NOTE *Pathology*

Hepatocellular Carcinoma in a Hokkaido Brown Bear (*Ursus arctos yesoensis*)

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ABSTRACT. Hepatocellular carcinoma with metastases to lymph nodes and adrenal glands was found in a 26-year-old male Hokkaido brown bear (*Ursus arctos yesoensis*). Left hepatic lobe was largely replaced by well-differentiated neoplastic cells, whereas poorly differentiated tumor cells had proliferated in part of the left hepatic lobe, in scattered nodules in the remainig liver tissue, and at the metastatic sites. Immunoreactivity for hepatocyte antigen (hepatocyte paraffin 1 antibody) and alpha-fetoprotein was observed in both well- and poorly differentiated neoplastic cells in the liver and metastatic foci. To our knowledge, this case is the first report of hepatocellular carcinoma in brown bears.

KEY WORDS: brown bear, hepatocellular carcinoma, immunohistochemistry.

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Hokkaido brown bear (*Ursus arctos yesoensis*) is a subspecies of the brown bear, found in Hokkaido, Japan. Brown bears (*Ursus arctos*) belong to the genus *Ursus*, which includes American black, Asiatic black, polar, sun, and sloth bears [14]. Most hepatic neoplasms in *Ursus* are biliary carcinomas and only 2 cases of hepatocellular tumors have been reported in polar bears [5, 9, 11]. The purpose of this paper is to report the first case of hepatocellular carcinoma in a Hokkaido brown bear.

A 26-year-old male Hokkaido brown bear (*Ursus arctos yesoensis*) had decreased activity and appetite, and became dehydrated and astatic. Fluid therapy improved the symptoms but the bear remained astatic. Blood test revealed anemia and an increased number of leukocytes (Table 1). Serum biochemistry abnormalities included increases in urea nitrogen, γ-glutamyltransferase, and a decrease in cholesterol (Table 1). Two weeks after the clinical onset, the bear died. The bear had spent his whole life in 1 facility: body weight at 15 years old was 328 kg and had gradually decreased to 142 kg at death.

Grossly, the left hepatic lobe was largely replaced by a multinodular firm mass, 26 × 25 × 20 cm in size, and solid white nodules, up to 3.5 cm in diameter, were scattered throughout the remaining hepatic lobes (Fig. 1). The surface of the mass in the left lobe adhered to the greater omentum (Fig. 1), and cut surfaces revealed multinodular yellowish tissue compartmentalized with the fibrous septum, which frequently underwent degeneration and hemorrhage (Fig. 2). Surrounding hepatic parenchyma was partially discolored. Two white nodules, 2.5 cm and 0.7 cm in diameter, were found in the right adrenal gland and similar nodules, up to 0.5 cm in diameter, were in the pancreas and pancreatico-duodenal lymph node.

Multiple ecchymoses were found in the gastric mucosa. In the heart, the septal cusps of both atrioventricular valves were thickend. A large number of ascarids were present from the esophagus to colon, and from the right nasal cavity to bronchi. Skin sores had developed on the right cheek, shoulder, elbow, lateral side of the chest and knee. Focal discoloration with fibrosis was detected in the right gastroc-

Table 1. Clinical pathology data of the affected Hokkaido brown bear

Parameter	Result	Reference interval[a)]
Erythrocytes (× $10^6/\mu l$)	4.03	4.17–8.93
Hematocrit (%)	25	36.8–62.7
Hemoglobin (g/d*l*)	8.7	11.7–59.4
Leukocytes (× $10^3/\mu l$)	22.4	2.9–15.3
Platelets (× $10^6/\mu l$)	557	134–719
Total protein (g/d*l*)	8.5	5.7–8.8
Albumin (g/d*l*)	3.3	2.7–5.4
Calcium (mg/d*l*)	7.9	6.6–11.5
Phosphorus (mg/d*l*)	6.0	3.2–9.1
Sodium (mEq/*l*)	146	123–150
Potassium (mEq/*l*)	3.8	3.6–6.1
Chloride (mEq/*l*)	105	94–112
Creatinine (mg/d*l*)	0.7	0.5–3.9
Urea nitrogen (mg/d*l*)	70	4–43
Cholesterol (mg/d*l*)	98	172–1030
Total bilirubin (mg/d*l*)	0.3	0.0–1.4
Direct bilirubin (mg/d*l*)	0.1	0.0–0.1
ALT (IU/*l*)	36	10–101
AST (IU/*l*)	167	25–203
ALP (IU/*l*)	95	4–210
GGT (IU/*l*)	579	16–176[b)]

a) Reference intervals are cited from data from brown bears [Ref. 14] except for GGT.
b) Reference interval of GGT is cited from data from polar bears [Ref. 1].
ALT, alanine aminotransferase, AST, aspartate aminotransferase, ALP, alkaline phosphatase, GGT, γ-glutamyltransferase.

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nemius muscle.

Histologically, the mass in the left hepatic lobe was composed of the proliferation of hepatocellular neoplastic cells arranged in trabeculae of various thicknesses (Fig. 3). Necrosis was present within the broad trabeculae. Fibrin exudated in the sinusoid-like spaces between the trabeculae (Fig. 3). The neoplastic cells had a round to ovoid nucleus and relatively broad, eosinophilic cytoplasm, which was often vacuolated (Fig. 3 inset). Neoplastic growth was largely compartmentalized with fibrous stroma. The partial proliferation of neoplastic cells with more atypical morphology was present. The cells had a more pleomorphic nucleus

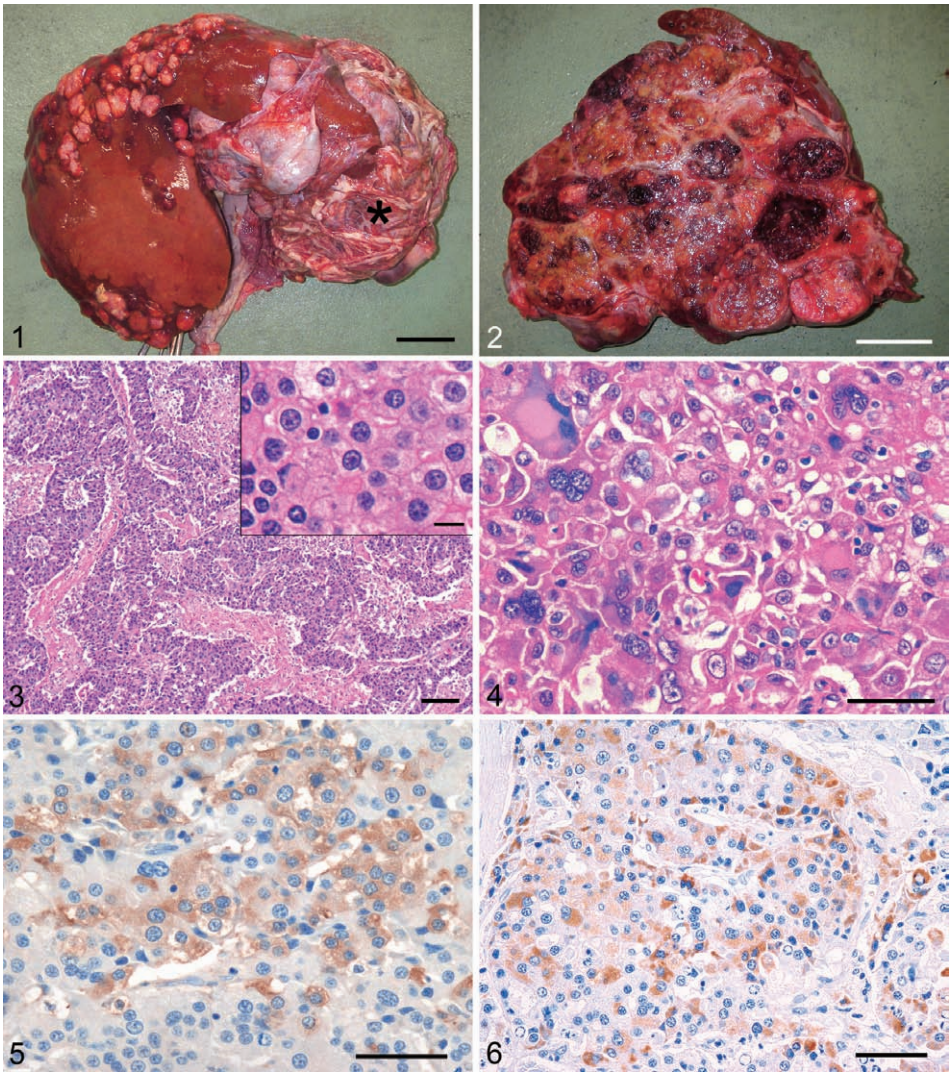


Fig. 1. Left hepatic lobe adhered to the greater omentum (asterisk) and scattered nodules in the remaining lobes. Bar=5 cm.

Fig. 2. Cut surface of the left hepatic lobe. The hepatic parenchyma was replaced by yellowish neoplastic tissue with degeneration and hemorrhage, compartmentalized by fibrous septum. Bar=5 cm.

Fig. 3. Neoplastic cells in the left hepatic lobe, proliferating in haphazardly arranged trabeculae of various thicknesses. Dilated sinus-like spaces contain exudated fibrin. Hematoxylin and eosin (HE). Bar=100 μm. (Inset) Higher magnification. Neoplastic cells with round nucleus and relatively broad, eosinophilic cytoplasm, resembling hepatocytes. HE. Bar=10 μm.

Fig. 4. Neoplastic cells in the hepatic lymph node, with severe nuclear atypia and anisocytosis. Many multinucleated cells are present. HE. Bar=50 μm.

Fig. 5. Immunoreactivity for AFP in well-differentiated neoplastic cells in the left hepatic lobe. Immunohistochemistry for AFP counterstained with hematoxylin. Bar=50 μm.

Fig. 6. Immunolabeling with hepatocyte paraffin 1 antibody in well-differentiated neoplastic cells in the left hepatic lobe. Immunohistochemistry with hepatocyte paraffin 1 antibody counterstained with hematoxylin. Bar=50 μm.

and eosinophilic, polyhedral cytoplasm with anisocytosis. Multinucleated neoplastic cells were frequently found. The cells were arranged in sheets of various sizes, most of which had central necrosis. Vascular invasion of the neoplastic cells was observed more frequently in these regions. The proliferation of similar poorly differentiated neoplastic cells was also found in scattered nodules in the residual hepatic lobes, hepatic, pancreatico-duodenal, and cranial mesenteric lymph nodes, and both adrenal glands (Fig. 4). Histopathological findings in other organs included multifocal esophageal ulcer and gastric erosion, suppurative rhinitis in the nasal conchae, focal scar in the left ventricle, focal infarcts with fibrosis in the kidneys, hemosiderin deposition in the intraabdominal lymph nodes and renal tubules, and degeneration and necrosis in the right gastrocnemius and semimembranous muscles. White nodules in the pancreas were nodular hyperplasia. Thickened cardiac vulves were comprised of myxomatous change, accompanied by superficial ulcertive endocarditis.

Immunohistochemical examination was performed by the avidin-biotin-peroxidase complex method (Vectastain Elite ABC Kit; Vector Laboratories, Burlingame, CA, U.S.A.) using a monoclonal antibody against human hepatocyte (hepatocyte paraffin 1, clone OCH1E5; Dako, Glostrup, Denmark) and a polyclonal antibody against human alpha-fetoprotein (AFP; Nichirei Biosciences, Tokyo, Japan). Positive reaction for AFP and hepatocyte paraffin 1 was scattered in both well- and poorly differentiated neoplastic cells in the liver and metastatic sites (Figs. 5, 6). Non-neoplastic hepatocytes were negative for AFP. Diffuse staining with hepatocyte paraffin 1 antibody was observed in non-neoplastic hepatocytes but not in biliary epithelium or stromal cells.

Based on macroscopic, histological and immunohistochemical examinations, the tumor was diagnosed as hepatocellular carcinoma with metastases to lymph nodes and adrenal glands. Referring to the histological classification of hepatic tumors in WHO International Histological Classification of Tumors of Domestic Animals [6], well- and poorly differentiated neoplastic growth likely corresponded to trabecular and solid type, respectively. Tumors in brown bears reported in the English literature are limited, but include osteosarcoma, lymphosarcoma, mammary carcinoma and cardiac schwannoma [2, 8, 10, 12, 16]. To our knowledge, the present case is the first report of hepatic neoplasm in brown bears.

AFP is an oncofetal protein and has been considered to be specific but insensitive for human and canine hepatocellular carcinomas [3, 4, 7]. Hepatocyte paraffin 1 is a monoclonal antibody raised against formalin-fixed human liver tissue, and sensitively reacts with neoplastic and non-neoplastic hepatocytes in formalin-fixed and paraffin-embedded sections in humans and dogs [4, 13, 15]. The staining property was diffuse, granular and cytoplasmic, and detected in all to scattered cells in the individual tumor growth [4, 13, 15], which was consistent with the result in the present case. In the reports on hepatic tumors in Ursus bears, no immunohistochemical examinations were performed [5, 9, 11]. Based on the result in the present case, immunohistochemistry for human AFP and hepatocyte paraffin 1 might be applicable to the diagnosis of hepatic malignancies in bears as well as humans and dogs.

The cause of hepatocellular carcinoma in this brown bear was unknown. In a report on polar bears, the authors summarized the possible causes of hepatic neoplasm in bears: diets contaminated by aflatoxin, continual feeding of high protein diets, viral infections, clonorchid fluke infection, and industrial chemical exposure [9]. Clinicopathologically, anemia in this bear might have been associated with neoplastic hemorrhage. The increase in serum γ-glutamyltransferase and hypocholesterolemia was considered to be due to severe hepatic damage. Increased urea nitrogen probably reflected clinical dehydration. The gradual weight loss might not have solely been the result of hepatic neoplasm in this case; however, we should consider neoplastic diseases, including hepatocellular carcinoma, in the differential diagnosis of aged brown bears with wasting conditions.

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