Combined Hepatocellular and Cholangiocellular Carcinoma in a Dog

Atsushi SHIGA¹*, Kinji SHIROTA²**, and Makoto ENOMOTO³)

¹Department of Veterinary Pathology and ²Research Institute of Biosciences, Azabu University, 1–17–71 Fuchinobe, Sagamihara, Kanagawa 229–8501 and ³Biosafety Research Center, Foods, Drugs and Pesticides, 582–2 Shioshinden, Fukude-cho, Iwata-gun, Shizuoka 437–1213, Japan

(Received 11 September 2000/Accepted 5 January 2001)

NOTE Pathology

**CORRESPONDENCE TO: Dr. SHIROTA, K., Research Institute of Biosciences, School of Veterinary Medicine, Azabu University, 1–17–71 Fuchinobe, Sagamihara, Kanagawa 229–8501, Japan.

A transitional type of combined hepatocellular and cholangiocellular carcinoma developed in a 12-year-old male Yorkshire terrier dog. The tumor was histologically composed of both hepatocellular carcinoma and cholangiocellular carcinoma components, and both elements were closely intermingled. Intraluminal mucin accumulation in cytokeratin-positive tubular/glandular structures was observed within the cholangiocellular carcinoma components and this feature was useful histological marker for a differential diagnosis of hepatocellular carcinoma.

**NOTE: A transitional type of combined hepatocellular and cholangiocellular carcinoma developed in a 12-year-old male Yorkshire terrier dog. The tumor was histologically composed of both hepatocellular carcinoma and cholangiocellular carcinoma components, and both elements were closely intermingled. Intraluminal mucin accumulation in cytokeratin-positive tubular/glandular structures was observed within the cholangiocellular carcinoma components and this feature was useful histological marker for a differential diagnosis of hepatocellular carcinoma.

Combined hepatocellular and cholangiocellular carcinoma (combined HCCC) is a primary hepatic tumor with a hepatocellular carcinoma (HCC) and a cholangiocellular carcinoma (CCC) within the same neoplastic tissue and is quite rare in humans [1, 4]. Allen and Lisa [1] classified combined HCCCs into three histological types: (1) double cancer showing separate neoplastic masses, each composed of either HCC or CCC; (2) combined type with contiguous masses with different characters that may mingle as they grow; and (3) mixed type showing an individual mass displaying both features. On the other hand, Goodman et al. [4] proposed a new classification: (1) type I (collision tumor), apparently representing a coincidental occurrence of both HCC and CCC in the same patient; (2) type II (transitional tumor) having areas of intermediate differentiation and an identifiable transition between HCC and CCC, and (3) type III (fibrolamellar variant of HCC, but also containing mucin-producing pseudoglands.

The entity of the combined HCCC has been established in the field of human pathology with several detailed histopathological and immunohistochemical descriptions [4, 5, 7, 10]. As in humans, combined HCCCs are extremely rare tumors in dogs. Cases of a double cancer type and a mixed type of canine combined HCCC have been reported [11, 12]. However, the details of their characteristic histopathological features have been not fully investigated. We describe here histological, immunohistochemical and ultrastructural features of a possible case of canine combined HCCC.

A 12-year-old male Yorkshire terrier dog with chief signs of depression and distended abdomen was hospitalized in the Veterinary Teaching Hospital of Azabu University. Laparotomy was performed to resect the abdominal mass that had been detected by ultrasonography. No distant metastasis was noted by X-ray examination.

At surgery, a large nodular mass (60 × 40 × 50 mm in size) was found in the left hepatic lobe. In addition, many white nodules varying in size (from 5 × 5 mm to 30 × 20 mm) were widespread in other lobes of the liver, with one involving the gallbladder. Complete resection of all the nodules was impossible because of their extensive distribution. The central part of the resected left lobe was occupied by a white mass containing a large cavity and many small cysts filled with a turbid brownish mucus substance. The mass was well demarcated from the surrounding liver tissue, but without encapsulation. No detailed clinical data were obtained after surgery.

The resected liver tissues were fixed in 10% phosphate-buffered formalin, processed routinely, and embedded in paraffin. Paraffin sections 4 µm thick were cut and stained with hematoxylin and eosin (HE), alcian blue, periodic acid-Schiff (PAS) with diastase digestion, and Watanabe’s silver. Immunohistochemical examination using paraffin sections was carried out by the streptavidin-peroxidase method with a HISTOFINE kit (Nichirei, Tokyo, Japan). Primary antibodies used were as follows: anti-human cytokeratin antibody (Böehringer Mannheim Biochemicals, Mannheim, Germany, which recognizes bile duct type cytokeratin), anti-human carcinoembryonic antigen (CEA) antibody (Dako, Glostrup, Denmark), anti-human α-smooth muscle actin (α-SMA) antibody (Bioscience Inc., Bethlehem, PA, U.S.A.), anti-human desmin antibody (Dako), and anti-porcine vimentin antibody (Dako). All the primary antibodies were mouse monoclonals. Secondary reaction using biotinylated anti-mouse IgG supplied with the kit and further application of peroxidase-labeled streptavidin-biotin were conducted. Immunoreactivity was visualized...
with 3,3’-diaminobenzidine in 0.1 M Tris HCl buffer (pH 7.6) plus hydrogen peroxide. The sections were counterstained with Mayer’s hematoxylin.

For electron microscopy, small blocks (1 mm³) of the largest mass were fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in Epoxy resin. Ultra-thin sections were cut and double-stained with uranyl acetate and lead nitrate.

Histologically, the tumor mass and nodules were composed of moderately differentiated HCC and CCC, and both components were often intermingled (Fig. 1a). The tumor showed tubular or pseudoglandular structures in the area facing a large cavity or cysts. The HCC component showed a thick trabecular growth pattern and occasionally underwent squamous metaplasia or single-cell keratinization. Most tumor cells showed nuclear and cellular atypism and anisonucleosis. Three or four mitotic figures were seen per high-power microscopic field. The CCC components had tubular/glandular structures showing moderately differentiated cellular and structural atypism. Intraluminal mucin accumulation in the tubular structures was demonstrated by alcian blue staining (Fig. 1b). PAS reaction revealed glycogen storage in the CCC as well as HCC cells. Tumor cells infiltrated into the surrounding liver tissue by the sinusoidal or replacing mode of invasion. Reticulin fibers surrounded the trabecular or tubular tumor cell clusters and were not seen around individual tumor cells. No cirrhotic or fibrotic changes were found in the surrounding non-neoplastic liver tissue.

The results of immunohistochemical examination are summarized in Table 1. Cytokeratin-positive cells with a diffuse cytoplasmic staining pattern were characteristically observed in all CCC components (Fig. 2a). Cytokeratin-positive cells showing a focal cytoplasmic staining pattern were scattered in the HCC components (Fig. 2a). Positive reaction for vimentin was observed diffusely in a portion of CCC cells and stromal cells, and this was also seen rarely in the HCC areas. CEA were present diffusely in the tubular structures (Fig. 2b) and focally in the large bile duct-like structures (Fig. 2c) of CCC components. In the surrounding
liver tissue, CEA were positive at the apical surface of bile duct cells. Interestingly, expression of desmin was rarely detected in some HCC cells showing a trabecular growth pattern (Fig. 2d). Moreover, α-SMA-positive tumor cells were widely distributed in both the HCC and CCC components (Fig. 2e).

Electron microscopic examination revealed that tumor cells possessed a few microvilli on their apical cell surface and were accompanied with basement membrane-like materials at their circumference (Fig. 3). Tumor cells contained several types of filaments including tonofilaments and microfilaments, and abundant organelles such as mitochondria of varying size, rough endoplasmic reticulum, and ribosomes. Tumor cells containing microfilaments with electron-dense patches were rarely observed (Fig. 3). Fat droplets and glycogen granules were frequently observed in many of the tumor cells. Intercellular junctional complexes were not definitely demonstrated.

In the present case, the tumor was composed of both HCC and CCC components and they were intermingled, indicating that this case seemed to correspond to the transitional type of combined HCCC according to the classification of Goodman et al. [4].

Positive reaction for CEA with a diffuse cytoplasmic staining pattern was reported in the CCC components in human combined HCCC [4]. In the normal human liver, CEA was positive at the luminal surface of bile duct cells [4]. In the present canine case, tubular CCC cells were positive for both bile duct type-cytokeratin and CEA, as in humans.

| Table 1. Immunohistochemical findings in tumor and normal liver tissues |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
|                                 | Tumor | Normal liver |                |                |
|                                 | HCC   | CCC   | Hepatocytes | bile duct epithelium |
| Cytokeratin                     | +     | ++    | –             | ++             |
| CEA                             | –     | +/++  | –             | –              |
| α-SMA                           | +     | +     | –             | –              |
| vimentin                        | +     | +/++  | –             | –              |
| desmin                          | +     | +     | –             | –              |

Staining pattern: a) cytoplasmic, and b) apical surface.

HCC: Hepatocellular carcinoma component, and CCC: cholangiocellular carcinoma component.

+++ Wide distribution of positive cells, +: focal distribution of positive cells, and – negative.

Fig. 3. Electron micrograph of HCC cells. A tumor cell filled with cytoplasmic filaments possessing focal electron-dense patches (arrows). Basement membrane was observed in the surrounding area (arrowheads), Bar= 4 µm.
Hepatocytes during the developmental stages are known to express both desmin and α-SMA antigen [6]. In the present case, some of the tumor cells were positive for both desmin and α-SMA and had microfilaments with focal densities. These filaments were considered to be myofilaments because they showed an orderly parallel arrangement and focal densities along their coarse. In addition, the tumor cells in the present case also showed abundant tonofilaments. Tonofilaments are known to characterize epithelial features of tumor cells and to be present in tumor cells of HCC, CCC [9] and hepatoblastoma [8]. Vimentin and bile duct-type cytokeratin expressions were scattered in HCC components. These findings in primary hepatic tumors may be interpreted in the context of their immaturity [2]. Such phenotypic variations of tumor cells in the present case may represent the stem cell nature of the neoplastic cells.

Combined HCCC must be distinguished from the pseudoglandular type of HCC. Tumor cells of canine HCC are negative for bile duct type-cytokeratin [3] and no mucin production was demonstrated within pseudoglands in the pseudoglandular type of HCC [4]. The demonstration of intraluminal mucus accumulation and the presence of bile duct type-cytokeratin in the present canine case was useful for a differential diagnosis between pseudoglandular type of HCC and combined HCCC. This tumor is an extremely rare primary hepatic tumor in dogs, and studying it may provide significant information regarding the features of canine hepatic stem cells.

REFERENCES