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NOTE

Pathology

Combined hepatocellular-cholangiocarcinoma in a cow

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ABSTRACT. We examined a 10-year-old cow in which about half of the liver was displaced by malignant tissue consisting of hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC). Cytokeratin (CK) 18 and 7 were expressed in the latter. Metastasis was present in the hepatic, pancreaticoduodenal and mediastinal lymph nodes, where malignant cells had hepatocellular features, but more pleomorphic and atypical than in the primary lesion. Areas composed solely of CC cells or less-differentiated HCC cells were observed. In contrast, well-differentiated HCC cells were almost always admixed with the other two types, and may have had the ability to transform into CC cells and to dedifferentiate into less-differentiated cells. This report suggests that CK18 is an excellent marker for biliary differentiation in cattle.

KEY WORDS: cattle, combined hepatic carcinoma, cytokeratin 18, eosinophilic hyaline globule

In humans, combined hepatocellular-cholangiocarcinoma (HCC-CC), a rare but increasingly recognized primary malignant neoplasm of the liver, unequivocally shares features of both hepatocellular carcinoma (HCC) and cholangiocarcinoma (CC) [22]. Typical histology of HCC shows a trabecular or pseudoglandular growth pattern, bile in the canaliculi, and carcinoma cells resembling hepatocytes with fat, Mallory bodies or hyaline globules in the cytoplasm [1, 22]. In contrast, the classic histopathology of CC is characterized by desmoplastic stroma, glandular structures, mucin production and expression of cytokeratin (CK) 19 and CK7 [22]. Combined HCC-CC is postulated to result from collision of HCC and CC, dedifferentiation and redifferentiation of malignant hepatocytes or cholangiocytes, or malignant transformation (divergent differentiation) of hepatic progenitor cells [22]. There are very few reports of this type of carcinoma in domestic animals [9, 15] or birds [16, 21]. An abattoir survey revealed that tumors of the liver and biliary system made up 3.1% of all tumors collected from cattle [3]. Another survey indicates that hepatocellular and biliary neoplasms account for 10% of all neoplasms in cattle, 31% in sheep and 4% in pigs [2], but aged miniature pet pigs can be predisposed to HCCs [5]. The current study reports a case of combined HCC-CC in a cow, in which well-differentiated HCC cells could be distinguished from CC cells and less-differentiated HCC cells by immunostaining for CK18 or CK7.

Complete loss of appetite was noted in a 10-year-old Japanese Black cow. This remained unchanged despite administration of ampicillin. Five days later, the animal was depressed and somewhat emaciated, with abnormal liver function values. It was euthanized next day because of the poor prognosis. At necropsy, the subcutaneous tissue was yellowish in color. The liver and rumen were partially adherent to the diaphragm, and the former was greatly enlarged with a hard consistency. The left and quadrate lobes were largely replaced by white tissue, the external surface of which was irregular and nodular (Fig. 1). On cut sections, the tissue was partially nodular, and bile ducts were distended and filled with pus-like fluid. The right and caudate lobes had yellowish orange cut surfaces, with conspicuous interlobular connective tissues. The gall bladder was markedly enlarged, containing bile with fine, white to black stones. The hepatic and mediastinal lymph nodes were greatly swollen, and the texture of the former was hard.

Tissue samples from the liver, spleen, kidney, heart, lung, digestive organs, lymph nodes and brain were fixed in 10% buffered formalin, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (HE), Giemsa, trichrome and mucicarmine. Immunostaining was performed by the streptavidin-biotin complex/horseradish peroxidase (SAB) method on selected histological sections using a commercially available kit (Nichirei, Tokyo, Japan). The primary antibodies utilized were mouse monoclonal antibodies to CK7 (clone Ks7.18, 1:10, Acris Antibodies, Herford, Germany), CK18 (clone Ks18.04, prediluted,

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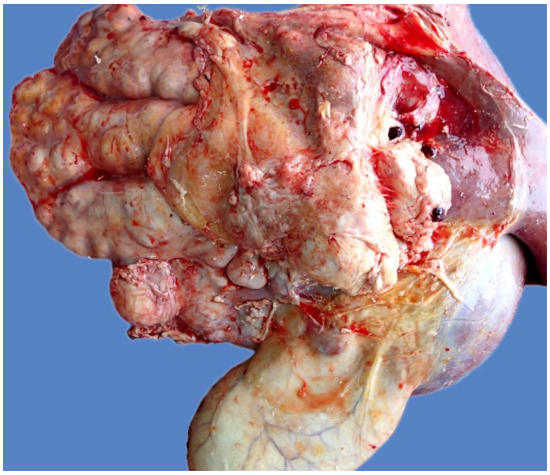

Fig. 1. The visceral aspect of the liver shows the left and quadrate lobes replaced by white tumor tissue, and the exceedingly enlarged gall bladder.

Progen Biotechnik, Heidelberg, Germany), hepatocyte specific antigen (Hep) (clone OCH1E5, 1:25, Dako A/S, Glostrup, Denmark), and a rabbit polyclonal antibody to CK19 (1:100, Abcam, Cambridge, UK). Antigen retrieval was by enzymatic digestion with 0.05% pepsin at 37°C for 25 min (CK18, CK19), or microwave heating in 10 mM citrate buffer, pH 6.0 at 90°C for 9 min (CK7, Hep). For histological comparison, tissues from a case of very well-differentiated HCC in a 2-year-old Japanese Black cow were treated in the same manner and examined histologically as a control.

Histology revealed that the left half of the liver was replaced by abundant very dense collagenous connective tissue with accumulation of numerous lipid-laden macrophages and smaller numbers of neutrophils (xanthogranuloma) in some parts. Neoplastic and residual hepatic tissues were also observed, with most of these cells being necrotic. However, areas of intact neoplastic cells were observed in some places, and these were comprised of HCC, CC, or both.

In HCC areas, neoplastic hepatocytes were arranged in trabeculae or solid nests with or without central necrosis (Fig. 2A and 2B), and stromal fibrosis was absent or mild. The cells were polygonal in shape, having round to oval nuclei with medium-sized nucleoli and moderately condensed chromatin. The cytoplasm was eosinophilic or pale eosinophilic and abundant, occasionally with lipid droplets or eosinophilic hyaline globules. Intercellular or intracytoplasmic bile canaliculus-like structures were visible (Fig. 2C), and focal lesions with central necrosis appeared to have pseudoglands (Fig. 2B). Mucin production was absent or very low in most canaliculus-like and pseudoglandular structures. Very large, atypical mononuclear or multinuclear HCC cells were rare in the HCC areas. Thirty one mitoses per 10 high-power fields (HPFs) were observed.

The CC component was characterized by tubules of neoplastic cholangiocytes with stromal fibrosis (Fig. 2D and 2E), and mucin was present on the apical surface or in the cytoplasm. The cells were cuboidal or columnar in shape with round to oval nuclei, and showed more condensed chromatin and less prominent nucleoli than neoplastic hepatocytes. The cytoplasm was dense and amphophilic. Although very rare, the presence of eosinophilic hyaline globules and bile canaliculus-like structures was confirmed. In CC and combined areas (Fig. 2F), mucin production was observed in CC cells facing the lumen but not in HCC cells (Fig. 2G). Mitotic figures were 84/10 HPFs in CC areas, and 138/10 HPFs in combined areas.

In metastatic lesions (hepatic, pancreaticoduodenal and mediastinal lymph nodes), widespread necrosis and fibrosis were observed. The neoplastic tissue consisted mainly of large to giant HCC cells, and atypical single or multiple nuclei were detected in some giant cells. The HCC cells frequently contained eosinophilic hyaline globules in the cytoplasm (Fig. 2H). Variously sized canaliculus-like lumina, not or only faintly stained for mucicarmine, were conspicuous. Intracytoplasmic or intraluminal pale greenish brown globules were present but rare. Mitotic figures were more frequent than in the liver (168/10 HPFs).

In the right half of the liver, interlobular fibrosis with hyperplastic pseudo-bile ductules, dilatation of central veins and centrilobular fatty degeneration of hepatocytes were observed. Eosinophilic hyaline globules were occasionally seen in hepatocytes, but were all very fine.

Immunohistochemical results are presented in Table 1. In the liver, well-differentiated HCC cells were largely negative for CK18 and CK7 (Fig. 2I), but staining was seen in a few tumor cells lining pseudolumina (Fig. 2J) or in large, atypical HCC cells. Nearly all CC cells stained positively for CK18 (Fig. 2K) and more weakly for CK7 (Fig. 2L). In metastatic lesions, HCC cells were positive for CK18 and weakly reactive to CK7. CK19 was well expressed in all of the tumor cell types (Fig. 2M). The staining pattern was usually diffuse cytoplasmic in tumor cells stained for CK7, CK18 and CK19, but tended to be submembranous in weakly stained cells. In non-neoplastic hepatic tissues, normal bile ducts and hyperplastic bile ductules were consistently positive for these markers. However, weaker labeling was seen in hepatocytes stained for CK18, and the staining pattern was submembranous. Immunoreactivity for Hep appeared with a coarsely granular pattern in the cytoplasm of hepatocytes, but was absent from the other cell types.

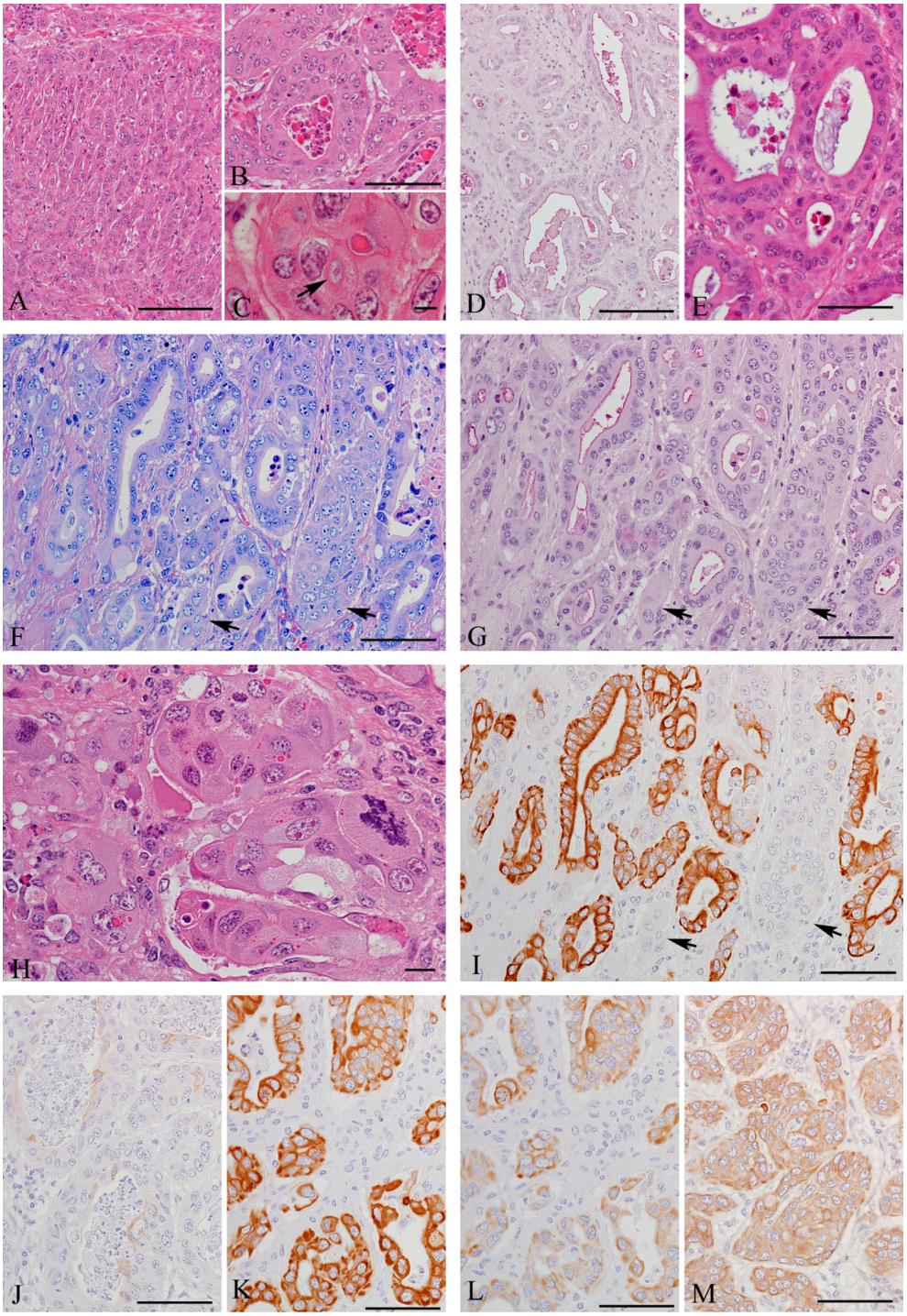

Fig. 2. Histology and immunohistochemistry. (A) Liver, hepatocellular carcinoma (HCC) area. Neoplastic hepatocytes are arranged in microtrabeculae. HE. Bar=100 μm. (B) Liver, HCC area, showing solid nests of HCC cells with centrally located necrotic cells. HE. Bar=50 μm. (C) Liver, HCC area. An arrow indicates a bile canaliculus-like structure formed between neoplastic hepatocytes. HE. Bar=5 μm. (D) Liver, cholangiocarcinoma (CC) area. All neoplastic tubules are accompanied by collagenous stroma, and show mucin production. Mucicarmine stain. Bar=100 μm. (E) Liver, CC area. This field consists of CC cells forming monolayered tubules, and no HCC element is visible. HE. Bar=50 μm. (F) Liver, combined area. The cytoplasm of CC cells are liable to be more strongly stained than that of HCC cells (arrows), although it is difficult to distinguish unequivocally between these two cell types. Giemsa. Bar=50 μm. (G) Liver, combined area. Mucin is visible in CC cells forming lumina, but not in HCC cells (arrows). Mucicarmine stain. Bar=50 μm. (H) Pancreaticoduodenal lymph node. Metastatic HCC cells are variable in size, and eosinophilic hyaline globules are visible in the cytoplasm. HE. Bar=10 μm. (I) Liver, combined area. Serial section adjacent to Fig. 2F and 2G shows that CC cells exhibiting tubule formation and mucin production are stained for CK18 but HCC cells are negative (arrows). Note the intimate admixture of CC and HCC cells. SAB. Bar=50 μm. (J) Liver. In this HCC area, cells facing pseudolumina exhibit weak immunoreactivity for CK18. SAB. Bar=50 μm. (K) Liver, This field is composed of only CC cells, expressing CK18. SAB. Bar=50 μm. (L) Liver. As in Fig. 2K, CC cells react positively but weakly for CK7. SAB. Bar=50 μm. (M) Liver, HCC area. Nearly all HCC cells are positive for CK19. SAB. Bar=50 μm.

Table 1. Immunohistochemical results

Cell type	CK18	CK7	CK19	Hep
CC cell	+++	+++ (w)	+++	–
Well-differentiated HCC cell	+ (w)	+ (w)	+++	–
Less-differenitated HCC cell	+++	+++ (w)	+++	–
Hyperplastic cholangiocyte	+++	+++ (w)	+++	–
Interlobular bile duct cell	+++	+++ (w)	+++	–
Hepatocyte	+++ (w)	+++ (w)	+++	+++
HCC cell of the control case	+ (w)	–	+++	+

CC: cholangiocarcinoma; HCC: hepatocellular carcinoma. +++: >90% of cells positive; ++: 90–10% of cells positive; +: <10% of cells positive; –: negative. w: weakly staining.

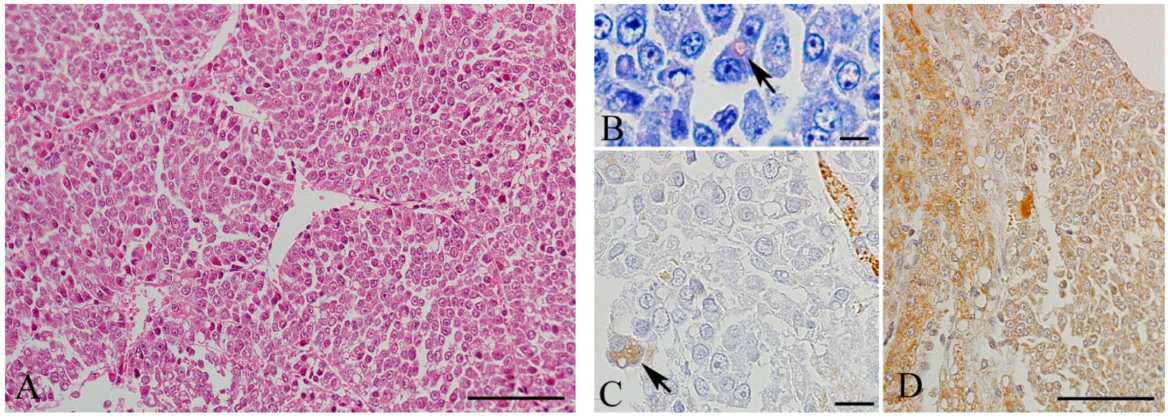

Fig. 3. Histology and immunohistochemistry of the control case. (A) Liver. Solid nests or lobules are surrounded by bare sinusoidal capillaries. HE. Bar=50 μm. (B) Liver. A bile canaliculus-like structure is visible between neoplastic cells (arrow). Giemsa. Bar=5 μm. (C) Liver. Finely granular staining for Hep is observed in a neoplastic hepatocyte (arrow), but strong coarsely granular staining in residual hepatocytes. SAB. Bar=10 μm. (D) Liver. CK19 positive staining is stronger in hepatocytes (left) than in neoplastic cells. SAB. Bar=50 μm.

In the control case of HCC, the neoplastic cells showed a sheet-like, insular or cord-like growth pattern, and tumor cell nests were separated by sinusoidal capillaries or delicate strands of vascular connective tissue (Fig. 3A). The cells were homogeneous in size and polygonal in shape, having eosinophilic cytoplasm frequently with lipid droplets. The presence of bile canaliculus-like lumina was confirmed (Fig. 3B), but mucin production was absent. Mitoses were relatively rare. Immunohistochemically, CK18 or Hep expression was exceedingly rarely found (Fig. 3C). Neoplastic cells showed CK19 positivity (Fig. 3D).

In the liver of the current case, well-differentiated HCC cells were characterized by eosinophilic cytoplasm, eosinophilic hyaline globules and bile canaliculus-like structures. On the other hand, CC cells showed tubular structures with mucin production [22]. On the basis of these histological and histochemical findings, a diagnosis of combined HCC-CC was made [22]. This diagnosis was definitively established by the immunohistochemical finding that HCC cells and CC cells revealed negativity and positivity for CK18 and CK7, respectively. In humans, CK19 and CK7, which are specifically expressed in CC and cholangiolocellular carcinoma (CLC) cells, are helpful markers for distinguishing between CC or CLC (a subtype of CC) and HCC [8, 10], although HCC with these biliary markers has also been reported [18]. In the present study, CK18 proved to be most useful for identifying these cells in the liver, because CK7 was more weakly expressed in CC cells and CK19 was expressed in both CC and HCC cells. In addition, nearly all tumor cells were negative for CK18 in the control case of HCC. Expression of CK18 is observed in bovine CLC, a disease taking an intermediate form between HCC and CC and which is closely related to peripheral hepatocytes and proliferating pseudocholangioles (unpublished data). The paucity or absence of CK18 and CK7 expression in bovine HCC cells is thought to be a reflection of weak or rare expression in normal hepatocytes, and also to be associated with loss of immunophenotypic markers caused by neoplastic transformation. Remarkable phenotypic discrepancies are observed between rat and mouse oval progenitor cells [7], and there are species differences in the histopathology of human and animal tumors [6, 12, 14]. It should be noted that not only histological but also immunohistochemical features in human tumors do not always correspond to those in bovine counterpart neoplasms.

Unlike in the differentiated HCC component and the control case of HCC in the present study, neoplastic cells have more differentiated morphology and are accompanied by more developed sinusoids in canine well-differentiated HCCs [19]. Such differences may explain the negativity of Hep in bovine HCC cells, because this marker is expressed in a large proportion of canine HCCs but not in more aggressive forms [4]. Taking into account the fact that CK19 is a marker for hepatic progenitor cells and immature hepatocytes in dogs [4, 20], the presence of CK19 in bovine hepatocytes suggests that multipotency and high proliferation potency may be preserved in these cells, and that HCCs derived from such cells are liable to be more atypical and

aggressive than in dogs.

In human medicine, CK19 is a cholangiocyte marker, but CK19-positive HCC cases have been reported, and tend to be cytologically less differentiated and to metastasize more readily to lymph nodes than CK19-negative cases [18]. Similarly, the expression of the hepatic progenitor cell marker CK19 in canine HCC is linked with a poor prognosis, and this type of HCC is characterized by marked cellular and nuclear pleomorphism [4, 19, 20]. Analogously, in the current case, CK18 served as a marker for CC, but was also expressed in the less-differentiated HCC component. CK18 is considered to be an excellent marker for distinguishing between well-differentiated HCC and other types of hepatic carcinoma (CC, CLC and less-differentiated HCC) in cattle.

In areas composed almost exclusively of well-differentiated HCC cells, a few tumor cells with weak immunolabeling for CK18 and CK7 were interpreted as showing incomplete differentiation toward CC cells. Considering this and the complete absence of the HCC element (CK18-negative cells) in areas of CC, well-differentiated HCC cells preserving multipotency similar to normal hepatocytes [11, 13] may be capable of showing metaplasia (transdifferentiation) to CC cells, unable to redifferentiate toward the hepatic lineage. In contrast, less-differentiated HCC cells seemed to be anaplastic, unable to metamorphose to CC cells, given the absence of CC cells in the metastatic lesions [11, 13].

In humans, xanthogranulomatous cholecystitis is characterized by marked infiltration of lipid-laden histiocytes with severe fibrosis, and is etiologically related to gallstones [17]. Similar histological findings were observed in the hepatic parenchyma of the present case, and may be associated with biliary tract obstruction caused by the hepatic tumor. Severe fibrosis in the liver may have facilitated metaplasia of HCC cells to CC cells, because an increase of collagen can induce metaplasia of hepatocytes to bile duct epithelium in the rat [13]. As indicated in the control case, bovine HCCs are usually not accompanied by fibrosis or cirrhosis.

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