Ground-glass Cells in Hepatocellular Carcinoma

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Stromeyer, F. Wayne, Ishak, Kamal G., Gerber, Michael A., and Mathew T.: Ground-glass cells in hepatocellular carcinoma. Am J Clin Pathol 74:254-258, 1980. Hematoxylin and eosin-stained sections representing ten cases of hepatocellular carcinoma showed many tumor cells with ground-glass cytoplasm identical to that found in hepatocytes containing hepatitis B surface antigen (HBsAg). However, the aldehyde fuchsin stain was negative, as were the immunoperoxidase stains for HBsAg and core antigen (HBcAg). Electron microscopically, the ground-glass appearance corresponded to the presence of non-membrane-bound amorphous or fibrillar inclusions. Immunohistochemically, the ground-glass material reacted with antisera to human fibrinogen, suggesting synthesis of this protein by the carcinoma cells. Although the ground-glass appearance in hepatocellular carcinomas may sometimes be associated with HBsAg, special stains or techniques are necessary to confirm its presence. (Key words: Ground-glass cells; Hepatocellular carcinoma; Hepatitis B surface antigen; Fibrinogen.)

ALTHOUGH an association between hepatitis B infection and hepatocellular carcinoma appears to be established, components of the hepatitis B virus have only recently been identified in the carcinoma cells (reviewed by Gerber and Thung). "Ground glass" change, a hallmark of the presence of hepatitis B surface antigen (HBsAg) in the cytoplasm of hepatocytes was recently identified in a hepatocellular carcinoma. The presence of HBsAg in the carcinoma cells was confirmed by aldehyde fuchsin and immunoperoxidase stains and by immunofluorescence; this phenomenon appears to be very rare, since it was found to occur in only one of 130 consecutive cases of hepatocellular carcinoma from a hospital in Hong Kong.

We have studied ten examples of hepatocellular carcinoma in which numerous ground-glass cells were present. Although the cytoplasm of the cells had an appearance identical to that found in HBsAg-containing hepatocytes, special stains for HBsAg were negative.

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Materials and Methods

Ten cases of hepatocellular carcinoma with ground-glass cells were studied at this Institute during the past two years. Hematoxylin and eosin-stained sections from all cases were evaluated. In eight cases with adequate material, additional sections were stained with periodic acid-Schiff (PAS) after diastase digestion, mucicarmine, alcian blue, and a modification of the aldehyde fuchsin method for HBsAg. Formalin-fixed tissues from two cases (Patients 7 and 8) were prepared for electron microscopy and stained with uranyl acetate and lead citrate.

Immunohistochemical studies for the identification of six antigens were performed on paraffin sections of formalin-fixed tissues from three cases (Patients 3, 7, and 9). The unlabeled antibody enzyme method with peroxidase-antiperoxidase complexes was employed to demonstrate HBsAg, hepatitis B core antigen (HBcAg), alpha-fetoprotein, alpha-1-antitrypsin, and carcinoembryonic antigen. The antisera used, the technic, and the controls have been described previously. Fibrinogen was detected by the direct fluorescent antibody method on deparaffinized trypsin-digested sections using fluoresceinated sheep antihuman fibrinogen. The specificity of the reaction was proved by absorption of the sheep antiserum with human fibrinogen.

Results

Selected clinical and histologic data are recorded in Table 1. Of six patients for whom nonneoplastic hepatic tissue was available, two had cirrhosis. The aldehyde fuchsin stain was negative in the nonneoplastic tissues of all six patients. Two of five patients had positive serologic tests for HBsAg. One patient (Patient 2) who had a negative HBsAg test had a posi-
tive serologic test for anti-HBcAg. Another patient (Patient 9) had used an oral contraceptive for seven years.

The tumors were classified according to various histologic patterns described by Peters.14 Many tumor cells contained well-delineated, pale, eosinophilic homogeneous or slightly vesicular inclusions with an appearance resembling ground glass (Fig. 1). The nuclei of the affected cells were often compressed against the plasma membrane. Occasionally the cytoplasm of tumor cells was diffusely involved by the ground-glass change. The glass-grass areas of the cytoplasm did not stain with the PAS or aldehyde fuchsin stains.

Alpha-fetoprotein, alpha-1-antitrypsin, carcinoembryonic antigen, HBsAg, and HbcAg were not detected in the ground-glass cells of the three tumors studied by immunoperoxidase methods; however, the ground-glass material reacted strongly with fluo-

Table 1. Selected Clinical and Histologic Findings*

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Cirrhosis</th>
<th>Serum HBsAg</th>
<th>Tumor Pattern</th>
<th>Periodic Acid-Schiff</th>
<th>Aldehyde Fuchsin</th>
<th>Hyaline Globules</th>
<th>Mallory Bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1, 49, M</td>
<td>0</td>
<td>-</td>
<td>Trabecular</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Patient 2, 74, M</td>
<td>+</td>
<td>-</td>
<td>Trabecular</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Patient 3, 26, M</td>
<td>-</td>
<td>0</td>
<td>Lamellar fibrosis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Patient 4, 70, F</td>
<td>-</td>
<td>0</td>
<td>Giant cell</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 5, 53, F</td>
<td>0</td>
<td>0</td>
<td>Trabecular</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 6, 70, F</td>
<td>+</td>
<td>+</td>
<td>Trabecular</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Patient 7, 7, M</td>
<td>-</td>
<td>0</td>
<td>Lamellar fibrosis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Patient 8, 37, F</td>
<td>0</td>
<td>-</td>
<td>Macrotrabecular</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Patient 9, 28, F</td>
<td>-</td>
<td>0</td>
<td>Lamellar fibrosis</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Patient 10, 51, F</td>
<td>0</td>
<td>+</td>
<td>Macrotrabecular</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

* + = positive or present; - = negative or absent; 0 = information or material unavailable.

hepatitis, chronic active hepatitis, cirrhosis, and the carrier state), is not diagnostic of hepatitis B infection, however. It has also been found in association with therapy with certain drugs.16,21 Immunohistochemical methods and/or special staining technics (aldehyde fuchsin, orcein) are necessary to confirm the presence of HBsAg in liver cells.

Although hepatitis B antigens have been demonstrated in hepatocellular carcinoma, the HBsAg-positive tumor cells usually do not have a ground-glass appearance6,15 with the exception of one case described recently.22

The present study provides evidence that the finding of ground-glass cells in hepatocellular carcinoma does not always indicate the presence of HBsAg. Of ten carcinomas with ground-glass cells, three from patients with evidence of hepatitis B infection, none were positive for HBsAg with the aldehyde fuchsin stain. Additionally, the electron-microscopic appearance of the ground-glass cells in this series was entirely different from that found in other cases associated with HBsAg or drug therapy. The ground-glass appearance in the latter instances is accounted for by hyperplasia of the smooth endoplasmic reticulum. If related to hepatitis B infection, HBsAg particles may be found within the cisternae of the endoplasmic reticulum. In the present series, the ground-glass appearance corresponded to non-membrane-bound amorphous or fibrillar material. This material did bear an ultrastructural resemblance to other inclusions described in hepatocellular carcinoma cells of man and experimental animals,7,19,11,17 although these cells were not stated to have had a ground-glass appearance by light microscopy.

To our knowledge the ultrastructural appearance of fibrinogen is not known. The fine structure of fibrin (that forms extracellularly) is compact bundles with a fine fibrillary pattern that may have axial periodicity at approximately 230-A intervals.18 The ultrastructural appearance of the ground-glass cells in the present
study does not resemble that of fibrin. Ultimate proof that some or all of the amorphous material is fibrinogen will require immunoperoxidase localization at the ultrastructural level, as has been accomplished for albumin, for example.12 It is of interest that fibrin deposits have been found in the immediate vicinity of many spontaneous tumors of animals and man, including human carcinomas, sarcomas, melanomas, and various lymphomas.4,10 In the case of the transplantable line 10 hepatocarcinoma, the tumors become enveloped in fibrin-gel within hours of implantation.4 The possible tumor-protective effects of the fibrin are thoroughly discussed by Dvorak and colleagues.4 It is assumed that the extracellular fibrin deposits derive from the host’s plasma fibrinogen.4 In view of our findings, a possible tumor-protective role of fibrinogen secreted by some hepatocellular carcinomas cannot be excluded.

Immunohistochemical studies of three cases of the present series showed that the ground-glass material represented accumulation of fibrinogen in carcinoma cells. This finding favors increased production of fibrinogen rather than release of an antifibrinolytic agent by some hepatocellular carcinomas associated with hyper- or dysfibrinogenemia.1,2

In the present study, in addition to the ground-glass cells, some tumor cells contained PAS-positive cytoplasmic globules that resisted diastase digestion. Immunoperoxidase stains confirmed the presence of alpha-1-antitrypsin at the periphery of these globules, as has been previously reported.13 In two cases, Mallory bodies were identified in some of the tumor cells. It is of interest that ground-glass tumor cells found in three of the ten cases occurred in an extremely rare type of hepatocellular carcinoma variously referred to as “eosinophilic carcinoma with lamellar fibrosis”14 and “polygonal cell carcinoma with fibrous stroma.”15 The type of tumor accounted for less than 2% of the 225 hepatocellular carcinomas studied by Peters.14 If the high incidence of ground-glass tumor cells in hepatocellular carcinoma with increased stromal fibrosis can be confirmed by future studies, then a search for these cells in biopsy material would be important, since patients with this histologic variant appear to have an improved survival.5,14

Although the ground-glass appearance in hepatocytes or cells of hepatocellular carcinoma can suggest the presence of HBsAg, special stains or techniques should be used for definitive identification. The clinical implications of ground-glass tumor cells, particularly in relation to hyper- or dysfibrinogenemia and to prognosis, remains to be determined. The usefulness of fibrinogen as a marker for hepatocellular carcinoma requires the study of many more cases by immunohistochemistry.

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References


