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Case report

Well-differentiated hepatocellular carcinoma associated with long-term survival

Report of two cases

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Summary. Two cases of well-differentiated hepatocellular carcinoma (HCC) with focal biliary differentiation are presented. The distinct histological features of these neoplasms and the unusually protracted clinical course of 8 and 10 years distinguish them from previously described pathological categories of primary hepatic tumors. Electron microscopic and immunohistochemical findings support a dual hepatic and bile duct differentiation of the tumor cells. If additional examples of this tumor are found to be associated with a similarly prolonged symptom-free survival, the distinction of this entity from traditional, rapidly fatal HCC becomes important. Less aggressive therapeutic options may be entertained.

Key words: Hepatocellular carcinoma – Immunohistochemistry – Prolonged survival

Introduction

Long-term survival after the diagnosis of primary liver cancer is rare. Although occasional patients have small, resectable tumors and are potentially curable (Kishi et al. 1983) survival with primary hepatocellular carcinoma (HCC) rarely extends for a year after diagnosis in untreated cases, with a mean survival of 4 months (Nagasue et al. 1984). In particular, recurrence of previously resected tumors has an extremely poor prognosis, with most patients dying within 1 year of recurrence (Hsu et al. 1988). The prognosis is equally dismal in patients with combined hepatocellular-cholangiocarcinoma (HCC-CC) (Goodman et al. 1985) and, with rare exceptions, with pure cholangiocarcinoma (CC) (Rockwell et al. 1966). In this tumor the survival improves, albeit slightly, the more distal the lesion is located within the biliary tree (Silverman et al. 1988). The major exception to these generalisations is the fibrolamellar variant of HCC, which has a mean survival of 32–73 months and a 5-year survival as high as 63% (Berman et al. 1980, 1988; Craig et al. 1980).

We report two cases of HCC dissimilar from the fibrolamellar variant, with distinctive histologic findings, immunohistochemical and ultrastructural features indicative of both hepatocellular and cholangiocellular differentiation, and survival with persistent or metastatic disease for 8 and 10 years after diagnosis.

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Case reports

Case 1. A 54-year-old post-menopausal Mexican female emigrated to the United States at age 15. She had no known hepatitis exposure, but was treated for a parasitic infection as a child. She had a 3-month trial of oral contraceptives in the distant past. In 1977, she developed vague, mid-epigastric and right upper quadrant discomfort. Ultrasound revealed a right hepatic cyst. Subsequent computerized tomography (CT) scan of the liver with a celiac angiogram revealed a defect compatible with malignancy. Serology for hepatitis B was negative. Serum alpha-fetoprotein (AFP) was negative, while all other liver function tests including prothrombin time (PT), alkaline phosphatase, bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and albumin were normal. The patient underwent an exploratory laparotomy with drainage of the right hepatic cyst and a left hemi-hepatectomy for a localized tumor. The remainder of the parenchyma appeared normal. Careful exploration revealed an enlarged right ovary, which was excised. Pathologic examination revealed well-differentiated HCC with focal biliary differentiation confined to the left lobe of the liver and surrounded by normal hepatic parenchyma, and a fibrothecoma of the right ovary. The post-operative course was uneventful. Yearly follow-up with serum liver enzymes and AFP, as well as abdominal-pelvic CT scans were normal until July 1984, when the patient developed mid-epigastric discomfort and was found to have a 10-cm neoplasm located in the omentum, which was excised. The histology and electron microscopy (EM) were identical to the original tumor.

At follow-up in September 1986, physical examination revealed a periumbilical mass. At laparotomy, multiple tumor implants in the residual omentum were removed. Pathologic examination was consistent with the original tumor. In March 1988, a routine CT

revealed a soft tissue pelvic mass posterior to the uterus. Again, liver function tests were within normal limits, serology for hepatitis B was negative and AFP was normal. The patient underwent total abdominal hysterectomy and left salpingo-oophorectomy for removal of a tumor that replaced the left ovary and had spread into the broad ligament and anterior cul-de-sac. Exploration of the abdomen demonstrated two isolated serosal implants on the sigmoid and transverse colon which were also removed. Pathology was identical to the original hepatic tumor.

*Case 2.*A 37-year-old white man was noted to be hepatitis B antigen positive when he volunteered to donate blood in 1975. He had no history of blood transfusions or symptoms at the time. In 1980, he experienced fevers up to 102° F for several weeks. Of note was an elevated AFP to 480 IU/ml, and other liver function tests within normal limits. Exploratory laparotomy, revealed extensive replacement of the liver by tumor diagnosed as HCC on microscopic examination. The surrounding parenchyma was normal. No resection was attempted. He was treated for 5 years with adriamycin. In 1985, the patient developed congestive heart failure and was treated with digoxin, furosemide and captopril. In May 1987, he experienced life-threatening ventricular arrhythmias, which were eventually controlled with amiodarone. Over the ensuing months, he had frequent episodes of upper gastrointestinal bleeding from rupture of esophageal varices requiring multiple transfusions and ten attempts at sclerotherapy. He was admitted in January 1988 for mesocaval anastomosis. Abnormal liver function tests included ALT of 1252 μmol/l, AST of 1216 μmol/l, albumin 24 g/l, bilirubin 57.8 μmol (direct 39.1 μmol/l) and AFP of 19000 IU/ml. At laparotomy, numerous perihepatic adhesions and the likelihood of bleeding complications precluded a repeat biopsy. Following a successful shunt procedure, the patient was discharged.

Materials and methods

Specimens available from case 1 consisted of tissue from the left ovary (1988), abdominal wall (1986), omentum (1984) and liver (1977). Case 2 material was the wedge liver biopsy from 1980. All specimens were paraffin-embedded, serially sectioned at 6 μm and stained with hematoxylin and eosin, and mucicarmine. Tissue for immunohistochemistry was fixed in alcoholic zinc-formalin (case 1, 1988 tissue) and 10% phosphate-buffered formalin (case 1, 1984 tissue; case 2, 1980 tissue). For EM, 1 mm^3 tissue fragments were fixed in Karnovsky's fixative and processed by routine methods.

Immunohistochemical staining was carried out by the peroxidase antiperoxidase method. Paraffin-embedded sections were cut at 6 μm and incubated at 50–60° C for approximately 1 h, deparaffinized, and washed in running water. Endogenous peroxidase activity was quenched by 30-min incubation in 0.5% hydrogen peroxide in methanol. The sections were sequentially incubated at room temperature with swine blocking serum for 30 min and dilutions of primary antibodies for 60 min. Following incubations with the polyclonal primary antisera (alpha-1-antitrypsin 1:300; alpha-1-antichymotrypsin 1:300; AFP 1:200; neuron specific enolase (NSE) 1:200 and carcino-embryonic antigen (CEA) 1:500, Dako, Santa Barbara, Calif.), a secondary bridging swine anti-rabbit immunoglobulin antiserum (Dako) at 1:60 dilution was applied for 30 min and followed by peroxidase antiperoxidase complex (Cappel, West Chester, PA 1:300, 30 min) and 0.2% diaminobenzedine (DAB). The sections were counterstained with hematoxylin. The monoclonal antibodies (AE1/AE3, Boehringer, Mannheim, FRG, 1:300; CAM 5.2, Becton Dickinson, Mountain View, Calif. 1:5, epithelial membrane antigen, Dako 1:100; chromogranin, Boehringer, 1:1000; vimentin, Dako, 1:60, alpha-human chorionic gonadotropin (HCG), Sigma St. Louis, Mo 1:100) were sequentially followed, after appropriate washes in Tris buffer, by peroxidase-conjugated rabbit anti-mouse (Dako, 1:40, 30 min) and then by peroxidase-conjugated swine anti-rabbit immunoglobulin (Dako, 1:60; 30 min). The antigens were localized using DAB, and the sections counterstained with hematoxylin. Sections stained for keratin (AE1/AE3, CAM 5.2), alpha 1-antichymotrypsin and and alpha-1-antirypsin were pretreated with trypsin (0.025%, Sigma T-8128, 20 min, 37° C). Controls, always negative, consisted of omission of primary antibody.

Results

Both tumours arose on a background of normal hepatic parenchyma. The recurrent tumors in case 1 (only a wedge biopsy was performed in case 2) were soft, homogeneous, light tan with areas of hemorrhage. The masses appeared well-circumscribed but not encapsulated.

Sections from the original and metastatic tumors in both cases revealed a neoplasm composed of monotonous epithelial cells with occasional variability in cell size. The cells were small, cuboidal, with a rounded centrally positioned nucleus and a finely granular, chromatin pattern. Most had centrally or peripherally positioned small nucleoli and mitoses were very rare. The cells were arranged in both a trabecular pattern separated by scant, loose fibroconnective tissue (Fig. 1A),

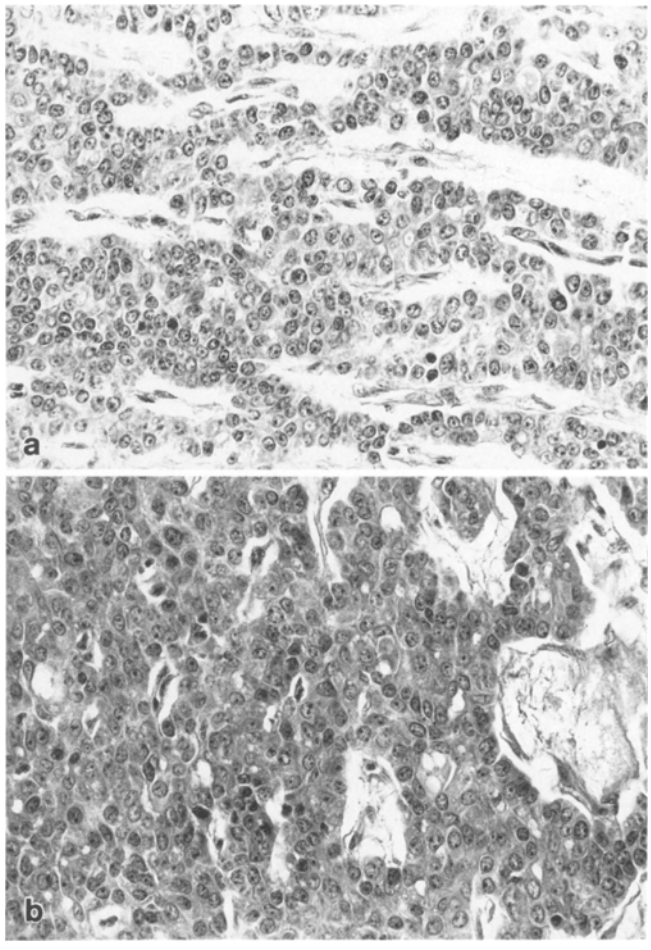

Fig. 1A, B. Case 1. Metastatic hepatocellular carcinoma to the ovary. Notice the uniformity of the neoplastic cells, the lack of mitoses and the trabecular (**A**) and sheet-like (**B**) arrangement with focal formation of pseudoglands. H & E, ×100

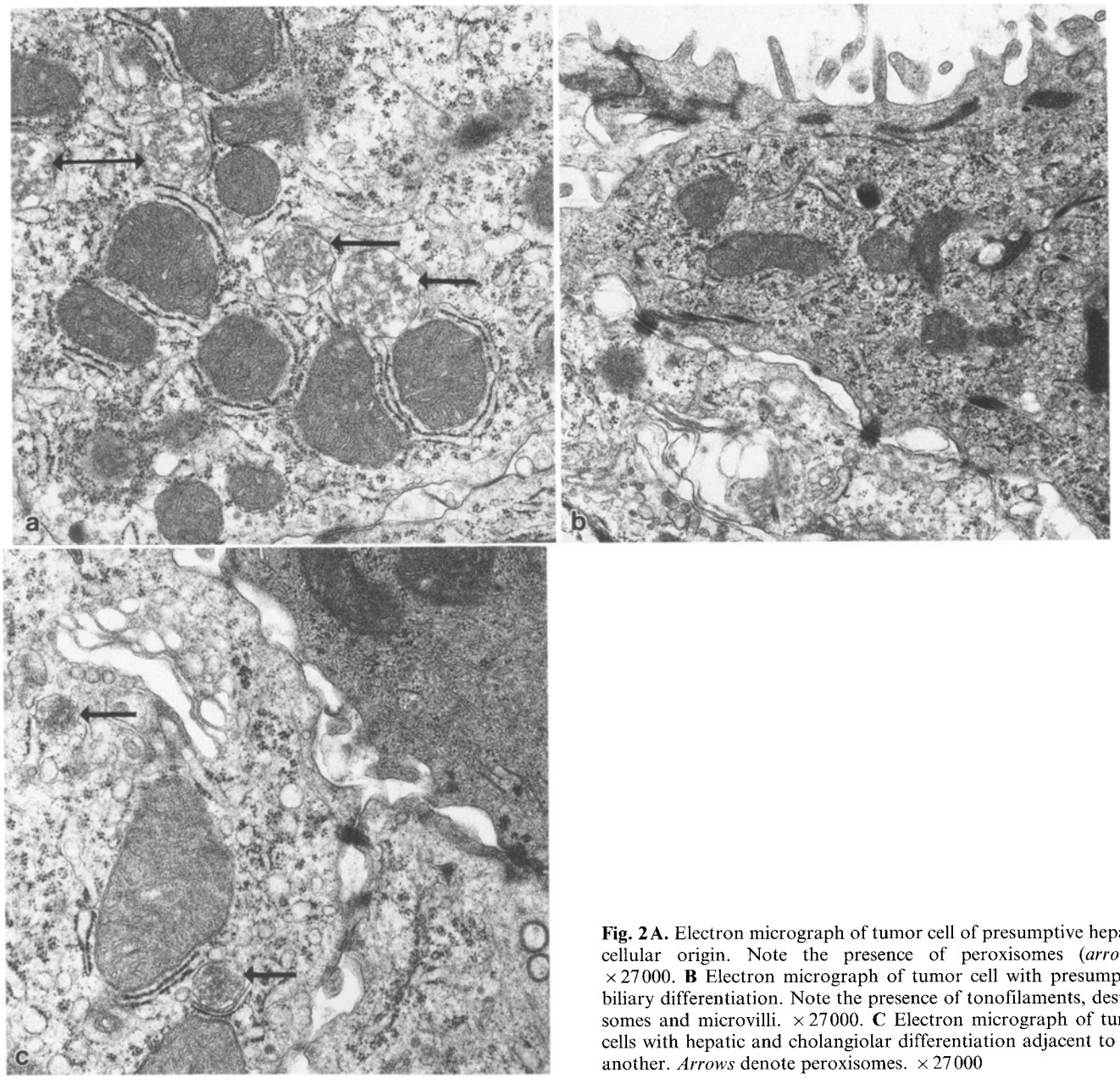

Fig. 2A. Electron micrograph of tumor cell of presumptive hepatocellular origin. Note the presence of peroxisomes (*arrows*). ×27000. **B** Electron micrograph of tumor cell with presumptive biliary differentiation. Note the presence of tonofilaments, desmosomes and microvilli. ×27000. **C** Electron micrograph of tumor cells with hepatic and cholangiolar differentiation adjacent to one another. *Arrows* denote peroxisomes. ×27000

and in cohesive, solid sheets of monotonous tumor with focal pseudoglandular formation (Fig. 1B). These two patterns were intimately admixed. The cytological and architectural features in these neoplasms were somewhat reminiscent of neuroendocrine tumors. There were rare foci of hemorrhagic necrosis. The eosinophilic material within the pseudoglandular areas was focally mucicarmine positive. No bile production was seen. The liver tissue surrounding the tumor appeared normal in both cases.

On EM two cell types were identified among the tumour cells. One had abundant organelles including mitochondria, Golgi, rough and smooth endoplasmic reticulum, and peroxisomes. Although ubiquitous, peroxisomes are most frequently found in liver and kidney cells, and in this context are strongly suggestive of hepatocellular differentiation (Fig. 2A). Other cells had well-developed desmosome, tonofilaments, and microvilli with microfilamentous cores (Fig. 2B). These findings suggest biliary epithelial cell differentiation (Ordonez and Mackay 1983). Bile canaliculi were observed, and the presumed hepatocellular cells and biliary tract cells were frequently found adjacent to one another (Fig. 2C). Although light microscopic findings raised the question of neuroendocrine differentiation, dense core granules were not seen by EM.

A dual population of keratin-positive cells was observed immunohistochemically. Distributed throughout the tissue were CAM 5.2-positive, AE1/AE3-negative cells which appeared most abundant in areas of the tumor with a trabecular architecture (Fig. 3A). In a more select distribution were AE1/AE3-positive cells, which

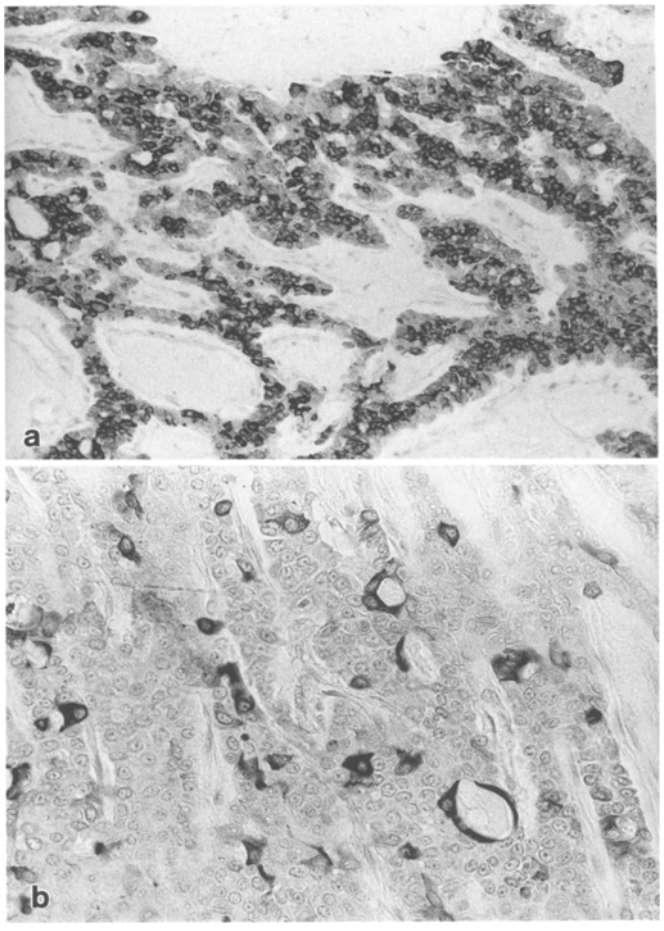

Fig. 3. A Case 1. Trabecular pattern of metastatic hepatocellular-carcinoma with diffuse staining with monoclonal antibody CAM 5. 2. Peroxidase-antiperoxidase method, counterstained with haematoxylin, ×100. **B** Case 1. Diffuse pattern of metastatic hepatocellularcarcinoma with selective immunocytochemical staining with AE1/AE3 in individual, scattered tumour cells and in those lining pseudoglandular areas. Peroxidase-antiperoxidase method counterstained with haematoxylin, ×100

were most frequently seen in pseudoglandular conformation (Fig. 3B). There was focal positivity for alpha-1-antitrypsin. Tumour cells were negative for alpha-1-antichymotrypsin, alpha-HCG, NSE and chromogranin. Alpha-fetoprotein was also negative, a finding not uncommon for HCC (Koelma et al. 1986, Chedid et al. 1990), especially in well-differentiated tumours (Brumm et al. 1989). Focal cytoplasmic positivity for CEA in the pseudoglandular areas was found in both cases. There was weak membrane staining for this antigen in trabecular zones. Scattered cells throughout the tumor had cytoplasmic positivity for vimentin.

Discussion

The neoplasms outlined appear to be an unusual morphological type of primary hepatic cancer arising in non-cirrhotic liver, with the behaviour of a low-grade malignancy. The fact that they are malignant tumors is confirmed by the metastases in one case and the liver replacement in the other. Immunohistochemistry together with EM proved useful in determining the differentiation of tumor cells.

Two monoclonal antibodies to keratin were used; a cocktail of AE1 and AE3, and CAM 5.2. The antibody AE3, which reacts primarily with high-molecular-weight keratins (Battifora 1988) represents 10% of the cytokeratin AE1/AE3 cocktail and does not react with normal or neoplastic bile duct cells or hepatocytes (Lai et al. 1989). Moreover, since formalin fixation somehow alters the epitope it recognizes (Battifora 1988), its contribution to the staining profile of these formalin-fixed tissues is probably negligible. The antibody AE1 recognizes members of the low-molecular-weight keratins (Moll et al. 1982; Tseng et al. 1982) and labels many normal epithelia, including bile duct but not hepatocytes (Lai et al. 1989). The second antibody, CAM 5.2 (raised against a colon carcinoma cell line) recognizes high-molecular-weight keratins, and stains both biliary epithelium and hepatocytes (Johnson et al. 1988). Since both hepatocytes and bile duct epithelium seem to retain their distinct cytokeratin profiles in liver disease including malignant transformation (Lai et al. 1989), we considered AE1/AE3 positivity indicative of biliary differentiation, while CAM 5.2 positivity was thought to stain cells with either hepatocellular or biliary differentiation.

The neoplasms presented illustrate features at the light-ultrastructural and immunohistochemical level that support our contention that they represent an unusual variant of well-differentiated HCC exhibiting focal cholangiocellular differentiation. Features indicative of the latter are the pseudoglandular areas with focal mucin production, ultrastructural presence of microvilli with microfilamentous cores, selective reactivity to AE1, and focally to CEA, by immunohistochemistry, in these areas. The hepatocellular origin is supported by the trabecular architecture seen on light microscopy with a diffuse immunoperoxidase reaction in these areas to high-molecular-weight keratins, and by the abundance cytoplasmic peroxisomes seen by EM.

The cytokeratin immunoreactivity pattern of well-differentiated, pure HCC has been shown to be almost exclusively that of high-molecular-weight keratin positivity (Van Eyken et al. 1988). Since these tumors are, by conventional cytologic and histologic criteria, well-differentiated neoplasms, the finding of focal low-molecular-weight keratin reactivity further supports the contention of cholangiocellular phenotype of some of the cells (Balaton et al. 1988). Cytoplasmic positivity to CEA has been reported only in CC or in mixed HCC-CC (Brumm et al. 1989; Ganjey et al. 1988). The focal membrane ("canalicular") staining observed in our cases in the trabecular areas was interpreted as cross-reaction of polyclonal anti-CEA antibody with biliary glycoprotein I (Ganjey et al. 1988). This artefact can be abolished with the use of monoclonal anti-CEA antibodies (Koelma et al. 1986). Although rare cells showed cytoplasmic vimentin positivity in both trabecular and pseudoglandular areas, the majority of tumor cells were negative. Indeed vimentin is usually co-expressed with cytokeratin only in poorly differentiated HCC (Brumm et al. 1989).

Alpha-HCG was also negative in both cases, as expected (Brumm et al. 1989).

Despite the long-term survival with recurrent disease, neither tumour displayed the histologic appearance characteristic of the fibrolamellar variant of HCC (Berman et al. 1980, 1988; Craig et al. 1980). Rather, they resembled neuroendocrine neoplasms morphologically. However, unlike these tumors and dissimilar from a reported case of HCC with carcinoid differentiation (Barsky et al. 1984), the two cases in this report did not react with anti-chromogranin or anti-NSE antibodies and had no dense-core granules by EM.

Consideration was also given to a previously described CC with unique histologic features and a 15-year survival (Foucar et al. 1979). In this report, the tumor exhibited the true glandular differentiation characteristic of well-differentiated adenocarcinomas with basal lamina and interglandular connective tissue. Immunohistochemical studies were limited to AFP, which was negative. The absence of basal lamina and stromal separation of glands in the current cases and, most importantly, the predominant hepatocellular differentiation, suggest that the two entities are distinct.

Combined HCC-CC have been classified by Goodman et al. (1985), into "collision tumors", with coincidental occurrence of HCC and CC, and "transitional tumors" in which intermediate differentiation with areas of transition between the two types of tumor are found. The latter type is rare, comprising less than 5% of primary liver cancer. The subtle architectural transitions between trabecular and pseudoglandular patterns without intervening stroma is quite distinct from the tubular adenocarcinoma surrounded by a desmoplastic reaction, a characteristic component of the "transitional tumor" subcategory of HCC-CC described by these authors. Moreover, reported survival for these combined tumors was typically measured in months (Goodman et al. 1985).

The neoplasms reported herein also bear a resemblance to those morphological variants described by Kondo and Nakajima (1987), whose study focused on a morphological evaluation of the types of pseudoglandular pattern seen in HC with very different histology. However, ultrastructural, immunocytochemical findings or clinical follow-up were not reported, thus making a comparison with our material difficult.

The long-term survival associated with this neoplasm renders its distinction from the traditional rapidly fatal HCC an important one in the selection of therapeutic options.

The first patient (case 1) underwent several cytoreductive surgical procedures without chemotherapy over a 10-year period, while the second patient received only chemotherapy. Both survived despite recurrence or continued presence of malignant disease. Although the role chemotherapy played in the second patient is open only to speculation, the possibility of controlling disease with repeated surgical procedures (thus avoiding the potentially life-threatening complications of long-term chemotherapy) may result in prolonged survival. These tumors thus appear to have both pathologic and epidemiologic differences from the usual HCC, which typically arise in cirrhotic livers and have a dismal prognosis. If additional examples of such neoplasms are found to have a similarly protracted clinical course, then it may be worthwhile to recognize them as a distinct clinicopathologic subset within the broad group of HCC.

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