Primary Liver Carcinoma in Genetic Hemochromatosis Reveals a Broad Histologic Spectrum

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Key Words: Hepatocellular carcinoma; Cholangiocarcinoma; Genetic hemochromatosis; Biliary microhamartoma; Von Meyenburg complex

Abstract

Hepatocellular carcinoma (HCC) is a well-known complication of genetic hemochromatosis (GH). However, the frequency of primary liver carcinoma (PLC) with biliary differentiation, such as cholangiocarcinoma (CC) and combined hepatocellularcholangiocarcinoma (CHCC), in GH remains unclear. We analyzed the histologic type of 20 PLCs occurring in the background of GH; all patients were homozygotic for the C282Y mutation. Ten were depleted of iron by successive phlebotomies, while the remaining 10 were untreated. Histologically, 13 cases were classified as HCC, 3 as CC, and 4 as CHCC. Immunohistochemical detection of Hep Par 1, cytokeratin 19 (CK19), and MUC1 supported this classification; PLC with biliary differentiation was immunoreactive for MUC1 in 86% (6/7) of cases and for CK19 in 100% (7/7) of cases. The nontumoral liver exhibited no cirrhosis or extensive fibrosis in 6 cases. Von Meyenburg complexes were present in 11 cases and intraparenchymal bile duct adenomas in 3. These data suggest that PLCs in patients with GH present a wide histologic spectrum, with tumors showing frequent biliary differentiation; may arise on a nonfibrotic or a cirrhotic liver; and often are associated with Von Meyenburg complexes and to a lesser extent with bile duct adenomas.

Hemochromatosis is a common inherited disorder of Caucasians in whom the incidence of expressed disease is 1 in 300 to 400.1,2 The hemochromatosis gene (HFE) has been identified on the short arm of chromosome 6,3 and 80% to 90% of patients are homozygous for the C282Y mutation.4-6 Major complications of genetic hemochromatosis (GH) are cirrhosis and hepatic carcinoma. Primary liver carcinoma (PLC) can account for 45% of deaths in people with GH.7 The relative risk for the development of PLC in people with GH with cirrhosis has been calculated at more than 200.8,9 Large series describing the histologic characteristics of PLC occurring in GH are rare. The major study reported by Deugnier et al10 demonstrated that iron-free foci (IFF) may be markers of an early stage of hepatocellular carcinoma (HCC) in GH and that such tumors may develop in people without cirrhosis. These data have been confirmed by others.11,12 Most liver tumors in GH correspond to classic HCC. However, rare isolated cases of cholangiocarcinoma (CC) have been reported.10,13,14 The incidence of liver tumors with cholangiolar differentiation remains unclear.

Therefore, we used histomorphologic and immunohistochemical analyses to detail fully the pathologic features of 20 cases of PLC as well as the adjacent nonneoplastic liver tissue in GH.

Materials and Methods

Between March 1988 and April 2000, 40 histologically proven PLCs developing in pathologically suspected GH were observed. As iron overload could be encountered in cirrhosis of various causes, our study focused exclusively on
20 cases in which the C282Y mutation in the HFE gene had been confirmed. The others were excluded owing to either absent C282Y homozygous mutation or insufficient material. Among the 20 retained cases, the material available for study comprised 3 fine-needle biopsy specimens and 15 surgical partial hepatectomy specimens, while the remaining 2 cases were from total hepatectomies for liver transplantation. All specimens were fixed in 4% formaldehyde and embedded in paraffin. Between 2 and 20 paraffin blocks were available for each case (mean, 6 blocks). Tissue sections were stained with H&E, Sirius red, and trichrome green. Perls stain was performed on nontumor tissue to evaluate the iron load and the presence of IFF.

Our study consisted of analyzing tumoral and nontumor tissue. In nontumor tissue, we classified the following: (1) the degree of fibrosis according to the METAVIR classification (scores ranging from 0 to 4)\textsuperscript{15}; (2) a histologic hepatic iron index (HHII) determined by the ratio of total histologic iron score (sum of hepatocytic, sinusoidal, and portal iron scores ranging from 0 to 60) to age, defined by Deugnier et al\textsuperscript{16}; (3) the presence or absence of small and/or large cell dysplasia; (4) the presence or absence of IFF; and (5) the association or not of biliary anomalies such as Von Meyenburg complexes (VMCs; or biliary microhamartomas) and/or bile duct adenomas (BDAs). Owing to the high frequency of subcapsular BDAs, only those located at more than 1 cm away from the Glisson capsule were taken into consideration. The VMCs were taken into account only when there was more than 1 present in a biopsy specimen or more than 3 in a surgical resection specimen.

Liver tumors were classified by standard H&E stain into HCC, CC, and combined hepatocellularecholangiocarcinoma (CHCC). HCC was defined by the presence of bile production and/or a trabecular growth pattern with endothelial-lined cell plates separated by little or no intervening stroma. They were classified according to the World Health Organization classification into trabecular, trabeculoacinar, or acinar and graded according to the Edmondson classification into 4 subgroups (I-IV). CC was characterized by definite gland formation associated with/or without mucin production, together with fibrous stroma. CHCC demonstrated distinct areas and/or intermingling features of HCC (trabecular and/or pseudoglandular patterns) and cholangiocarcinoma foci (gland formation and mucin production). A minimum amount of glandular differentiation (at least 30%) was required to classify a tumor as CHCC. Pseudoglandular growth patterns in HCC were excluded as evidence of biliary differentiation. To confirm the presence of HCC and CC elements, histochemical and immunohistochemical analyses were performed in all cases with the alcian blue technique and with the following primary antibodies: cytokeratin 19 (CK19) (DAKO, Glostrup, Denmark), Hep Par 1 (DAKO), and MUC1 (Novocastra, Peterborough, England). The expression of the p53 protein also was analyzed using the DO7 antibody (DAKO). After microwave pretreatment with citrate buffer, a 3-step immunoperoxidase technique was performed. A positive immunoreaction was determined if at least 10% of the cells were stained with DO7 antibody and 30% of cells with MUC1 and CK19 antibodies. Alcian blue stains were considered positive if more than 10% of tumoral cells or tumoral glandular lumens exhibited a strong staining.

Genomic DNA was extracted from frozen or paraffin-embedded tissues. DNA fragments were amplified by polymerase chain reaction using primers and reaction conditions as described by Feder and colleagues.\textsuperscript{3} The polymerase chain reaction products from all samples were treated with the restriction enzyme Rsal to identify the C282Y mutation.

The control group was composed of 60 PLCs of viral origin (30 hepatitis B virus and 30 hepatitis C virus [HCV]) and 40 PLCs of alcoholic or unknown origin. There were 88 men and 12 women, and 85 were Caucasian. The mean ± SD age of the patients was 55.6 ± 13 years. All cases were surgically resected and did not show significant differences in terms of age, race, and sex with the patients with GH. Specimens were processed for macroscopic and microscopic techniques in the same way as the GH cases to determine the tumor type and the presence of biliary anomalies in the adjacent nontumoral parenchyma. The mean number of blocks (6.8 blocks) did not differ significantly from the GH series. An immunohistochemical study of liver tumors of the control group was performed using the CK19 and MUC1 antibodies.

**Results**

**Clinicopathologic Findings**

All patients included in the study were homozygous for the C282Y mutation. They were all Caucasian men. The mean ± SD age of the patients was 63 ± 9 years. In 10 patients, the liver tumor revealed GH. In the other 10, the diagnosis of the disease was made 2 to 25 years (median, 11.2 years) before the discovery of a macroscopic nodule. Patients in this latter group were treated regularly by phlebotomy and followed up by abdominal ultrasound together with regular assay of alpha-fetoprotein levels. Hepatitis B and C virus serologic test results available for 12 cases were negative. Chronic alcoholic abuse was noted in 1 patient.

Twenty-one macroscopic tumors were identified in 19 patients. Two of these patients showed 2 independent nodules corresponding in each case to HCC and BDA. The remaining patient from the 20 cases exhibited multiple nodules of HCC in the surgically resected liver. Tumor size ranged from 2.4 to 11.5 cm (median, 6.95 cm). The series of
PLCs included 13 HCCs (65%), 4 CHCCs (20%), and 3 CCs (15%) [Table 1]. HCCs were trabecular in 9 cases, trabeculoacinar in 3 cases, and acinar in 1 case. One HCC showed a clear cell pattern. Nine HCC were grade II according to Edmondson, 3 were grade III, and 1 was grade IV. All CCs were intrahepatic and showed a nodular macroscopic pattern. An abundant stroma was found in all of them. All CHCCs revealed contiguous and intermingling HCC and CC components. The neoplastic cells, usually eosinophilic, were organized in a trabecular or glandular pattern with a stromal reaction of variable intensity [Image 1]. Mucus production was present in all CHCCs, whereas bile production was detected in only 1 case.

Nonneoplastic liver showed extensive fibrosis or cirrhosis in 14 cases, equivalent to scores F3 and F4 according to the METAVIR grading system. Preservation of central veins in most hepatic nodules was observed. Five patients had mild to moderate fibrosis (scores F1 and F2), and 1 patient had no fibrosis (score F0). Three typical intraparenchymal BDAs were observed in our series, with a size ranging from 1.5 to 3.1 cm [Image 2]. Two were detected macroscopically at a distance from the principal tumors, whereas 1 was included within the main tumor mass. All BDAs were associated with VMCs and PLCs of the HCC type. Eleven cases (10 surgical resection specimens and 1 biopsy specimen) showed VMCs (55%), which were numerous in 3 cases. These VMCs were associated in 3 cases with PLC showing biliary differentiation (CC and CHCC). Although in one of the latter cases, the CC colonized some adjacent VMCs [Image 3], there was no clear evidence of transition between these two lesions. The 10 untreated patients showed high HHIIs (>0.2), whereas all the untreated patients had HHIIs of less than 0.15. In cases with high HHIIs, both BDAs and VMCs exhibited no iron overload (in contrast with bile ducts present in the fibrotic tissue) and only 2 (20%) exhibited IFF. Three patients showed both small and large cell dysplasia. No cases showed histologic evidence of alpha_1-antitrypsin deficiency.

The comparative study of the control groups of 60 PLCs of viral origin (30 HCV cases and 30 hepatitis B virus cases), as well as 40 PLCs of alcoholic or undetermined origin, revealed that all PLCs corresponded to HCC except for 4 (2 CCs in HCV-positive patients and 2 CHCCs in an alcoholic and an HCV-positive patient). The cirrhotic parenchyma in these 4 cases revealed no or mild iron overload (score, 2). Among these control groups, 4 liver specimens showed some VMCs (associated with intraparenchymal BDA in 2 cases). Thus, PLCs with biliary differentiation (P < .001), VMCs (P < .001), and intraparenchymal BDAs (P < .01) seem significantly more frequent in GH than in other etiologies.

![Image 1](https://academic.oup.com/ajcp/article-abstract/116/5/738/1758354)

**Image 1** Histologic characteristics of a hepatocellular carcinoma showing features of both hepatocellular carcinoma (top) and cholangiocarcinoma (bottom).

![Image 2](https://academic.oup.com/ajcp/article-abstract/116/5/738/1758354)

**Image 2** Gross pathology of a deep bile duct adenoma that developed in a cirrhotic parenchyma.

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**Table 1**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>No. (%) of Malignant and Benign Liver Lesions</th>
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<tbody>
<tr>
<td>Hepatocellular carcinoma</td>
<td>13 (65)</td>
</tr>
<tr>
<td>Hepatocholedangiocarcinoma</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Cholangiocarcinoma</td>
<td>3 (15)</td>
</tr>
<tr>
<td>Von Meyenburg complex</td>
<td>11 (55)</td>
</tr>
<tr>
<td>Deep bile duct adenoma*</td>
<td>3 (15)</td>
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</table>

* Two were detected macroscopically.
Immunohistochemical Findings

All HCCs except 1 (12/13 [92%]) showed a homogeneous positivity for Hep Par 1 antibody and were negative for both MUC1 and CK19 antibodies. Two (67%) of 3 and 3 (100%) of 3 CCs expressed MUC1 and CK19 respectively. Table 2. Immunohistochemical analysis underlined the hybrid features in 3 (75%) of 4 CHCCs showing diffuse positivity for both MUC1 and CK19, as well as for Hep Par 1. The remaining CHCC did not express Hep Par 1. There was immunoreactivity for the p53 protein in 3 (23%) of 13 HCCs, 1 (33%) of 3 CCs, and 3 (75%) of 4 CHCCs. In the control group, expression of CK19 and MUC1 was present in all CCs and CHCCs, and in 4 (4%) of 96 and 2 (2%) of 96 HCCs, respectively.

Discussion

The most interesting result of our study was the broad histologic spectrum of PLC in patients with GH, including HCC, CC, and CHCC. Furthermore, the adjacent nontumoral liver tissue frequently showed tumor-like bile duct lesions, such as VMCs, as well as BDAs. These lesions could represent a potential predisposing factor in the pathogenesis and development of malignant tumors with multiple divergent differentiation despite the absence of morphologic signs of transition characterized in our series.

As end-stage cirrhosis arising from various causes often is complicated by parenchymal siderosis that may mimic hemochromatosis, the confirmation of homozygous C282Y mutation in the HFE gene seems to be essential to diagnose GH. All patients were homozygous for this mutation. The frequency of PLC with cholangiolar differentiation (35%) observed in our series seems to be much higher than that reported by Deugnier et al,10 who found that only 1 CC (5%) was demonstrated within the 19 histologically analyzed tumors. Such a prevalence also seems higher than that associated with other causes (viral or alcoholic).

As much controversy still exists in the literature concerning what is accepted as evidence of divergent differentiation, we performed additional immunohistochemical staining procedures to demonstrate the presence of both HCC and CC components. All CCs showed diffuse positivity for CK19, whereas all HCCs except 1 (92%) were positive for Hep Par 1 antibody. In 75% of cases of CHCC, immunohistochemical analysis confirmed the double differentiation of the tumor (areas positive for all Hep Par 1, CK19, and MUC1 antibodies). The failure to detect immunoreactivity using the Hep Par 1 antibody in a typical HCC, as well as its negativity in 1 CHCC, may be attributed to a low sensitivity of this immunohistochemical assay. It has been evaluated as 80% to 92% in previous series.17,18 The use of a more sensitive technique, such as in situ hybridization, to detect albumin messenger RNA expression could be of value in such cases.19 The wide histologic spectrum of hemochromatosis-associated liver carcinomas shown in our series supports their development from hepatocytes, as well as from biliary cells, or more likely from multipotent stem or progenitor cells. Some authors have suggested that CHCCs originate from progenitor cells possessing a dual potential of differentiation.20-22

Although an increased intrahepatic iron concentration has been shown to induce tissue injury and damage as well

<table>
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<tr>
<th>Antibody</th>
<th>Hepatocellular Carcinoma (n = 13)</th>
<th>Cholangiocarcinoma (n = 3)</th>
<th>Hepatocholangiocarcinoma (n = 4)</th>
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</thead>
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<td>Cytokeratin 19</td>
<td>0 (0)</td>
<td>3 (100)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Hep Par 1</td>
<td>12 (92)</td>
<td>0 (0)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>MUC1</td>
<td>0 (0)</td>
<td>2 (67)</td>
<td>4 (100)</td>
</tr>
<tr>
<td>DO7</td>
<td>3 (23)</td>
<td>1 (33)</td>
<td>3 (75)</td>
</tr>
</tbody>
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* Data are given as number (percentage). See the “Materials and Methods” section for antibody sources.
as fibrosis, the exact mechanism of iron-induced carcinogenesis remains to be clarified. It is known that iron may be a carcinogenic factor involved in both cancer initiation (by induction of oxidative stress) and promotion (by facilitating tumor growth). However, iron depletion in patients with GH does not prevent the development of HCC, regardless of whether cirrhosis is present. As reported in isolated cases in the literature and in our series, VMCs (and to a lesser extent BDAs) are relatively common in the livers of people with GH. Thus, a putative carcinogenic effect of iron on biliary cells may explain the occurrence of CC. However, although iron accumulation was observed in biliary cells of the portal tracts, such accumulation was not observed within VMCs and BDAs in PLCs occurring in patients with GH. While a certain relationship exists between IFF and the development of PLC, a similar relationship cannot be confirmed between biliary anomalies and the development of PLC with biliary differentiation. It should be noted that such biliary anomalies also are encountered in classic HCC. Furthermore, in contrast with a recent report suggesting that cholangiocarcinoma may arise from VMCs, we did not observe morphologic signs of transition between these lesions in our series.

The underlying molecular alterations remain unclear. Interestingly, a similar spectrum of morphologic appearances has been reported in PiZ alpha1-antitrypsin deficiency with both bidirectional differentiation of liver tumors and VMCs. These similarities raise the question whether common oncogenic mechanisms exist for these metabolic liver disorders that both show lesions suggesting ductal plate malformations. Controversies exist concerning the possible relationship between these two inborn errors of metabolism. In no case in our series did we observe morphologic signs in favor of alpha1-antitrypsin deficiency in the nontumoral parenchyma.

The analysis of the nontumoral liver in our series revealed great diversity regarding liver fibrosis, ranging from no fibrosis to frank cirrhosis. In 30% of our patients (6/20), PLC developed with no extensive fibrosis or cirrhosis. This higher frequency when compared with that reported by Deugnier et al (16%) may be explained by an early diagnosis and/or a prolonged phlebotomy treatment. It also confirms that liver tumors could arise in noncirrhotic livers and that iron depletion does not prevent the development of liver tumors. By contrast, a low prevalence of IFF (10%) and dysplastic cell changes (15%) has been characterized in our study.

Because a high frequency of p53 gene mutations has been reported in HCC occurring in patients with GH, we evaluated the expression profile of its protein. Although the percentage of nuclear positivity for p53 in typical HCC (23%) is similar to those reported in viral and alcoholic causes, it seems higher in PLC with cholangiolar differentiation (33% in CC and 75% in CHCC). These need confirmation in a larger series with characterization of the mutations.

Primary liver carcinoma shows a wide histologic spectrum in patients with GH, including HCC, CC, and CHCC. Associated with these tumors, frequent VMCs and BDAs were present in liver adjacent parenchyma. In patients in whom a definite cause for chronic liver disease is missing, the presence of such morphologic biliary anomalies would
encourage investigation of a possible metabolic disorder such as hemochromatosis or PiZ alpha1-antitrypsin deficiency as the origin of the tumor.

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References