version 18 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Associations between clinicopathologic factors and DBC1 expression were tested using a chi-square test or Fisher's exact test. Survival rates were calculated using the Kaplan-Meier method and analyzed using the log-rank test. Univariate and multivariate Cox proportional regression analyses of survival were performed. Factors that were significant on univariate analysis for either RFS or OS were included in the multivariate model. A P value of < 0.05 was considered statistically significant.