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Original Article

Investigation of diagnostic utility and expression profiles of stem cell markers (CD133 and CD90) in hepatocellular carcinoma, small cell dysplasia, and cirrhosis

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ABSTRACT

The aim of this study was to investigate the expression rates of CD133 and CD90 in cirrhosis–dysplastic nodule–HCC (Crh–DN–HCC) sequence related to the etiologic background.

Thirty-five HCC, 8 small cell dysplasia (SCD), and 63 cases of cirrhosis having different etiologies were collected. Immunohistochemical expressions of CD133 and CD90 were evaluated.

CD133 positivity was higher in HCC cases with chronic hepatitis B and CD90 with chronic hepatitis C. The highest staining rate was seen in poorly differentiated HCC cases. Only one SCD case and almost half of the cirrhotic cases which were stained for CD133 were associated with hepatitis B; none was stained for CD90. Increased CD133 expression indicated a significantly shorter overall survival rate. No significant relationship was detected between the expression rates of CD133 or CD90 and other parameters.

In this study, which investigates the immunohistochemical expression profiles of CD133 and CD90 through Crh–DN–HCC sequence, the highest staining rate was detected in HCC. It is rational to try to elucidate the earliest events in hepatocarcinogenesis by studying SCD. It is important to be aware of this issue in daily practice, which will provide a deeper insight into the understanding of the effects of CSCs in the progression and management of HCC.

Introduction

Hepatocellular carcinoma (HCC), one of the common cancers worldwide, still continues to increase [5]. Men are more frequently affected than women. Etiologically, chronic liver disease due to hepatitis B and C viruses, aflatoxin exposure, alcohol abuse, steatohepatitis, smoking, and drugs are the well-known risk factors. Poor prognosis is usually due to the delay in its diagnosis, the background of cirrhosis in the liver, and resistance to therapy.

Small cell dysplasia/change (SCD) is a proliferative lesion that characterizes the high-grade dysplastic nodules, and it has been found to be associated with HCC development. Several lines of evidence, such as morphological features reminiscent of HCC or presence of foci of HCC in a high-grade dysplastic nodule, suggest that high-grade dysplastic nodules characterized with SCD are precursors of HCC. The sequence between liver cirrhosis and SCD and HCC was first suggested by Japanese investigators [9,27], as dysplastic nodules represent premalignant lesions in chronic liver diseases, and recent findings obtained from explanted cirrhotic livers have confirmed this hypothesis [13].

With the help of accumulating data concerning the cancer stem cells (CSCs) and their critical role in the genesis, progression, metastasis, and recurrence of cancer, we are able to gain a deeper insight into what is happening at the side of carcinogenesis, especially the development of effective and specific treatment modalities. We now know that 40% of HCC are thought to arise from progenitor or stem cells [32], and they have a critical role in the development and progression of HCC. It is stated that there are three major types of liver stem cells: (i) dedifferentiated hepatocytes, (ii) hepatic and oval cells, and (iii) bone marrow cells. Although many stem cell markers have been reported in the literature, especially CD133, CD90 (Thy-1), and EpCAM appeared as more specific antigenic markers for HCC stem cells [15,22,24,31]. CD133 (AC133, Prominin 1) is a five transmembrane cell surface glycoprotein that in 1997 was identified for the first time in stem cells derived from fetal bone marrow, fetal liver, or peripheral blood [34], and is known as the primitive hematopoietic and neuronal cell marker. It localizes to the plasma membrane protrusions at the apical

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surface of cells [3]. CD90 is a 25–37 kDa glycosyl phosphatidylinositol anchored glycoprotein expressed mainly in leucocytes. It is involved in cell–cell and cell–matrix interactions [17], and it is also found to be expressed in hepatic stem/progenitor cells [7].

Although there is increasing evidence of CSCs in the development of HCC, the role of CSCs in cirrhosis–dysplastic nodule–HCC (Crh–DN–HCC) sequence is not clear and needs more investigation. This issue may be important in order to find out the earliest detectable level of antigenic stem cell marker expression and to help in the accumulation of data on the lesions, not only the cancerous but also the precancerous ones, related to the liver progenitor or stem cells. In daily practice, it is also important to be willing to and to be able to use these stem cell markers in order to specify HCC cases. This can be reached by using immunohistochemistry, which is standardized properly in many countries. We think that this point of view will make us notice the developmental nuances in progression and behavior of HCC related to CSCs. In this study, we investigated the expression rate and staining patterns of CD133 and CD90 in cirrhosis, small cell liver dysplasia and HCC in order to find out if there is a relationship in this developmental sequence, similar to the adenoma–carcinoma sequence in colon cancer, and also if there is a significant difference between the lesions according to the etiologic background.

Materials and methods

Patients and tissue specimens

We collected a total of 35 HCC specimens, which consisted of 3 total resections, 7 partial resections, 1 wedge resection, and 24 needle core biopsies; 8 needle core biopsies involving SCD, and 63 cases of cirrhosis having different etiologies, which consisted of 7 total resections, 1 partial resection, 3 wedge resections, and 52 needle core biopsies. Selection of tissue specimens for the study was done by considering tissue surface area of at least 1 cm in length to 0.1 cm in width, optimal tissue fixation and processing with the help of hematoxylin and eosin-stained sections, and artifacts especially in chemoembolized HCC cases. There was one patient whose HCC, SCD and cirrhotic liver samples were available. Apart from this case, there was no overlapping in HCC, dysplasia and cirrhosis cases. The cirrhotic background in the HCC cases was examined and noted separately. Among the patients with HCC, there were 30 men and 5 women with ages ranging from 41 to 87 years (mean: 64 years). The patients having small cell liver dysplasia included 6 men and 2 women, their age ranging from 59 to 79 years (mean: 69 years). Cirrhotic patients were composed of 41 men and 22 women between 18 and 78 years of age (mean: 57 years). In HCC cases, tumor size was recorded as the greatest dimension of the tumor, ranging between 1.5 and 20 cm. For the statistical examination, tumor sizes were divided into two categories: <5 cm and ≥5 cm. All diagnoses of HCC were confirmed again by histopathological examination. Tumor grading was performed according to the Edmondson–Steiner nuclear grading system [4]. The Ethics Committee of Ministry of Health approved the study protocol.

Immunohistochemistry

All tissue specimens were fixed in 10% formalin and embedded in paraffin, and cut into 4 μm sections. These were routinely processed with deparaffinization by applying xylene and rehydratation using graded alcohol solutions.

In this study, two stem cell markers, CD133 and CD90, were investigated. One of the primary antibodies was rabbit antihuman polyclonal CD133 (dilution 1:200, Abcam, clone: ab19895). The other primary antibody was CD90 (dilution 1:25, Sigma, clone: anti Thy-1, lot no: B35104). Antigen retrieval was performed for 10 min at 95 °C and then for 5 min at 72 °C in Tris–EDTA, with pH 9 in a microwave oven. Endogenous peroxidase inactivation was done by using hydrogen peroxide for 15 min. The sections were incubated overnight at 37 °C in a humidified container, 1 h for CD133 and 2.5 h for CD90. Amino ethyl carbazole was used as the chromogen and counterstained with Mayer's hematoxylin. Immunostained slides were evaluated by a blinded hepatopathologist. Expressions of both CD133 and CD90 were evaluated by counting 5 random fields at high magnification (400×), as done before by other investigators [15]. Then, a percentage of the total number of hepatocytes was noted for each immunohistochemical marker. Fetal liver tissue and Purkinje cells in cerebellar tissue were used as external positive controls for CD133 and CD90, respectively (Figs. 1 and 2).

While evaluating the expressions of CD133 and CD90, cases were divided into four groups in order not to miss even a focal expression area that might be related to the different lesions having different etiologic backgrounds. Regarding this issue, cases with staining >50% were considered as high (score 3), 50–25% as moderate (score 2), <25% as low (score 1), and no staining as negative (score 0).

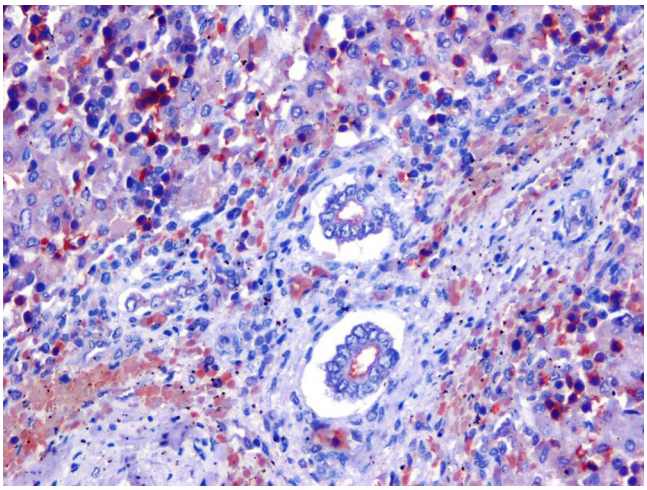

Fig. 1. External positive control for CD133. Luminal epithelial staining of bile ducts in 20 weeks of fetal liver (CD133, 400×).

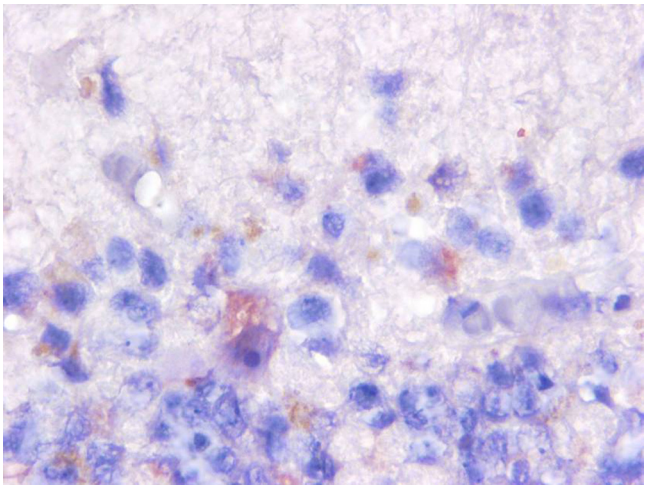

Fig. 2. External positive control for CD90. Cytoplasmic staining of cerebellar Purkinje cells (CD90, 400×).