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CASE REPORT

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Biliary cystadenocarcinoma with two types of tumour cells

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Abstract We report a rare case of biliary cystadenocarcinoma that occurred in the left hepatic lobe of a 62-year-old man and measured 20 cm in its greatest dimension. The neoplastic epithelium consisted of two types of cells: (1) cells with clear cytoplasm containing abundant mucin, and (2) cells with eosinophilic cytoplasm, which in some areas formed nodules with hepatocytoid features (polygonal cell shape, large nuclei with prominent nucleoli, and pseudoglandular structures). Histochemical stains revealed the presence of cytoplasmic mucin in the hepatocytoid areas, whereas immunohistochemical stains clearly showed a biliary phenotype (diffuse positive staining for "biliary type" cytokeratins, rare foci of positive staining with antibody to human hepatocytes (HEP-PAR1), absence of staining for α-fetoprotein, and no evidence of canalicular pattern of staining with polyclonal antibody to carcinoembryonic antigen). These findings indicate that areas reminiscent of hepatocellular carcinoma may occur in biliary cystadenocarcinomas. Histochemical and immunohistochemical stains are useful in reaching a definitive diagnosis in such cases.

Keywords Biliary cystadenocarcinoma · Carcinoma · Liver · Malignant tumour

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Introduction

Biliary cystadenocarcinoma (BCCA) is a rare hepatic tumour that may arise within its benign counterpart, biliary cystadenoma [3, 4, 13]. BCCA typically consists of a large, multilocular cyst lined by a stratified neoplastic epithelium, which in some areas forms tufts and papillary structures [1, 2, 3, 4, 13]. The epithelium may be columnar, cuboidal or squamous. The cyst is surrounded by a pseudocapsule of fibrous tissue that may be invaded by tumour cells. The majority of these tumours occur in women of middle age and do not cause symptoms until they reach a large size [1]. In a series of nine cases, Nakajima et al. [9] classified BCCA into two categories: (1) noninvasive tumours (limited to the cyst wall) with good prognosis following surgical resection; and (2) invasive tumours (extending to the hepatic parenchyma or neighbouring organs) with bad prognosis. We describe herein the morphologic and immunohistochemical features of a case that contained areas reminiscent of hepatocellular carcinoma.

Clinical history

This 62-year-old Romanian man presented to N.G. Lupu Hospital (Bucharest, Romania) with complaints of right upper abdominal pain, cough, diarrhoea and weight loss (6 kg in 6 months). Ultrasound and computed tomography (CT) scan revealed a large tumour in the left hepatic lobe that extended to the hilum (Fig. 1). He was transferred to Fundeni Hospital to be evaluated for surgery. At the time, laboratory findings were: haemoglobin 15.5 g/dl, haematocrit 44%, white cell count 6,400 per cubic millimeter (granulocytes 62%, lymphocytes 25%, monocytes 9%, eosinophils 4%), platelets 244,000 per cubic millimetre, glucose 94 mg/dl, urea 27 mg/dl, creatinine 0.6 mg/dl, cholesterol 220 mg/dl, aspartate aminotransferase 16.9 U/l (normal range 14–59 U/l), alanine aminotransferase 12.5 U/l (normal range 10–72 U/l), alkaline phosphatase 23.1 U/l (normal range 38–126 U/l),

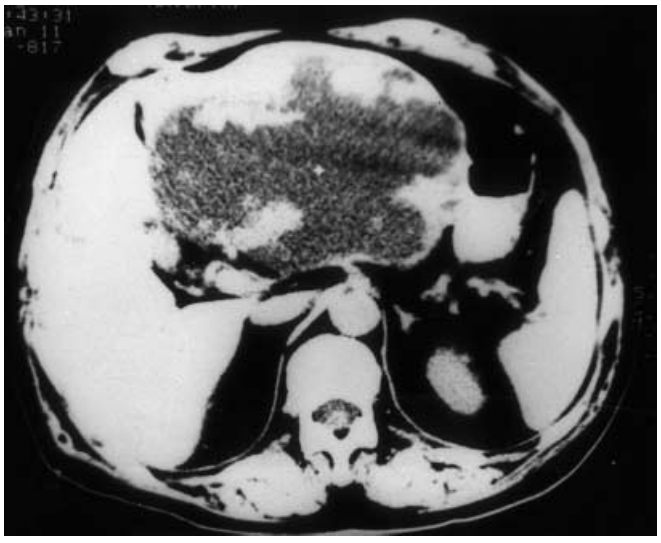


Fig. 1 Computed tomography (CT) scan of the abdomen showing a large tumour replacing the left hepatic lobe and extending to the hilum

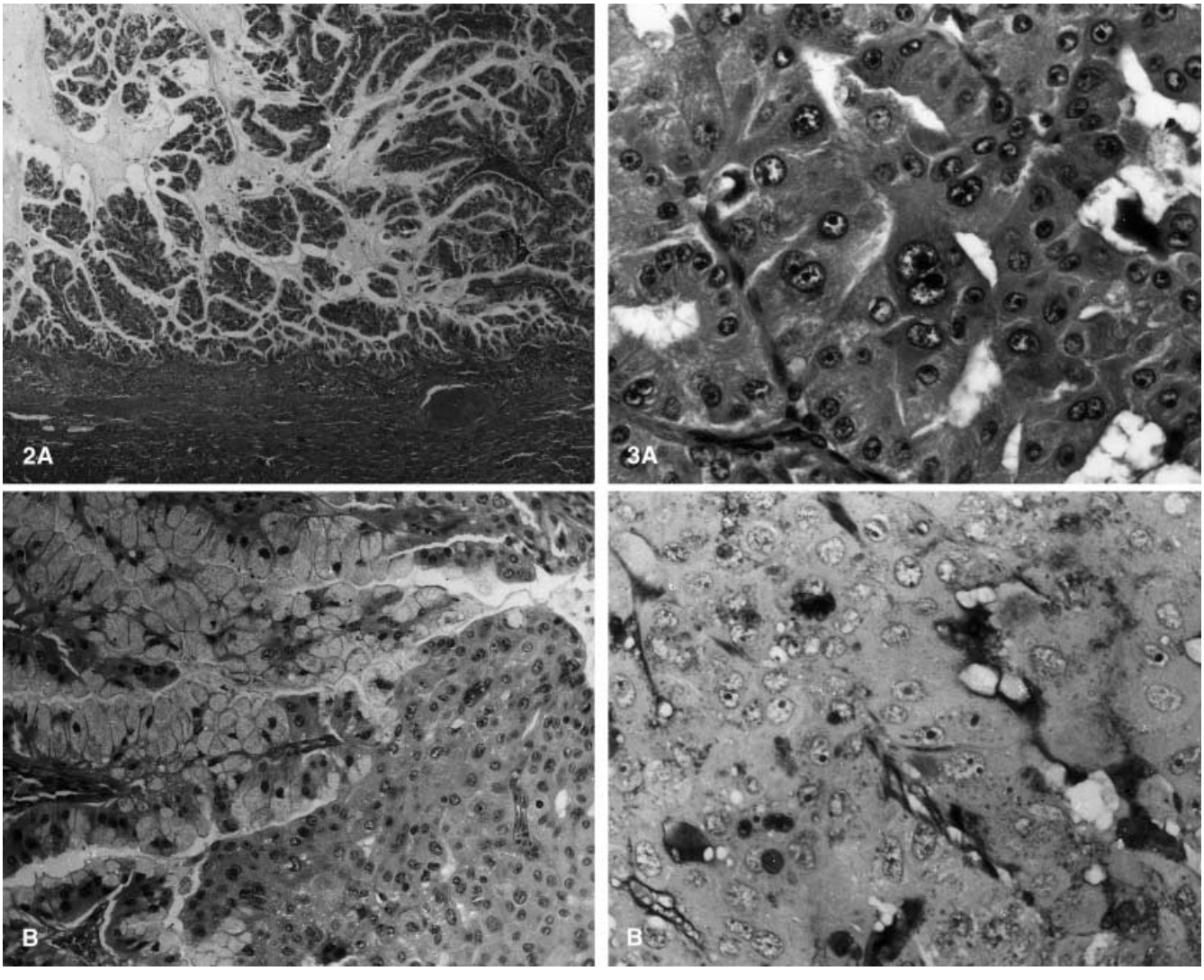
total bilirubin 1.1 mg/dl, total protein 7.0 g/dl, albumin 4 g/dl and globulins 3.0 g/dl. Left hepatic lobectomy and cholecystectomy were performed. The patient did well postoperatively and was discharged on the 40th postoperative day. He received 2 g 5-fluorouracil in four successive days (0.5 g per day). He is alive and well, 16 months following surgery.

Materials and methods

Representative tissue sections were fixed in 10% formalin and embedded in paraffin. Four-micron thick paraffin sections were

Fig. 2 **A** Low power view of the cyst wall showing papillary fronds lined by tumour cells. The fibrous pseudocapsule is seen in the lower part of the field (×40) **B** Two types of tumour cells are easily recognised on the basis of cytoplasmic staining (clear versus eosinophilic). Occasional cells with intermediate features are also seen. Cells with eosinophilic cytoplasm form a nodular aggregate (×200)

Fig. 3 **A** High power view of a type II cell nodule with features reminiscent of hepatocellular carcinoma (×400) **B** Periodic acid–Schiff (PAS)–diastase stain demonstrates the presence of mucin in tumour cells and pseudoglandular structures (×400)



stained with hematoxylin and eosin, periodic acid–Schiff (PAS), PAS–diastase, Alcian blue and phosphotungstic acid–hematoxylin (PTAH) stains. Additional paraffin sections were submitted for immunohistochemical stains, which were performed with a standard avidin–streptavidin peroxidase protocol. All antibodies utilised were monoclonal, with the exception of those to carcinoembryonic antigen (CEA) and α-fetoprotein, which were polyclonal. Antibodies to cytokeratin 7, cytokeratin 19, epithelial membrane antigen (EMA), CEA, α-fetoprotein and human hepatocytes (HEP-PAR1) were purchased from DAKO (Glostrup, Denmark); those to cytokeratin cam5.2 from Becton-Dickinson (San Jose, Calif.); and those to cytokeratin AE-1 from Zymed (San Francisco, Calif.).

Pathologic and immunohistochemical findings

The resected left hepatic lobe contained a well-demarcated, unilocular cystic tumour, which protruded at the surface of the liver and measured 20×18×15 cm. The cystic cavity contained mucinous fluid and showed a smooth gray–white lining. The wall showed nodular areas of thickening and measured 0.5 cm to 3.0 cm in thickness. The hepatic parenchyma surrounding the tumour appeared compressed, but otherwise unremarkable. No communication was identified between the tumour and the intra-hepatic or extra-hepatic bile ducts.

On histologic examination, the cystic cavity had a stratified lining of columnar cells that was surrounded by a pseudocapsule of fibrous tissue. The neoplastic epithelium showed considerable pleomorphism and formed tufts and papillary structures of variable size (Fig. 2a). Two types of tumour cells were identified (Fig. 2b):

1. Cells with clear cytoplasm and oval to elongated nuclei with inconspicuous nucleoli (“type I” cells). Large amounts of cytoplasmic mucin were evident on PAS–diastase and Alcian blue stains.
2. Cells with eosinophilic cytoplasm and round to oval nuclei that contained multiple small nucleoli or a single large nucleolus (“type II” cells). Scattered atypical mitotic figures were present.

A small number of tumour cells had features intermediate to those described above. These cells had oval nuclei and eosinophilic cytoplasms with apical clearing, due to the presence of cytoplasmic mucin.

Focally, the tumour cells formed nodules. These nodular areas were composed of type II cells in compact arrangement, which was interrupted by pseudoglandular structures and thin fibrovascular septa (Fig. 2b, Fig 3a). In these areas, the majority of type II cells had polygonal rather than columnar shape that, together with the nuclear features, resulted in a hepatocytoid appearance. However, PAS–diastase and Alcian blue stains demonstrated mucin within the cells and pseudoglandular structures (Fig. 3b). No definite glycogen granules were found on PAS stain. PTAH stain did not disclose any features suggestive of oncocytic change.

No evidence of pre-existing biliary cystadenoma was identified. On examination of multiple sections, no definite invasion of the pseudocapsule by neoplastic cells was seen. The hepatic parenchyma adjacent to the tu-

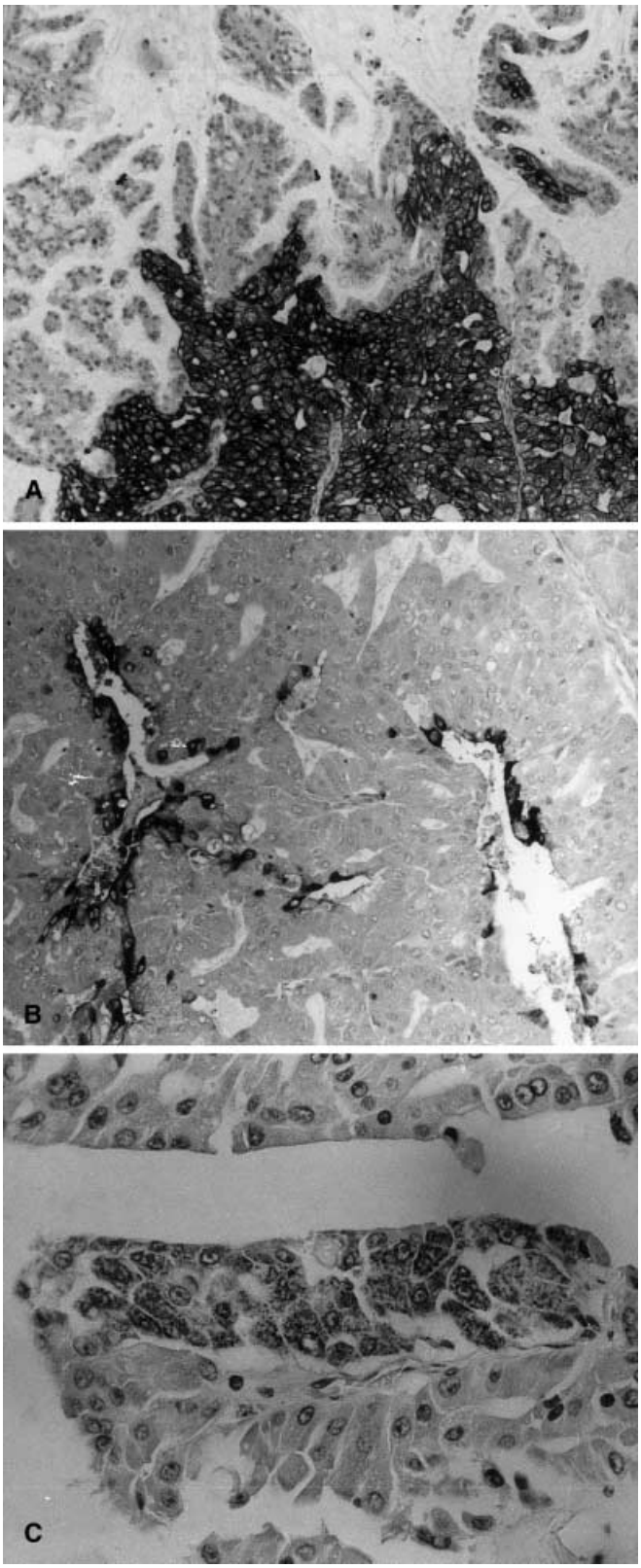


Fig. 4 Immunohistochemical findings **A** Type II cells are diffusely positive for cytokeratin AE-1. Type I cells are negative (×160) **B** Stains for epithelial membrane antigen (EMA) show focal positivity in type II cells around pseudoglandular structures (×160) **C** Small focus of antibody to human hepatocytes (HEP-PAR1) positivity in type II cells (×400)

Table 1 Summary of immunohistochemical findings ([f] focal, [d] diffuse, [g] around pseudoglandular structures, [c] canalicular)

Antibody	Tumour cells		Normal cells	
	Type I	Type II	Bile duct cells	Hepatocytes
Cytokeratin 7	Negative	Positive[f]	Positive[d]	Negative
Cytokeratin 19	Negative	Positive[d]	Positive[f]	Negative
Cytokeratin Cam5.2	Negative	Positive[d]	Positive[d]	Positive[d]
Cytokeratin AE-1	Negative	Positive[d]	Positive[d]	Negative
HEP-PAR1	Negative	Rare positive foci	Negative	Positive[d]
EMA	Positive[f]	Positive[f,g]	Positive[f]	Negative
CEA	Negative	Negative	Negative	Positive[c]
α-Fetoprotein	Negative	Negative	Negative	Negative

mour contained compressed sinusoids and mild to moderate inflammatory infiltrates in portal tracts. Mild steatosis was also present.

The immunohistochemical findings are summarised in Table 1 and illustrated in Fig. 4. Type I cells were focally positive for EMA and negative for all other markers. Tumour cells with intermediate features demonstrated, in addition, faint positivity for cytokeratins cam5.2 and AE-1. Type II cells were diffusely positive for cytokeratins cam5.2, AE-1 and 19, and focally positive for cytokeratin 7. Occasional type II cells around pseudoglandular structures were positive for EMA. Rare groups of type II cells were positive for HEP-PAR1. No staining for CEA or α-fetoprotein was seen.

Discussion

We report a rare case of BCCA containing two types of tumour cells: columnar mucin-producing (type I) cells, and columnar to polygonal (type II) cells with eosinophilic cytoplasm. A small subpopulation of tumour cells with intermediate features was also present. Mitotic activity was present in type II, but not type I cells. Although type II cells formed nodules with hepatocytoid features, they had immunophenotypic characteristics similar to those of normal biliary epithelium. Therefore, it is possible that type II cells represented precursors of type I cells.

We are aware of only one published case with similar microscopic features that was thought to represent BCCA with hepatocytic differentiation [11]. Like the present case, the tumour reported by Tomimatsu et al. [11] was unilocular and lacked evidence of a pre-existing cystadenoma. Apparently, it was entirely composed of cells similar to the type II cells reported herein. At the time, immunohistochemical stains were limited to α-fetoprotein and CEA. As in our case, the neoplastic cells were negative for both markers; however, it was not stated in the article whether the antibodies to CEA were monoclonal or polyclonal. Electron microscopy was used in that case and demonstrated bile canalicular structures between tumour cells. In our case, electron microscopy was not performed; nevertheless, extensive immunohistochemical stains provided evidence of biliary rather than hepatocytic differentiation of tumour cells.

The focal immunopositivity of type II cells for HEP-PAR1 should not be considered to represent definite evidence of hepatocytic differentiation. Although this antigen is predominantly expressed in benign and malignant hepatocytes, focal immunopositivity has also been observed in occasional adenocarcinomas of biliary, pancreatic and gastric origin [8, 12]. Such cases include, among others, five examples of cholangiocarcinoma (one of which was an intraductal papillary carcinoma) and two examples of mucinous cystadenocarcinoma of the pancreas.

BCCAs have histologic and immunohistochemical features that are practically identical to those of mucinous cystadenocarcinomas arising in the pancreas, ovary and retroperitoneum [15]. These malignant tumours and their benign counterparts (cystadenomas) occur more often in women and usually contain a cellular, "ovarian-like" stroma that may express estrogen and progesterone receptors [14, 15]. The stromal cells in the majority of the ovarian and pancreatic mucinous cystic tumours have also been found to express inhibin, a protein considered to represent a sensitive marker of ovarian origin [10, 15]. Evidently, the striking similarities among mucinous cystic tumours of biliary, pancreatic, ovarian and retroperitoneal origin suggest a common pathway of neoplastic development [15].

It should be noted, however, that a number of cases of BCCA, including the present one, do not contain ovarian-like stroma. In the series of Devaney et al. [3], ovarian-like stroma was present in 44 out of 52 cases of biliary cystadenoma (85%), and in only 6 out of 18 cases of BCCA (33%). In that series, which is the largest one thus far reported, the authors suggested that BCCAs lacking evidence of pre-existing cystadenoma with ovarian-like stroma, occurring in men, represent a subtype associated with poor prognosis [3].

We were tempted to call the tumour described herein a "hepatoid variant of BCCA." However, the term "hepatoid carcinoma" has been used for adenocarcinomas arising in various organs, such as the stomach, lung and ovary, that not only contain areas resembling hepatocellular carcinoma histologically, but also have evidence of hepatocytoid differentiation at a functional level [1, 5, 6, 7]. Such evidence includes production of α-fetoprotein and other substances elaborated by hepatocytes. In our case, the resemblance of type II cells to hepatocytes did not extend beyond the level of morphologic features.

Therefore, we conclude that BCCA may occasionally contain a subpopulation of tumour cells with hepatocytoid appearance, but biliary immunophenotype. Histochemical and immunohistochemical stains are useful in reaching a definitive diagnosis in such cases. This may be of practical importance, since the prognosis and treatment options for noninvasive BCCA differ from those of hepatocellular carcinoma.

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