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Hepatic carcinosarcoma: evidence of polyclonal origin based on microsatellite analysis

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ABSTRACT

Aims: Hepatic carcinosarcoma (HCS) is an aggressive tumor for which a consensus regarding the clonal origin has not yet been reached. The aim of the study was to identify the origin of the hepatocellular carcinoma (HCC) and sarcoma components in HCS.
Methods: We chose microsatellite technique containing loss of heterozygosity (LOH) and microsatellite instability (MSI) on three HCS patients who underwent curative resection confirmed by pathological examination. Tumors were firstly analyzed for Hep Par 1, CK18, CD10, CD117, SMA and vimentin expression by immunohistochemistry. LOH and MSI were then investigated. The incidence rate of LOH/MSI in all nine MS was calculated as the fractional allelic loss (FAL) index, which was internationally recognized standard. A FAL < 30% was representative of a monoclonal origin and a FAL ≥ 30% indicated a polyclonal origin.
Results: All patients were positive for HBsAg. Microscopic examination showed HCS containing two different cell types: a fibrosarcoma component with spindle cells and an HCC population of cells with a trabecular pattern. In the HCC tumor portions, Hep Par 1, CK18, CD10 were expressed while vimentin was not. In contrast, the spindle cell populations were positive for vimentin and negative for Hep Par 1, CK18, CD10. The highest frequencies of LOH and MSI were at the D16S505 (2/3; 66.7%), D17S831 (2/3; 66.7%) and D17S938 MS (2/3; 66.7%). The FALs for the three cases of HCS were 50% (4/8), 55.6% (5/9) and 33.3% (3/9), suggesting a polyclonal origin.
Conclusions: Immunohistochemistry and analysis of LOH and MSI strongly demonstrated that the three HCS samples were consistent with a polyclonal origin for all three cases.

1. Introduction

Carcinosarcoma is a type of tumor characterized by the presence of both malignant epithelial and mesenchymal components. Carcinosarcomas are most frequently observed in the breast, uterus, esophagus, larynx, lung, bladder and prostate. It is extremely rare, however, for carcinosarcomas to occur in the liver [1], and most incidences of hepatic carcinosarcoma (HCS) have been documented as case reports in the medical literature [2–4]. HCS is defined by the World Health Organization as a malignant tumor containing both carcinomatous and sarcomatous elements. The carcinomatous tissue can either be a hepatocellular carcinoma (HCC) or intrahepatic cholangiocarcinoma, while the sarcomatous component can be an osteosarcoma, chondrosarcoma, or rhabdomyosarcoma [5].

The pathogenesis and histogenesis for HCS are still controversial. Previous research has reported that the carcinomatous and sarcomatous components could originate from a single stem cell [6–8], whereas research from other groups supports the hypothesis that the epithelial and mesenchymal components occur as a result of a simultaneous malignant transformation event [9] or through a 'collision hypothesis' where two tumors merge [10]. Many researchers support a different hypothesis whereby carcinomatous cells give rise to the sarcomatous tissue in HCS [8,11,12]. Direct evidence from molecular biology for the above theories is still lacking and a clonal origin for HCS has not yet been observed. In the present study, we address the clonal origin of HCS from three cases using immunohistochemistry, loss of heterozygosity (LOH) and microsatellite instability (MSI) analyses.

2. Materials and methods

2.1. Samples and patients

All three patients with HCS underwent hepatic resection at the Eastern Hepatobiliary Surgery Hospital in The Second Military

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Medical University of China from January 2013 to August 2013. Blood samples were taken from each patient to measure levels of serum hepatitis B surface antigen (HBsAg), carcinoembryonic antigen (CEA), cancer antigen 19-9 (CA19-9) and alpha-fetoprotein (AFP) levels. The HCS samples were biopsied, formalin-fixed and paraffin-embedded in the Department of Pathology. Each patient provided written informed consent to participate in this study.

2.2. Immunohistochemistry

Representative 4 μm serial sections were obtained from 10% formalin-fixed, paraffin-embedded tissue blocks. Immunohistochemistry was carried out on serial tissue sections using the Dako EnVision system (Carpinteria, CA, USA). Briefly, slides were treated with 3% hydrogen peroxide for 10 min to block endogenous peroxidase activity. To increase immunoreactivity, microwave antigen retrieval was performed in citrate buffer (pH 6.0) for 5 min. The following primary antibodies were used: hepatocyte paraffin 1 (Hep Par 1; 1:50; Dako), cytokeratin 18 (CK18; 1:50; Changdao, Shanghai, China), CD10 (1:50; Maxim, Foochow, Fujian, China), CD117 (1:50; Changdao), SMA (1:50; Changdao) and vimentin (VI; 1:50; Changdao). Hep Par 1 and CK18 mark the cytoplasm of HCC, CD10 recognizes both the cytoplasm and cell membrane of HCC, CD117 recognizes the cytoplasm of gastrointestinal stromal tumors (GIST) and VI recognizes the cytoplasm of fibrosarcomas. Sections were incubated with primary antibodies in a humidified chamber at 4 °C overnight and then stained with an anti-mouse peroxidase-conjugated EnVision antibody at 37 °C for 30 min. Immunoreactions were visualized using 3,3′-diaminobenzidine tetrahydrochloride for 5 min at room temperature, followed by counterstaining with hematoxylin. For a negative control, sections were incubated with Tris-buffered saline in lieu of primary antibodies. Positive controls were prepared from tumor tissue sections that stained positively for Hep Par 1, CK18, CD10 or VI.

2.3. Microdissection and DNA extraction

Paraffin sections (10–12 μm thickness), containing both the HCC and fibrosarcoma components, in addition to unaffected neighboring liver tissue, were microdissected using sterile surgical blades under an inverted microscope (SZX2-FOF; Olympus, Tokyo, Japan), and each component was placed into a separate microfuge tube. The dissected tissues were then lysed in buffer containing proteinase K and digested at 56 °C overnight. Genomic DNA was isolated by phenol/chloroform extraction, precipitated, air dried and resuspended in TE buffer [13].

2.4. Selection of microsatellite (MS) markers

The following nine high-frequency MS markers, which have a high, frequent LOH rate in HCC, were selected for analysis: 8p23.3–p22 (D8S277) [14], 4q26 (D4S402), 8p21 (D8S264) [15], 16q24.1 (D16S505) [16], 16q21 (D16S514), 17p13.3 (D17S831), 17p13.1 (D17S938) [17,18], 8p23.1 (D8S520) [19], and 8q22 (D8S258). The chromosomal positions (4q, 8p, 13q, 16q, and 17p) and prime sequences for the nine MS markers are chosen from our previous research [26].

2.5. Polymerase chain reaction (PCR) of the MS markers

PCR was performed using recombinant Taq Gold DNA Polymerase (TaKaRa; Shiga, Japan). Each marker was amplified in a 10 μl reaction volume containing 10× PCR buffer (Mg^{2+} free), 0.6 μl 25 mmol/l $MgCl_2$, 0.2 μl dNTP mix (2.5 mmol each), 0.2 μl Taq Gold polymerase, 100 ng purified genomic DNA, 0.5 μl each forward and reverse primers (10 pmol/μl) and 6 μl ddH_2O. The PCR cycling conditions were as follows: initial denaturation cycle at 95 °C for 2 min, 6 cycles at 94 °C for 1 min, then 60 to 55 °C for 45 s, 72 °C for 90 s, 26 cycles at 94 °C for 1 min, 55 °C for 45 s, and 72 °C for 90 s with a final 10 min extension at 72 °C. All PCR products were then mixed with 2 μl loading buffer (100% formamide, 2 mM ethylenediaminetetraacetic acid and 2% dextran blue) and denatured at 95 °C for 10 min and quenched on ice for 5 min to change a homozygous product into a heterozygous product. The products were detected by PCR-single strand confirmation polymorphism silver staining and analyzed using Gene Scan Analysis software (FR-200A; Shanghai FURI Science & Technology Co., Ltd., Shanghai, China).

2.6. Analysis of results

Consistent with our previous report [20], amplification and detection of two heterozygous bands per MS locus from the microdissected noncancerous liver tissues indicated that the case was informative and could be used for MS analysis. LOH was classified as positive when the relative intensity of either band was reduced by more than 50% in the informative cases [20]. MS instability (MSI) was classified as positive when extra shadow bands appeared above or below each intense primary allelic band [21]. The MS incidence rate was calculated as a fractional allelic loss (FAL) index: FAL index = (total number of chromosomal loci with LOH/MSI)/total number of informative loci. A tumor with a FAL score <30% was considered monoclonal whereas a tumor with a FAL score ≥ 30% was considered polyclonal [20].

3. Results

3.1. Preoperative clinical characteristics

The clinicopathological characteristics of each patient are shown in Table 1. The mean age was 58 years (range: 56–60 years). The tumors ranged in size from 2.0 to 6.4 cm when examined pathologically. The tumor in case 1 was located on the left side of the liver while the tumors in cases 2 and 3 were located on the right side of the liver. Computed tomography (CT) examination of case 3 revealed a lesion 3 cm in diameter in the right lobe of the liver (Fig. 1A). All three cases were positive for HBsAg. The serum tumor biomarkers CEA and CA19-9 were negative and AFP levels were normal for case 1 and case 2. In case 3, however, AFP (31.4 μg/l) and CA19-9 (49.1 g/l) were elevated while CEA levels were within the normal range. Case 1 was treated surgically with a left hepatic tumor resection. Cases 2 and 3 underwent partial excision of the right hepatic lobes (Table 1).

3.2. Pathological characteristics

In cases 1, 2, and 3, liver specimens of 12.4 cm × 11.0 cm × 4.7 cm, 9.1 cm × 8.4 cm × 4.3 cm and 5.4 cm × 4.7 cm × 3.9 cm, respectively, all contained gray firm tumor masses of 8.0 cm × 3.6 cm, 4.2 cm × 3.1 cm and 3.2 cm × 2.7 cm, respectively, without an obvious envelope (Fig. 1B). No vascular invasion or satellite lesions were observed in case 2 or case 3, while case 1 did have vascular invasion.

Microscopically, all three HCS contained two tumor components, one with spindle cells containing large pleomorphic nuclei and the other with conventional, well-differentiated HCC arranged in a trabecular pattern, with large nuclei. The HCC component also presented with pathological mitotic figures. The two components were clearly intertwined (Fig. 1C), and no transformation zones were observed between them. Spindle cell encroachment on the hepatic sinusoid was observed. Moreover, in cases 1 and 2, invasion