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NOTE

Pathology

Hepatic neuroendocrine carcinoma with metastases to the lymph nodes in a sika deer (*Cervus nippon yakushimae*)

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ABSTRACT. A 26-year and 6-month-old male sika deer that was kept at the Showa Park, Tokyo, Japan, collapsed and died of severe disease wasting and severe tabefaction. Grossly, numerous masses, 0.3–1.0 cm diameter, were dispersed throughout the liver. The multiple masses were composed of tumor cells, which had hypochromatic nuclei and abundant faintly eosinophilic cytoplasm, arranged in nests of various sizes. Immunohistochemically, tumor cells were positive for cytokeratin, chromogranin A, synaptophysin and gastrin. Ultrastructurally, the cytoplasm of the tumor cells contained abundant membrane-bound electron-dense granules. A metastatic lesion was observed in the renal, hepatic and pancreatic lymph nodes. On the basis of these findings, this tumor was diagnosed as a neuroendocrine carcinoma with metastases to the lymph nodes.

KEY WORDS: hepatic neuroendocrine carcinoma, metastasis, sika deer

Hepatic neuroendocrine carcinomas (hepatic carcinoid) are extremely rare tumors in animals, but cases have been reported in several animals such as dogs [13], cats [1, 7, 9], cow [8], goat [17] and horse [3]. Hepatic neuroendocrine carcinoma can form small or large masses of multiple nodules scattered throughout the liver [5] and then metastasizes to the various organs, including the lungs and lymph nodes [1, 7, 8, 17]. Neuroendocrine markers such as chromogranin A, synaptophysin and neuron-specific enolase (NSE) are useful for the diagnosis of neuroendocrine carcinoma as well as the characteristic morphological features of the presence of argyrophilic granules positively stained with Grimelius stain and of membrane-bound neuroendocrine granules ultrastructurally [10].

Primary hepatic tumors, including hepatomas, hepatocellular carcinomas, cholangiomas, carcinoids, undifferentiated carcinomas and hemangiosarcomas have been reported in roe deer [4, 6, 11, 12, 15], white-tailed deer [14] and red deer hind [18]. Thus far, only one other case of a neuroendocrine tumor arising from the liver has been reported in a red deer hind, which was classified as a carcinoid tumor without metastasis [18]. However, immunohistochemistry was not conducted [18]. Thus, as far as we know, hepatic neuroendocrine carcinoma with metastases to the lymph nodes has not yet been reported in deer. In this report, we describe the histological, immunohistochemical and ultrastructural features of hepatic neuroendocrine carcinoma in a sika deer (*Cervus nippon yakushimae*).

A 26-year and 6-month-old male sika deer that was kept at the Showa Park, Tokyo, Japan, collapsed and died of severe wasting and tabefaction. The sika deer fed on root crops such as carrots and sweet potatoes, soybean meal and hay cubes. No detailed clinical examinations were performed. The sika deer was subjected to necropsy within 10 hr of death. The body weight of the dear at necropsy was 23.1 kg. Tissues collected during necropsy were fixed in 10% neutral buffered formalin, processed routinely

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Table 1. Primary antibodies used in the present case

Antibody	Clone	Dilution	Source	Antigen retrieval	Positive control tissue
Cytokeratin	AE1/AE3	1:200	Dako, Glostrup, Denmark	121°C for 15 min in citrate buffer, pH 6.0	Liver
Vimentin	V9	1:100	Dako	121°C for 15 min in citrate buffer, pH 6.0	Liver
Neuron-specific enolase	BBS/NC/VI-H14	1:200	Dako	121°C for 15 min in citrate buffer, pH 6.0	Pancreas
Synaptophysin	SRA-E5	1:100	TransGenic, Kumamoto, Japan	121°C for 15 min in citrate buffer, pH 6.0	Pancreas
Hepatocyte	OCH1E5	1:25	Dako	121°C for 20 min in citrate buffer, pH 6.0	Liver
Chromogranin A	Polyclonal	1:1,000	Dako	None	Pancreas
Insulin	Polyclonal	1:400	Biomed, Foster City, CA, USA	None	Pancreas
Gastrin	Polyclonal	Prediluted	Dako	None	Duodenum

and embedded in paraffin wax. Sections were stained with hematoxylin and eosin and Grimelius stains. Serial sections were then subjected to immunohistochemical analysis using primary antibodies listed in Table 1. After reaction with the primary antibodies, the sections were incubated with biotinylated goat anti-mouse IgG or anti-rabbit IgG antibodies (Dako Japan, Tokyo, Japan), followed by peroxidase-conjugated streptavidin. Finally, the sections were visualized following the addition of diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. The antibodies were validated by a positive reaction in tissue obtained from the pancreas, liver and duodenum in the present case and by a negative reaction due to replacement of the antibodies with a normal mouse or rabbit IgG. For electron microscopic examination, small pieces of the masses from the liver that were previously fixed in formalin were refixed in 1% osmium tetroxide in 0.2 M phosphate buffer and then embedded in epoxy resin. Ultrathin sections were examined using an electron microscope JEM-1011 (JEOL, Tokyo, Japan) after staining with uranyl acetate and lead citrate.

Grossly, numerous masses, measuring 0.3–1.0 cm in diameter, were randomly dispersed throughout the liver. Both the external and cut surfaces of the masses were homogeneously pale orange–red in color (Fig. 1). Microscopically, the multiple masses in the liver were composed of tumor cells arranged in nests of various sizes separated by delicate fibrovascular stroma (Fig. 2). The tumor cells also invaded the surrounding parenchyma. Tumor cells had small to intermediate, round hypochromatic nuclei and abundant faintly eosinophilic cytoplasm (Fig. 3). The frequency of mitosis in the tumor cells was 0–3 per high-power field (400×). The tumor cells were positive for argyrophilic granules with Grimelius stain (Fig. 3, inset). Tumor venous emboli were also observed in the liver. Metastatic lesions were observed in the renal, hepatic and pancreatic lymph nodes (Fig. 4). Immunohistochemically, tumor cells were mostly positive for cytokeratin (Fig. 5) and partially positive for chromogranin A (Fig. 6), synaptophysin (Supplementary Fig. 1), NSE (Supplementary Fig. 2) and gastrin (Fig. 7), but they were negative for vimentin and hepatocyte, also known as Hepatocyte Paraffin 1 (HepPar-1). The immunohistological features of the metastatic tumor in the lymph nodes were similar to that of multiple hepatic tumors (data not shown). Ultrastructurally, the cytoplasm of nearly all tumor cells examined contained moderate-to-abundant membrane-bound electron-dense granules (Fig. 8). Some spontaneous lesions, such as sarcocystosis in the tongue, heart and femoral muscle, aspiration pneumonia, testicular degeneration, suppurative keratitis and cortical cataract were noted. No tumor lesions were observed in other organs including the pancreas, gallbladder and digestive tract.

On the basis of the morphological, immunohistochemical and ultrastructural findings, this tumor was diagnosed as a neuroendocrine carcinoma with metastases to the lymph nodes. This tumor should be distinguished from hepatoblastoma and metastatic neuroendocrine carcinoma of the pancreas, gallbladder and digestive tract. Tumor cells of hepatoblastomas have argyrophilic granules and are positive for cytokeratin, HepPer-1, α-fetoprotein, NSE and synaptophysin but negative for chromogranin A [2, 3, 16]. In contrast, neuroendocrine tumors are negative for hepatocyte and α-fetoprotein [5]. By the detailed postmortem pathological examination, it was possible to rule out the presence of any tumor in the pancreas, gallbladder and digestive tract by the detailed pathological examination. Therefore, these differential diagnoses were excluded in the present case.

A number of neuroendocrine carcinomas arising from the liver are identified as hormone-nonsecreting tumors. However, it has been reported that in rare cases, some tumors can produce hormones such as gastrin, glucagon, insulin and somatostatin, indicating the presence of a functional neoplasm [1, 9, 13]. In cats, paraneoplastic signs of two types have been reported in hepatic neuroendocrine carcinomas: 1) necrolytic migratory erythema associated with a glucagon-producing type and 2) increased gastric acid secretion by gastrin production following gastrointestinal ulceration [1, 9]. A previous study in dogs reported the involvement of insulin and serotonin immunoreactivity [13], but the significance of insulin and serotonin expression remains unclear. In the present case, the neuroendocrine carcinoma could be considered as a nonfunctional neoplasm because there were no clinical signs, such as vomiting and bloody feces, or pathological findings, such as gastrointestinal hemorrhage and ulceration; however, some tumor cells were immunohistochemically positive for gastrin.

The cause of death in the present case may be related to aging as no histopathological lesions were found that could have been considered the cause of death. To the best of our knowledge, this is the first report of a hepatic neuroendocrine carcinoma with metastases to the lymph nodes in a sika deer.

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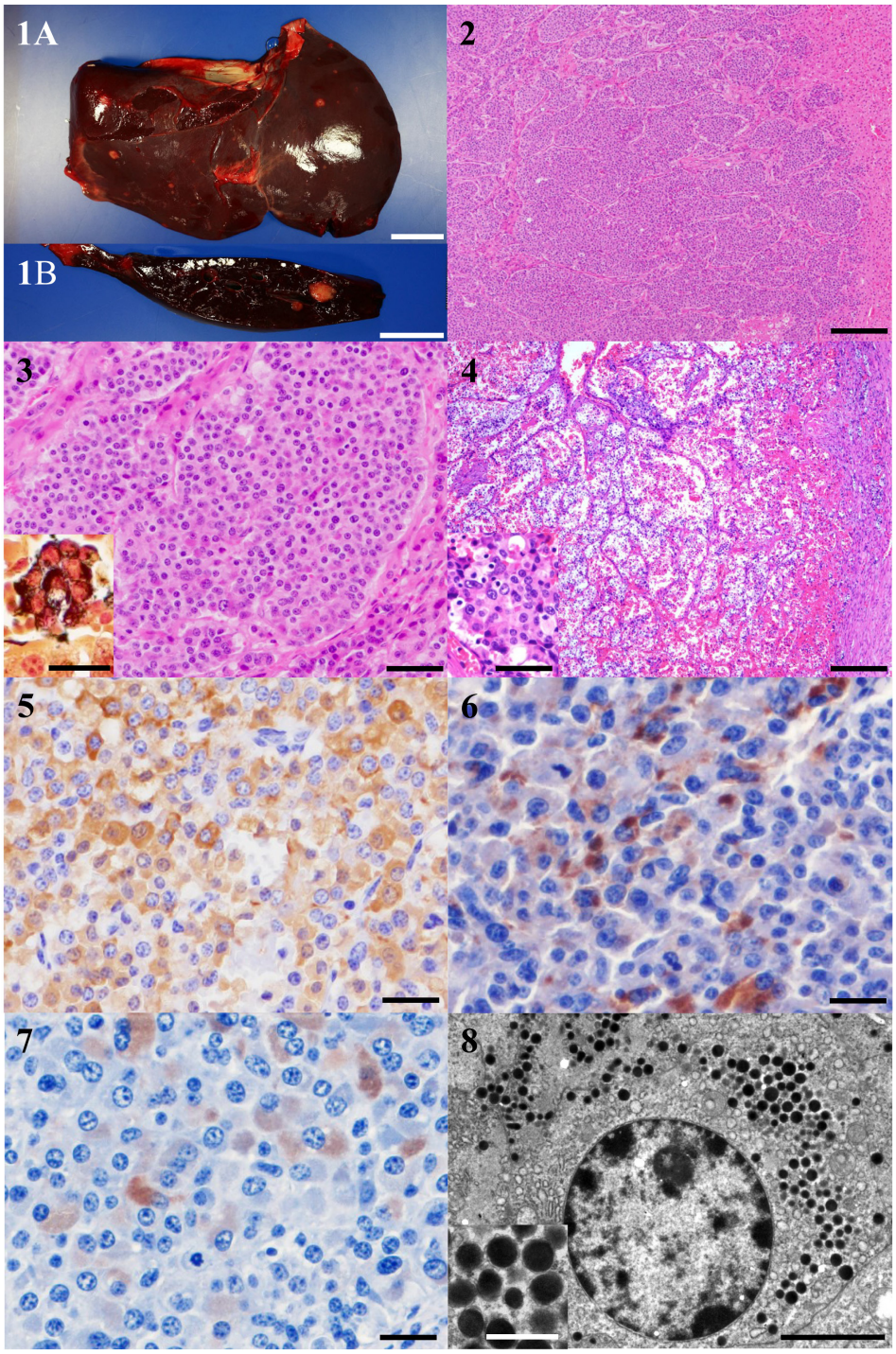

Fig. 1. The hepatic neuroendocrine carcinoma in a sika deer. (A) The masses, measuring 0.3–1.0 cm in diameter were randomly dispersed throughout the liver. The external surfaces were smooth and homogeneously pale orange–red in color (A and B). Bar=3.0 cm (A) and 2.0 cm (B).

Fig. 2. The mass is composed of tumor cells arranged in nests of various sizes separated by a delicate fibrovascular stroma. HE. Bar=200 μm.

Fig. 3. Tumor cells have small to intermediate, round hypochromatic nuclei and abundant faintly eosinophilic cytoplasm. HE. Bar=50 μm. Inset: Grimelius stain. Bar=20 μm.

Fig. 4. Metastases of tumor cells are observed in the pancreatic lymph nodes which contain numerous erythrocytes into the lymphatic sinus. HE. Bar=200 μm. Inset: HE. Bar=50 μm.

Fig. 5. Tumor cells from the liver were positive for cytokeratin. Immunohistochemistry. Bar=25 μm.

Fig. 6. A small number of tumor cells from the liver were positive for chromogranin A. Immunohistochemistry. Bar=25 μm.

Fig. 7. A small number of tumor cells from the liver were positive for gastrin. Immunohistochemistry. Bar=25 μm.

Fig. 8. Tumor cells contain abundant electron-dense granules. Bar=500 nm. Inset: membrane-bound granules. Bar=100 nm.

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