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A case of hepatocellular carcinoma consisting of two solitary poorly differentiated polyclonal intrahepatic tumor nodules

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Abstract: Two intrahepatic solitary tumors consisting of poorly differentiated hepatocellular carcinoma (HCC) were identified as polyclonal HCC nodules by analysis of the pattern of integration of hepatitis B viral DNA into nuclear DNA. After the removal of each nodule by partial liver resection, recurrent multiple tumors appeared within 10 months postoperatively. The findings in this case suggest that the effectiveness of reduction surgery for intrahepatic multiple tumors is limited in solitary multicentric HCC that consists of poorly differentiated HCC.

Key words: hepatocellular carcinoma, multicentric, reduction surgery

Introduction

How to distinguish between multicentric hepatocellular carcinoma HCC (MC-HCC)[1–6] and intrahepatic metastatic HCC (IM-HCC) is a recent subject of interest in the treatment of intrahepatic multiple HCC. Although the accurate determination of the clonality of multiple tumors as monoclonal or polyclonal depends on clonal[1–5] or gene analysis,[6] the histological criteria for this determination have made it feasible to detect the clinical entity of multiple HCC consisting of MC-HCC. However, since the histological criteria are based on the clinical experience that the metastatic HCC are less differentiated than the primary lesion,[7] the multiple tumors determined by these criteria are found to constitute a cluster of multicentrically developed well-differentiated HCC, which entity is apparently relatively slow growing compared to other HCC. It is possible that such entities as MC-HCC concomitant with IM-HCC, or a cluster of MC-HCC, which are all poorly differentiated HCC, also exist. In the present study, we report a case of the latter, confirmed by the finding of different patterns of integration of hepatitis B viral (HBV) DNA into the nuclear DNA of multiple tumors.

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Case report

A 52-year-old male was first found to have liver dysfunction at a health examination in June, 1991. In November, he experienced general malaise and loss of appetite, and sought treatment at a local hospital, where blood examination showed a high alpha-fetoprotein (AFP) level (10000 ng/ml) and ultrasonography (US) revealed a tumor in the medial segment of the liver. On March 2, 1992, he was referred to our department for surgical treatment.

He had a past history of appendectomy at the age of 22, and hemigastrectomy for duodenal ulcer at the age of 42. During the latter operation, he had received a blood transfusion. His mother and elder brother had died due to hepatocellular carcinoma (HCC). All of his three brothers and two sisters were hepatitis B virus (HBV) carriers.

At physical examination on admission, his general condition was good, and he was not icteric. The abdomen was flat. No ascites was noted, but an elastic hard tumor, about 6 cm in diameter, was palpable in the epigastric region.

The results of blood examination on March 3, 1992, were: white blood cells (WBC) 8300/mm^3; red blood cells (RBC) 4.40 × 10^6/mm^3; platelets (PLT) 125 × 10^3/mm^3; Hematocrit (Ht) 43.2%; total protein (TP) 7.0 g/dl; albumin (Alb) 3.7 g/dl; cholineesterase (Ch-E) 0.42 PH; zink sulfate turbidity test (ZTT)

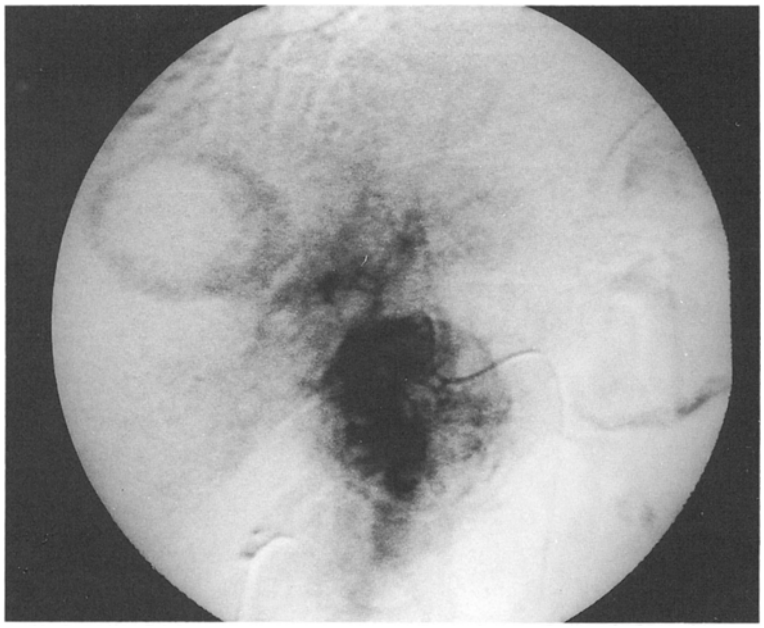

Fig. 1. Digital subtraction angiography (*DSA*) from the proper hepatic artery shows two tumor stains

14.4 KU; thymol turbidity test (TTT) 5.3 KU; total bilirubin (T.Bil) 0.8 mg/dl; alkaline phosphatase (ALP) 199 U/l; leucine aminopeptidase (LAP) 66 U/l; γ-guanosine triphosphate (γ-GTP) 74 U/l; lactic dehydrogenase (LDH) 420 U/l; glutamicoxaloacetic transaminase (GOT) 77 U/l; glutamic-pyruvic transaminase (GPT) 88 U/l; triglyceride (TG) 71 mg/dl; total cholesterol (T.Chol) 139 mg/dl; blood urea nitrogen (BUN) 14 mg/dl; creatinine (Crtn) 0.5 mg/dl; uric acid (UA) 2.3 mg/dl; creatinine clearance, 67 ml/min; C reactive protein (CRP) 4.8 mg/dl; prothrombin time (PT) 89.6%; hepatitis B surface antigen (HBsAg) +; hepatitis B envelope antigen (HBeAg) +; anti-HBe −; anti-hepatitis virus C (anti-HCV) −; carcinoembryonic antigen (CEA) 1.9 ng/ml; alpha-fetoprotein (AFP) 15 000 ng/ml; protein induced by vitamin K absence or antagonist II (PIVKA-II) 1.1 AU/ml; indocyanine Green 15 min-retention rate ($ICGR_{15}$) 4.6%.

Imaging diagnosis

Computed tomography (CT) revealed a low density area, measuring 5.0 × 4.5 cm, with a clear border in the medial segment (S4 tumor) and a low density area measuring 4.0 × 4.0 cm in the posterior-superior subsegment (S7 tumor). The peripheral region of both tumors was clearly enhanced, suggesting the presence of a tumor capsule. The interior of both areas showed partial non-enhancement, suggesting partial necrosis. Magnetic resonance imaging (MRI) similarly indicated the presence of two tumors, which, except for the partially necrotic portion, showed low intensity on T1-weighted images and high intensity on T2-weighted images. Celiac arteriography demonstrated the S4 and S7 tumors as tumor stains (Fig. 1). No vascular invasion was observed. Twenty mg of Epirubicin with 5 ml of lipiodol was infused from the proper hepatic artery. Subsequent CT showed lipiodol deposition in the two tumors; it was diffuse but not homogenous in the S4 tumor and showed peripheral accumulation in the S7 tumor (Fig. 2).

Under the preoperative diagnosis of two solitary HCC, partial resections that included each tumor were performed on March 17, 1992.

Operative findings

In the medial segment, an elastic round tumor (the S4 tumor) protruded caudally. The S7 tumor was a relatively hard mass. Each tumor was resected at the incisional line about 2 cm distant from the tumor edge, using a Cavitron Ultrasonic Aspiration System (CUSA) (Cavitron, Conn.) after heat-coagulation with a microwave tissue coagulator[8] along the incisional line, so that minute invisible tumor foci near the tumor could be eliminated.

Macroscopically, the tumors were classified as of the multi-nodular type; one tumor was of the single nodular type (the S4 tumor) and the other was of the single nodular type with proliferation into the surrounding area (the S7 tumor).[9] The S4 tumor, which measured 4.2 × 4.5 × 4 cm, was whitish, elastic, and hard, with a clear tumor capsule and a septum, and showed hemorrhage and an internal necrotic portion. The S7 tumor, 3.5 × 3.0 × 2.5 cm, presented findings similar to those for the S4 tumor, except for an area

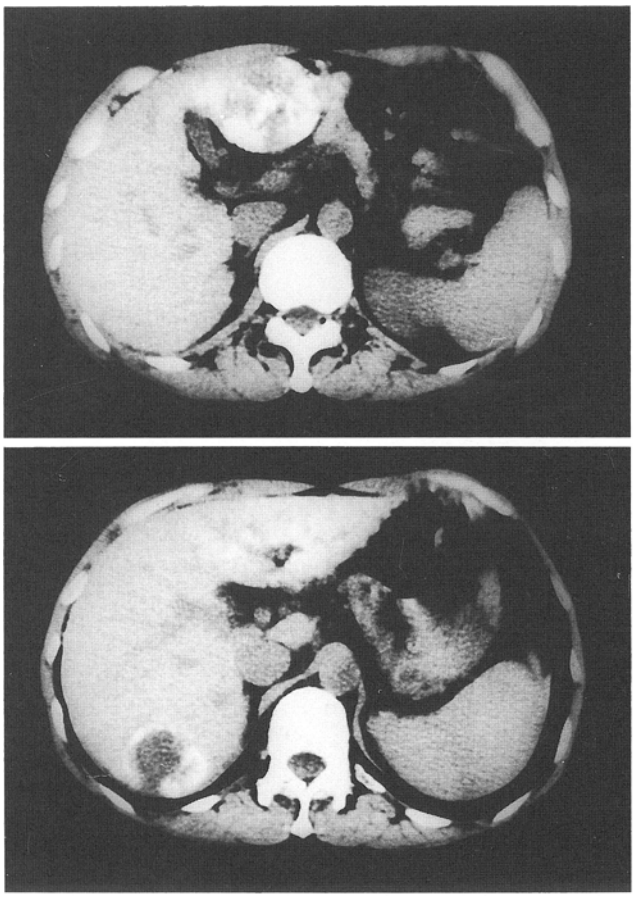

Fig. 2. Lipiodol computed tomography (*CT*) reveals lipiodol deposition in two round tumors: deposition is diffuse but homogenous in the S4 tumor (*above*) and shows peripheral accumulation in the S7 tumor (*below*)

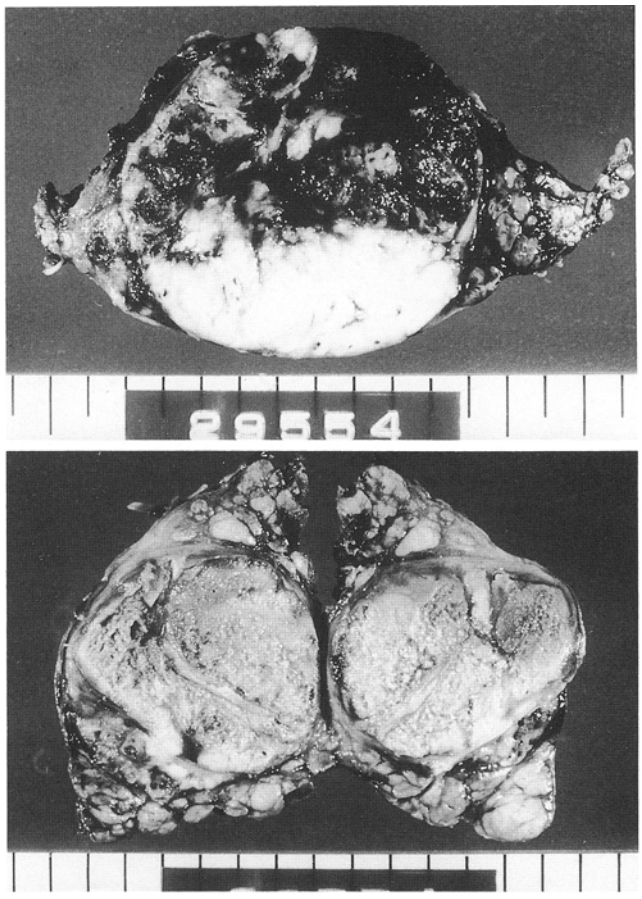

Fig. 3. Macroscopic features of sections of both tumors; *above*, the single nodular type (the S4 tumor) and, *below*, the single nodular type with proliferation into the surrounding area (the S7 tumor)

of small extracapsular proliferation (Fig. 3). The noncancerous portion of the liver was cirrhotic.

Microscopic findings

The S4 tumor was poorly differentiated HCC, showing dense proliferation of eosinophilic cytosol and large and irregular nuclei; it contained abundant vasculature. No distinct trabecular pattern other than partial nestic trabecular changes was observed. Tumor capsule invasion was prominent. The S7 tumor consisted of relatively more differentiated HCC than the S4 tumor, but was of similar character (Fig. 4). Based on the histological features, it was impossible to differentiate whether the relationship of the tumors was metastatic or multicentric.

Clonal analysis

Analysis of the pattern of integration of HBV DNA into the nuclear DNA[1–4] in both tumors was performed by Southern blot hybridization, as follows; samples (about 2 g) of the non-cancerous portion of the liver and of the S4 and S7 tumors were obtained from the surgical specimens soon after removal of the tumors. Care was taken to sample not the necrotized or heat-coagulated portion but the viable portion. The total DNA was extracted by digestion with sodium dodecyl sulfate (pH 8.0) and proteinase K, followed by phenol-chloroform extraction. After treatment with RNase, a 10-μg sample of purified DNA was digested with Hind III or Bam HI (Takara, Kyoto, Japan) overnight at 37°C, using 50 units of each enzyme. Each sample was applied to 0.8% agarose gel, electrophoresed, and then transferred to a nylon membrane (Hybond-N^+, Amersham, Buckinghamshire, UK). A full-length HBV-DNA (type adr) probe (Clonit, Milano, Italy) was labeled with [^{32}p] deoxycytidine 5-triphosphate, using a Multiprimer System (Amersham) and hybridization buffer (Amersham) containing 2×10^6 cpm/ml of denatured probe. The filter was then washed and antoradiographed. Since the patterns of integration of HBV-DNA into the host nuclear DNA

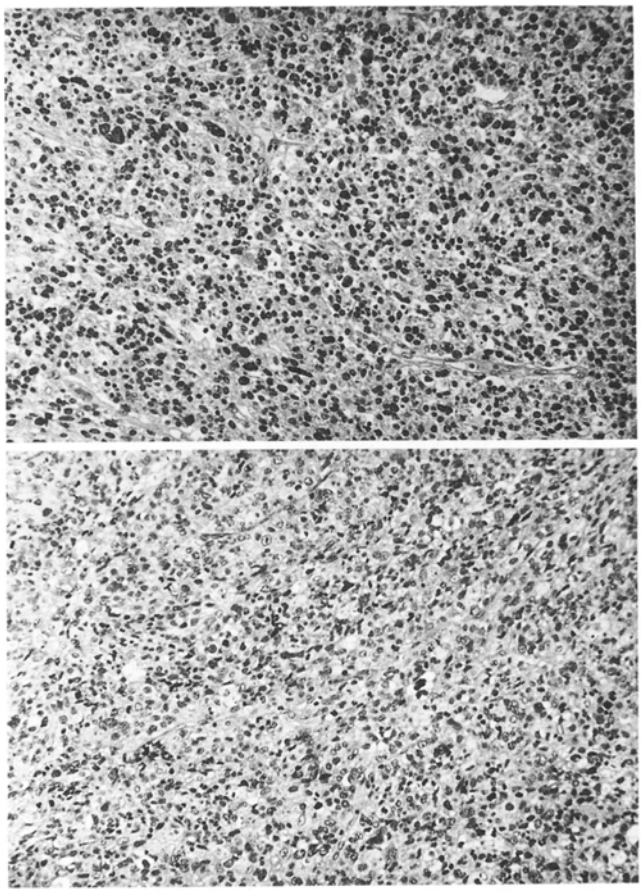

Fig. 4. Microscopic features of the two tumors; both are poorly differentiated HCC with tumor capsule invasion. *Above*, the S4 tumor and, *below*, the S7 tumor. H&E, ×75

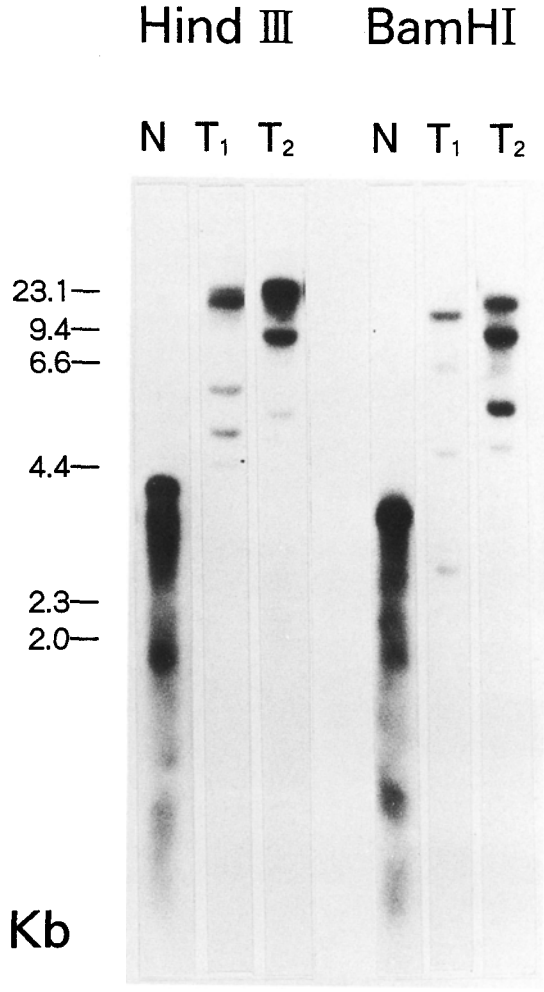

Fig. 5. Southern blot hybridization analysis of hepatitis B virus (*HBV*) DNA in DNA obtained from both tumors and from the noncancerous portion: *T1*, the S4 tumor; *T2*, the S7 tumor; *N*, the noncancerous portion. The integration patterns in lanes T1 and T2 are not the same, indicating that the S4 and S7 tumors have developed from different clones

in each intrahepatic lesion were different (Fig. 5), the lesions were confirmed as having a multicentric occurrence.

Postoperative course

The patient was discharged on May 24. CT performed on July 30 did not indicate any recurrence. AFP had decreased to 22 ng/ml on June 15 (highest preoperative level, 17000 ng/ml), and then remained at around 30 ng/ml until the end of August (Fig. 6). However, the AFP level increased to 67 ng/ml in November and 117 ng/dl in December. US performed in November did not reveal any tumor, but CT (January 19, 1993) revealed a low density area 3 cm in diameter in the medial (S4) segment and a low density area 1 cm in diameter in the anterior-superior (S8) subsegment. Ascites and pleural effusion were recognized. On February 10, 1993, he was admitted to our departement for treatment of recurrence.

The results of blood examination on the second admission (Feb. 12, 1992) revealed anemia (RBC $3.01 \times 10^6/mm^3$; Ht 29.6%) and hypoproteinemia (TP 5.2 g/dl; Alb 2.0 g/dl), although liver dysfunction was not severe. $ICGR_{15}$ was 16.3%, and prothrombin activity was 96.7%. However, renal dysfunction was suggested [BUN 24 mg/dl; Crtn 1.1 mg/dl; β_2-microglobulin 6.7 mg/l (normal range, 1.0–3.5 mg/l); creatinine clearance 39 ml/min] and urine examination revealed marked proteinuria and hematuria.

Because of the multiple recurrent tumors, of which at least four were demonstrated by lipiodol CT, and the renal dysfunction, operative treatment was abandoned, and intermittent chemoembolization, consisting of 10 mg of doxorubicin and 0.5 ml of lipiodol, has been performed once a week, since March 20, 1992, through a subcutaneous port connected to a catheter inserted into the proper hepatic artery.

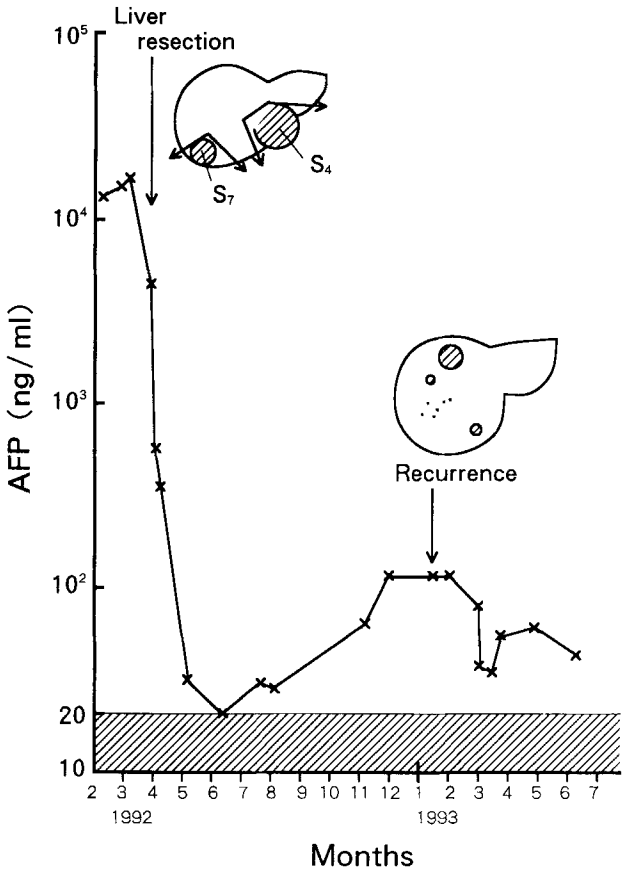

Fig. 6. The patient's postoperative course. The *shaded area* shows the normal range of alpha fetoprotein (*AFP*) levels

Discussion

In patients who are at high risk of HCC, i.e., those who have liver cirrhosis on HBV or HCV infection, there is the possibility simultaneous multicentric HCC. However, no well-established method for the determination of multicentric HCC has yet been developed, although some clonal and gene analyses, such as HBV DNA integration pattern analysis,[1–4] detection of loss of heterozygosity (LOH) of chromosome 16,[5] and p53 gene analysis[6] can be helpful. Therefore, the histological criteria for this determination, based on clinical experience indicating that metastatic HCCs are less differentiated than the primary lesion,[7] have been used, and the clinical entity known as MC-HCC has been discussed.

The histological guidelines for the determination of multicentric HCC[10] specify well-differentiated HCC; therefore, multicentric HCC comprising well-differentiated HCC nodules can be less malignant than intrahepatic metastatic HCC of less differentiated HCC. In a previous study, we demonstrated the clinical significance of tumor-mass reduction surgery for stage IV-A HCC,[11] since in some cases stage IV-A HCC is a cluster of multicentrically developed well-differentiated HCC, which is apparently a relatively slow growing HCC.

However, in the present patient, we determined that the two solitary intrahepatic tumors, which were similarly poorly differentiated HCC, were multicentric HCC, using clonal analysis of the pattern of HBV DNA integration into the nuclear DNA of the HCC. This finding suggests that multiple intrahepatic tumors can develop from the respective well-differentiated HCC of different clones, without evident metastatic lesions. Such multicentric HCC, consisting of poorly differentiated HCC, are more likely to have minute extracapsular infiltration, even though each tumor seems to be solitary. Therefore, in these patients, the tumor-mass reduction procedure may not extend the survival period. Thus, for the treatment of intrahepatic multiple HCC, it is of prime importance to determine whether each tumor is well-differentiated HCC, so that treatment appropriate to the tumor character, including liver resection, can be performed.

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