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ORIGINAL RESEARCH

Comparative study on proliferation activity in small hepatocellular carcinoma related to hepatitis virus B and C

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Abstract

AIM: To compare the proliferation activity of small hepatocellular carcinoma (HCC) related to hepatitis B virus (HBV) and hepatitis C virus (HCV).

METHODS: Sixty liver biopsy specimens were obtained from patients with small HCC (≤ 3 cm in diameter) and examined immunohistochemically using anti-proliferating cell nuclear antigen monoclonal antibody. Of the 60 specimens, 30 were HBV-related and 30 were HCV-related. The 60 patients providing the samples for study were matched by sex and morphologic features of the HCC specimens.

RESULTS: The labeling index of proliferating cell nuclear antigen was 7.9% in the HBV-related HCC specimens and 12.5% in the HCV-related HCC specimens. There was no statistically significant difference between the two groups ($P > 0.05$).

CONCLUSION: In the early phase, or small stage, of HCC, HBV-related HCC shows similar proliferating activity to that of HCV-related HCC; this finding suggests that in the early phase, HBV-related HCC has similar malignancy to HCV-related HCC.

Key words: Liver neoplasms; Carcinoma; Hepatocellular; Hepatitis B virus; Proliferating cell nuclear antigen

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INTRODUCTION

Human hepatocellular carcinoma (HCC) is one of the most common malignant tumors and its development is known to be closely associated with hepatitis B virus (HBV) infection and hepatitis C virus (HCV) infection[1,2]. However, no reports in the literature have yet described a comparative analysis of the tumor behaviors of these two kinds of HCCs.

Proliferating cell nuclear antigen (PCNA) is a nuclear protein related to cell proliferative activity[3], and its immunohistochemical detection is a useful adjunct to morphologic features, providing insight into the understanding of tumor behavior. In this study, we compared the proliferation activity of HBV-related small HCC with that of HCV-related small HCC by means of immunohistochemical staining using a monoclonal antibody against PCNA to provide information about their clinical features.

MATERIALS AND METHODS

Formalin-fixed and paraffin-embedded liver biopsy specimens were obtained from the archives of the Second Department of Internal Medicine at Kurume University School of Medicine, Japan. These specimens included 30 HBV-related HCCs (male:female ratio of 25:5; mean age of 57.6 years) and 30 HCV-related HCCs (matched by sex ratio and morphologic features; mean age of 60.1 years). The size of the tumors was < 3 cm in diameter and the number of tumors per patient ranged from 1 to 4. Histopathological diagnoses were made according to the General Rules for Cancer of the Liver (Liver Cancer Study Group of Japan)[4]. Of the 60 cases involved in this study, 32 were well-differentiated, 24 were moderately differentiated, and 4 were poorly differentiated.

Immunohistochemistry

Three μm thick sections were prepared from paraffin blocks and deparaffinized by soaking in a graded ethanol series, after which the processed sections were treated with 3% hydrogen peroxide to block endogenous peroxidase activity. Normal sheep serum was then applied for 20 min to reduce non-specific antibody binding. Monoclonal antibody to PCNA (clone 5A10) was applied as a 1:250 dilution and allowed to incubate for 1 h in a moist chamber. Biotinylated sheep anti-mouse IgG was then applied as the linker molecule and allowed to incubate for 30 min, followed by application of a streptavidin-horseradish peroxidase complex (Vector) and incubation for 1 h. All steps were carried out at room temperature and followed by washing in phosphate buffered saline (PBS). Diaminobenzidine-hydrogen peroxide was used as a chromogen and a hematoxylin counterstain was made. Sections were then processed in an alcohol gradient series, cleared in xylene, and mounted in DPX. A negative control was generated by replacing the primary antibody with PBS.

Assessment of PCNA

PCNA labeling indices were calculated after counting PCNA-positive nuclei per 500 nuclei in 2-5 fields of each HCC case at high power (× 200).

Table 1 Clinical parameters associated with prognosis

Parameter	HBV-related HCC	HCV-related HCC	*P* value
AFP in μg/L, mean	80.22	99.04	0.743
Survival duration in days, mean	1353.4	1341	0.947

Other clinical parameters

Alpha-fetoprotein (AFP) concentration and survival duration were analyzed retrospectively. Statistical analysis was made by Student's *t*-test.

RESULTS

Proliferating cell nuclear antigen LI

PCNA staining was confined to the nuclei. All identifiable staining was considered positive, regardless of the staining intensity. The PCNA LI was 7.9% in HBV-related HCCs and 12.5% in HCV-related HCCs. There was no statistically significant differences between these two groups ($P = 0.33 > 0.05$).

Other clinical parameters (Table 1)

DISCUSSION

HBV-related HCC and HCV-related HCC are clinically distinct. In the early phase, or small stage, of the HCC tumor development, these two kinds of HCC show similar growth rates, survival rates and prognosis, whereas in the advanced stage, the HBV-related HCC has poorer prognosis than that of HCV-related HCC (unpublished data). In this study, we retrospectively analyzed the survival duration of patients with small HCC who were treated with percutaneous ethanol injection therapy (PEIT) and confirmed that the survival duration was similar for the two forms.

Proliferation activity of tumors that is defined by PCNA immunohistochemical analysis has been reported to be related to metastatic potential, recurrence and overall prognosis[5,6]. Therefore, PCNA LI expression in the tumor reflects, at least partially, the degree of malignancy. The current study showed no significant difference in the expression of PCNA LI in patients with HBV-related small HCC and HCV-related small HCC; this finding suggests that in the early phase HBV-related HCC has similar malignancy to HCV-related HCC. It may therefore help to understand the clinical features of these two kinds of HCCs. Additionally, it has been previously reported that serum AFP values are not only of diagnostic value but also of prognostic significance in patients with HCC[7]. In the current study, we found that the serum AFP values were not significantly different between HBV-related small HCC and HCV-related small HCC. This finding is also in accordance with the clinical features of HBV-related HCC and HCV-related HCC in the early stage.

In conclusion, our results indicate that, in the early stage, the proliferation activity of HBV-related HCC is similar to that of HCV-related HCC. This information provides a better understanding of the clinical features of these two kinds of HCC.

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