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CASE REPORT

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Hepatocellular carcinoma associated with focal nodular hyperplasia

Report of a case with clonal analysis

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Abstract We describe a hepatocellular carcinoma partially surrounded by focal nodular hyperplasia in a 65-year-old female patient. In order to clarify the relationship of the hepatocellular carcinoma and the adjacent focal nodular hyperplasia, clonal analysis was conducted. The clonal analysis was based on the methylation pattern of the polymorphic X-chromosome-linked androgen receptor gene (HUMARA). The allelic bands from the amplification of the focal nodular hyperplasia and of the hepatocellular carcinoma showed a significant reduction in the intensity of one of the two alleles as compared with two alleles of equal intensity in the buff coat after *Hha*I digestion, which indicated that these two parts were monoclonal. However, the inactivated allele in the focal nodular hyperplasia and that in the hepatocellular carcinoma were not identical. Therefore, the focal nodular hyperplasia and hepatocellular carcinoma probably derived from the clonal expansion of two different clones.

Keywords Focal nodular hyperplasia · Hepatocellular carcinoma · Androgen receptor gene · HUMARA · Clonality

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Introduction

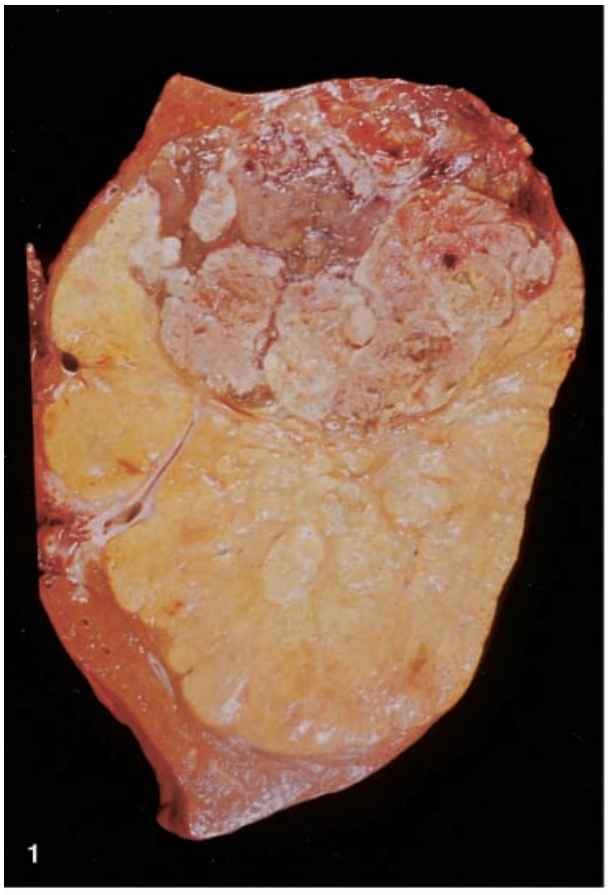
Focal nodular hyperplasia (FNH) is a common benign hepatic tumor. It is generally considered to be a hyperplastic response to an abnormal blood supply [13]. However, its nature and pathogenesis are still controversial. It has been recently shown to be a clonal proliferative disease using HUMARA (methylation pattern of the polymorphic X-chromosome-linked androgen receptor gene) analysis [2]. Its associations with fibrolamellar hepatocellular carcinoma (HCC) were also reported [1, 11, 12]. Although some reports indicated an association between FNH and HCC, most authors did not consider a pathogenetic correlation between them. In this report, we describe a FNH arising at the periphery of a HCC. Both lesions arose from clonal expansion of two different clones.

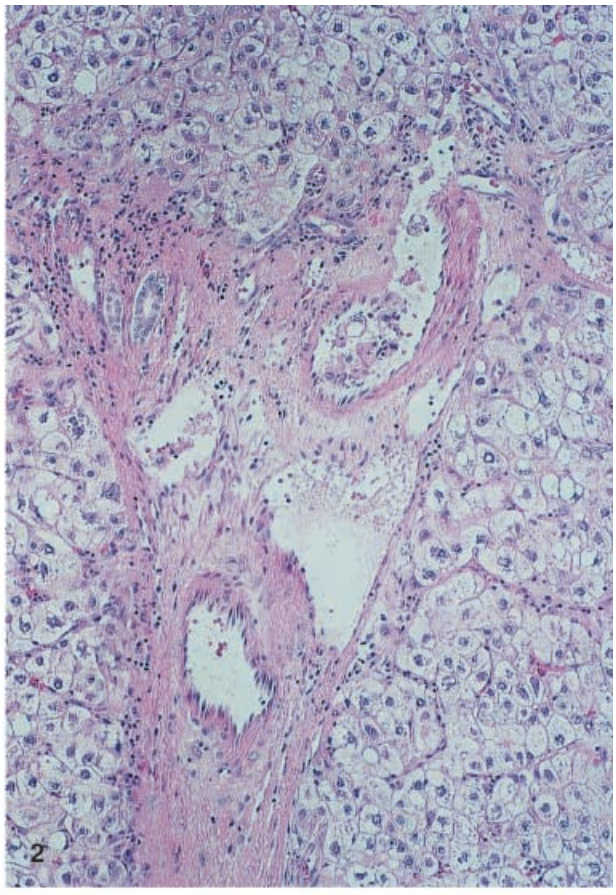
Clinical history

A 65-year-old Taiwanese female patient, with a history of pernicious anemia for several years, was noted to have hepatomegaly in a routine follow-up. Laboratory data were as follows: aspartate aminotransferase (AST) 240 U/l (normal <34 IU/l), alanine aminotransferase (ALT) 100 U/l (normal <36 IU/l), alkaline phosphatase 151 U/l (normal <96 IU/l), γ-glutamyl transferase 398 U/l (normal <96 IU/l), hemoglobin 9.4 g/dl, and white blood cell count 6800/cc. Serum α-fetoprotein was 2384 ng/ml (normal <5 ng/ml). Serum hepatitis B virus surface antigen and anti-hepatitis C virus antibody were negative. Image studies, including computed tomographic scan, abdominal ultrasound, and angiography, showed a huge hyperechoic, heterogeneous, hypervascular liver tumor occupying the right lobe and medial portion of the left lobe with central necrosis. The size of the spleen was within normal limits. There were no tumor thrombi within the portal veins. After the liver tumor was resected, the level of serum α-fetoprotein returned to the normal range.

Materials and methods

Representative sections were taken from the surgical specimen and fixed in formalin and embedded in paraffin. Histologic sec-





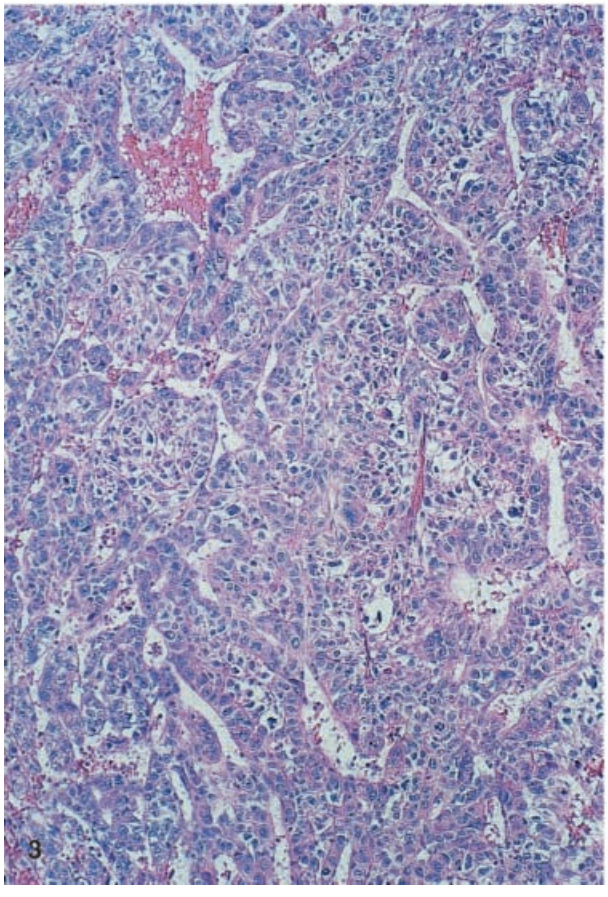


Fig. 1 The liver tumor was grossly composed of two different portions. The lower part was yellow and nodular, and the upper portion was brown with a large area of necrosis

Fig. 2 The yellow portion of the liver tumor was composed of large hyperplastic hepatocytes with short fibrous septa containing malformed vessels and bile ductules. ×200

Fig. 3 The brown portion of the liver tumor showed a classical hepatocellular carcinoma arranged in trabecular and acinar patterns. ×100

tions were stained with hematoxylin and eosin, periodic acid-Schiff with diastase digestion, Masson trichrome, reticulin, Perls'iron, and Victoria blue stains.

Polymerase chain reaction and clonal analysis

The liver tumor was grossly composed of two different parts, i.e., yellow and brown parts. Fresh tissue samples were dissected from the yellow and brown parts of the liver tumor. Peripheral blood of this patient was also available, and the buffy coat was collected for analysis. High molecular weight DNA was extracted and resuspended in 20 µl of water and split into two parts. One part was digested with 20 U *Hha*I restriction enzyme in a 40-µl reaction mixture at 37°C for 24 h.

Clonal analysis in the present study is based on HUMARA. The primer sequences used for the amplification of the HUMARA gene were 5'-TCCAGAATCTGTTCCAGAGC-3' and 5'-TGGGGAGAACCATCCTCACC -3', as previously described [6]. Each DNA sample (2 µl) was added to a 50-µl reaction mixture containing 100 ng of each primer, 200 µM dNTPs, and 0.3 U *Taq* polymerase in a standard polymerase chain reaction (PCR) buffer. One

of the primers was end-labeled with [γ-^{32}P] dATP. Thirty cycles of amplification were carried out using cycling parameters of 95°C for 30 s, 64°C for 30 s, and 72°C for 30 s. Each PCR product (2 µl) was added to an equal volume of formamide containing 0.1% bromophenol blue and 0.1% xylene cyanol, loaded onto a 7 M urea–polyacrylamide gel, and electrophoresed at 60 W for 4 h. The gel was dried and autoradiographed at –70°C.

Quantitative measurement of the PCR product bands was also performed by using a densitometer, as described by Paradis et al. [10]. In brief, the peak intensities of the two alleles (alleles 1 and 2) were measured for each specimen. A corrected ratio (CR) was first assessed by dividing the ratio (allele 1/allele 2) of the digested samples by *Hha*I by the ratio (allele 1/allele 2) of the non-digested sample. The use of CR corrects for the preferential amplification of one allele that might occur if the alleles differ markedly in length. A final clonality ratio was determined by dividing the CR of the lesional DNA by the CR of the non-lesional DNA. The ratio was inverted if necessary to obtain a value up to one. According to Paradis et al. [10], a final ratio of 1.5 corresponded to the presence of 25% clonal DNA in a polyclonal background. This value (1.5) was chosen as the threshold of sensitivity of a significant number of clonal cells.

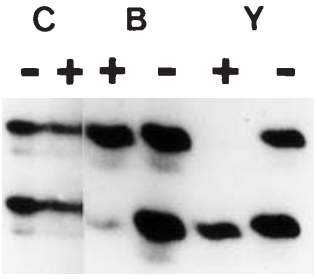


Fig. 4 Clonal analysis of the hepatocellular carcinoma (*B*) and the adjacent focal nodular hyperplasia (*Y*). "–" and "+" represent the absence or presence of prior *Hha*I digestion, respectively. Without *Hha*I digestion, two allelic bands with equal intensity were recognized in the three specimens. In the presence of *Hha*I digestion, the allelic bands from the focal nodular hyperplasia and the hepatocellular carcinoma showed a significant reduction in the intensity of one allele as compared with two alleles of equal intensity in the buffy coat (*C*), indicating monoclonal origins of these two lesions. However, the inactivated allele in the focal nodular hyperplasia and that in the hepatocellular carcinoma were not identical

Results

Pathologic findings

The resected liver tissue measured 20×18×12 cm and weighed 2900 g. There was a well-circumscribed but not encapsulated tumor mass (Fig. 1) measuring 20×14×11 cm. It was grossly composed of two different parts. The larger part of the liver tumor was yellow with a nodular appearance. The other part was brown and soft with a large area of necrosis. An aberrant vessel penetrated through the yellow portion to the brown part. However, no central scar was found in the yellow part. The non-tumor part had no obvious nodularity.

Microscopically, the yellow portion was composed of large hyperplastic hepatocytes with mild anisonucleosis. There were scattered fibrous septa and abnormal portal tracts with bile ductular proliferation and thick-walled arterioles (Fig. 2). Mallory bodies and fatty change were present. Reticulin stain showed that the liver cell plates were two to three cells thick. Focal Kupffer cell siderosis was identified with Perls' iron stain. The brown portion (Fig. 3) showed a classical HCC arranged in trabecular and acinar patterns with a large area of necrosis. No fibrous septum was present between the two portions of the liver tumor. The non-tumor part showed some fibrous septa but no cirrhotic change. There were grade III Kupffer cell siderosis and young hematopoietic cells in sinusoids.

The result of the clonal analysis is shown in Fig. 4. Without restriction enzyme digestion by *Hha*I, two allelic bands with equal intensity were observed in this patient, which indicated that the HUMARA gene of this patient was heterozygous and can be analyzed. After *Hha*I digestion, the allelic bands from the FNH and the HCC showed a significant reduction in the intensity of one of the two alleles compared with two allelic bands with equal intensity in the buffy coat. Thus, the HCC and FNH were interpreted as being monoclonal. However, the inactivated allele in the FNH and the one in the HCC were not identical. Therefore, the clonal origins of the FNH and HCC were different.

The final ratios of FNH and HCC using densitometric assessment of the PCR product bands were greater than ten, indicating the presence of 25% or more of clonal DNA. Thus, the quantitative analysis further confirmed the clonality of the FNH and HCC.

Discussion

The liver tumor described in this report was grossly composed of two different parts with different histologic features. The yellow part was composed of large hyperplastic hepatocytes with nodular abnormal architecture, malformed vessels, and bile ductular proliferation. Although the central scar was absent in this portion, the lesion fulfilled the morphologic diagnostic criteria of a FNH [8]. The large cell change in this lesion could represent an adaptive reaction to prolong cholestasis [7]. The brown portion was a classical HCC. The associations of HCC with adjacent FNH were rarely described [1, 11, 12]. In contrast to these reported cases, the HCC component in our case was not a fibrolamellar variant but a classical type. The FNH could have developed in our case secondary to the feeding artery of the HCC.

FNH has been demonstrated to be a clonal proliferating disease by HUMARA analysis [2]. Clonality can be assessed using the HUMARA assay when monoclonal cells comprise more than 25% of the total cell population in the specimen analyzed [10]. The results in the present study also showed that the FNH (yellow part) was monoclonal, as evidenced by a significant reduction in the intensity of one allele as compared with the two alleles of equal intensity in the buffy coat. However, a clonal disease is not invariably equivalent to a neoplastic process [3, 4]. There are two major mechanisms that could explain the occurrence of a uniform pattern of X chromo-

some inactivation in a non-neoplastic lesion. The first one is the patch concept [3, 4]. If, after cell division, progeny cells remain adjacent to each other, large patches of cells are formed, all of which contain an identical pattern of X chromosome inactivation. Lesions arising from two, three, or more cells within a patch will show the same pattern of X chromosome inactivation and appear clonal. Another explanation for the appearance of clonality is the selection hypothesis [3]. If one cell type within a multicellular lesion has a growth advantage, selective overgrowth of this cell type over time would result in a proliferation with an overall monoclonal pattern. Such a situation has been postulated for multinodular goiters of the thyroid [5]. Our results support that FNH is a clonal lesion. However, conflicting results were reported by Paradis et al. [9] using the same method. Therefore, the nature of FNH is still not settled.

In the present study, our results also showed that the inactivated allele in the FNH and the one in the HCC were different. These results indicated that the HCC and adjacent FNH probably developed through clonal expansion of different clones. Therefore, our study did not support that the HCC was the product of malignant transformation from the FNH.

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