Hepatoblastoma in a Dog

Atsushi SHIGA¹⁾, Kinji SHIROTA^{1,4)}, Takuo SHIDA²⁾, Takatsugu YAMADA^{3,4)}, and Yasuo NOMURA¹⁾

¹⁾Departments of Veterinary Pathology, ²⁾Veterinary Radiology, ³⁾Veterinary Internal Medicine, and ⁴⁾Research Institute of Biosciences, School of Veterinary Medicine, Azabu University, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 229, Japan

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ABSTRACT. A hepatoblastoma was found in a 13-year-old female Maltese dog. Histologically, the tumor showed a wide trabecular pattern and was frequently accompanied with vascular lake formation. Tumor cells were positive for cytokeratin and neuron specific enolase, but negative for chromogranin. Electronmicroscopically, tumor cells were accompanied with continuous basement membrane and had poorly developed desmosomes. Sinusoidal endothelia had fenestration and were surrounded by myofibroblast-like cells. To the best of our knowledge, this paper is the first report of morphological studies on canine hepatoblastoma. — **KEY WORDS:** canine, hepatoblastoma.

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Hepatoblastoma (HB) is the most common hepatic tumor of human childhood [22], but is extremely rare in domestic animals. Reported cases of HB in domestic animals are those in a horse [16], a bull [17], and dogs [24]. In laboratory animals, there are detailed descriptions on the HBs of mice [2, 9, 10]. However, a little pathological information on canine HBs has been available [24]. In this report, we describe histopathological, immunohistochemical, and electron microscopical findings on a HB in a dog.

A 13-year-old female Maltese dog without detectable clinical abnormalities was admitted to a local veterinary clinic for a physical examination and was pointed out to have hepatomegaly and cardiac murmur. Careful examinations at the Veterinary Teaching Hospital of Azabu University revealed a high blood ALP activity (659 IU/L), and serum α -fetoprotein (AFP) level measured by ELISA methods [26] ranged from 400 to 500 ng/ml during the next 2 years after the first admission.

A laparotomy was performed to clarify the nature of liver disorder of the dog. All except for the lateral left lobe had many white nodules measuring 4 to 50 mm in diameter. Larger nodules were depressed at the central portions. The cut surface of the nodules were homogeneous and white with some areas of hemorrhage of varying degree. The nodules were well demarcated from the surrounding liver tissue. No macroscopic abnormalities were found in the abdominal organs or cavity except for enlargement and hardening of the colonic and mesenteric lymph nodes. The lateral right lobe of the liver was excised for histopathological examinations.

For two years after the open biopsy, abnormal clinical signs such as enlargement of the liver and lymph nodes were not detected by X-ray examination, and serum ALT and ALP levels at the end of the second year were 30 and 117 (IU/L), respectively. However, the dog gradually showed emaciation and accumulation of ascites and died of respiratory disturbance due to heart failure. Necropsy was not performed. No metastasis was noted by X-ray examination.

The liver specimen was fixed in 10% phosphate-buffered formalin, processed routinely, and embedded in paraffin. Paraffin sections were cut at 3 μ m in thickness and stained with hematoxylin and eosin (HE), Grimerius method,

periodic acid-Schiff (PAS) reaction with or without diastase digestion, Orcein stain, and Watanabe's silver impregnation. Mitoses of tumor cells were counted and the data were compared with those of a canine hepatocellular carcinoma with distant metastasis.

Immunohistochemical examination was performed using labelled streptavidin-biotin method with a Histofine kit (Nichirei Corp., Tokyo, Japan). Primary antibodies used were as follows: anti-human cytokeratin antibody (Boehringer Mannheim Biochemica, Mannheim, Germany), anti-human neuron specific enolase (NSE) antibody (Dakopatts, Glostrup, Denmark), anti-bovine chromogranin antibody (Incstar Corp., Stillwater, MN, U.S.A.), anti- α -smooth muscle actin (α -SMA) antibody (Sigma Chemical Co., St. Louis, MO, U.S.A.), and anti-human factor VIII-related antigen (FVIII-RAG) antibody (Dakopatts, Glostrup, Denmark). Immunoreaction with primary antibodies was performed for 1 hr at 37°C. Thereafter, biotinylated anti-rabbit or mouse IgG antibody and peroxidase-labelled streptavidin were sequentially applied to the sections for 15 min each at room temperature. The reaction was visualized with 3,3'-diaminobenzidine in 0.1M Tris HCl buffer (pH 7.6) plus hydrogen peroxide. Sections were counterstained with Mayer's hematoxylin.

For electron microscopy, small blocks (1 mm³) of the tumor were fixed in 2.5% glutaraldehyde/0.1 M phosphate buffer for 2 hr at 4°C and post-fixed in 1% osmium tetroxide/0.2 M phosphate buffer for 2 hr at 4°C. The blocks were dehydrated in graded ethanol and embedded in Epoxy resin. Ultra-thin sections were cut and double stained with uranyl acetate and lead citrate and examined by a Hitachi H-300 transmission electron microscope at 75 kV.

Histologically, the nodules were consisted of compact proliferation of tumor cells in a wide trabecular pattern (Fig. 1). Many vascular lakes were present in the tumor tissues (Fig. 1). Necrosis and degeneration were rare. However, in the central portions of tumor nests, neoplastic cells fell into degeneration (Fig. 2a) and these portions occasionally connected to the vessels in the stroma (Fig. 2b). The tumor cells had a polarity: their nuclei were located at the opposite site of basal side (Fig. 2a). The nuclei of the tumor cells were round with relatively rich chromatin and did not show atypia or pleomorphism. The nucleus/cytoplasm ratio was

Fig. 1. The tumor tissue showing a wide trabecular pattern with many vascular lakes (arrows). HE stain, $\times 230$.

Fig. 2. a) High magnification of the tumor cell cord. Degeneration of tumor cells in the central area (arrows). b) Connection between degenerative area of tumor nest and stromal vessel (arrows). HE stain, $\times 920$

Fig. 3. a) Intrahepatic metastasis in the non-neoplastic liver tissue. b) Tumor tissue (arrows) within vessels (asterisk). HE stain, $\times 460$.

high. Mitoses of tumor cells were occasionally observed (mean number: 1.06/100 high power fields). The cytoplasm of tumor cells was weakly-eosinophilic, but contained no

Fig. 4. a) Reticulin fibers surrounding the tumor tissue. Watanabe's silver impregnation. $\times 230$. b) Elastic fibers in the stroma (arrowheads). Orcein stain, $\times 230$.

Fig. 5. Immunostaining with various antibodies. Counterstained with Mayer's hematoxylin, $\times 460$. a) Cytokeratin-positive tumor cells. b) NSE-positive tumor cells. c) α -SMA-positive stromal cells surrounding the tumor cell cord.

glycogen determined by PAS reaction. The argyrophilic granules were not demonstrated in the cytoplasm of tumor cells by Grimelius methods. Intrahepatic metastasis (Fig. 3a) and vascular invasion (Fig. 3b) of the tumor cells were occasionally observed. Tumor nests were separated from each other by sinusoids and abundant reticulin fibers (Fig. 4a). A small amount of elastic fibers (Fig. 4b) were observed in the stroma of tumor tissues by Orcein stain.

Immunohistochemically, some tumor cells were positive

Fig. 6. Tumor cells with numerous vacuoles of varying size, Golgi's complex (arrows), and mitochondria. Electron micrograph. Uranyl acetate and lead citrate stain, Bar = 4 μ m.

Fig. 7. Tumor cell (T) with continuous basement membrane (arrowheads). Sinusoidal endothelial cells with continuous basement membrane (arrows). Electron micrograph, Uranyl acetate and lead citrate stain, Bar = 1 μ m.

for cytokeratin (Fig. 5a) and NSE (Fig. 5b), but negative for chromogranin. Although no fibrous capsule was found in the periphery of the tumor in HE sections, α -SMA-positive cells were abundant at the margin and also in the stroma of the tumor (Fig. 5c). Sinusoidal endothelial cells (SECs) were positive for FVIII-RAg.

Electron microscopic examination revealed that the cytoplasm of the tumor cells was filled with many vacuoles having limit membranes (Fig. 6) and also contained a few mitochondria, Golgi's complexes, rough endoplasmic reticula (rER) and ribosomes. However, no neuroendocrine granules or glycogen particles were observed. Intercellular junctional complexes were rarely observed. Distinct and continuous basement membranes were formed around the tumor nest (Fig. 7). SECs had fenestrae and were accompanied with continuous basement membrane (Fig. 7). Myofibroblast-like cells surrounding discontinuous basement membrane were observed in the perisinusoidal areas.

As for primary hepatic tumors in dogs, hepatocellular

carcinoma [11, 23], cholangiocellular carcinoma [12] and hepatic carcinoid [13] have been reported in addition to HB [24]. We diagnosed the present case as HB based on morphological and immunohistochemical findings in comparison to those of other primary hepatic tumors (Table 1). Histologic features of the present case were similar to those of hepatic carcinoid. However, this case was differentiated from hepatic carcinoid because of absence of neuroendocrine granules. Vascular lake formation is thought to be a characteristic feature of human HB [6]. As for dogs, however, it seemed to be found in hepatocellular carcinoma as well as HB [20]. The connection between degenerative areas of central portion of tumor nest and neighboring stromal vessels may explain the process of vascular lake formation in this tumor.

As for ultrastructural findings, the presence of intercellular junctional complexes and absence of glycogen in tumor cells of the present case were similar to those in embryonal areas of human HB [15]. SECs in the tumor tissues of the present case preserved fenestration which was different from the cases of canine hepatocellular carcinoma [20]. The scarcity of degeneration or necrosis in this HB

Table 1. Comparison of immunohistochemical and ultrastructural findings between primary hepatic tumors

	Immunohistochemistry			Ultrastructure	
	cytokeratin (clone AE1/AE3)	NSE	glycogen	BM	NEG
Hepatocellular carcinoma	—	—	+	—	—
Cholangiocellular carcinoma	+	—	—	+	—
Carcinoid	—/+ ^{a)}	+	—	+	+
Hepatoblastoma	+	+	+/- ^{a)}	+	—
Present case	+	+	—	+	—

a) Depending on the histologic type. Abbreviation: BM: basement membrane, NEG: neuroendocrine granule, NSE: Neuron specific enolase. Literature cited: 1, 6, 13, 14, 18, 21, 25, 27.

compared with canine hepatocellular carcinoma may be explained by the presence of fenestrated SECs.

The present case did not show any remarkable clinical signs except for hepatomegaly. However, most of hepatic lobes had many neoplastic nodules varying in size at laparotomy and intrahepatic metastasis of tumor cells was histologically evident. As for human HB, it is known that a high mitotic activities of tumor cells are generally associated with poor prognosis [4, 19] and that necrosis or vascular invasion of tumor cells do not always influence prognosis [4]. Mitoses of tumor cells of one case of canine hepatocellular carcinoma in our laboratory were 1.34 (per 100 high power fields). Mitotic activities of tumor cells in the present case were considered to be high and this tumor was suspected to be malignant.

Serum AFP is the most reliable diagnostic marker for human HB [5, 8, 22]. AFP value of the present case was higher than that of normal adult dogs. However, its significance in differential diagnosis of canine HB is not clear because a higher AFP level is detected in a case of canine hepatocellular carcinoma and also in inflammatory diseases of the liver in dogs (unpublished data by coauthor T. Yamada).

Recently, the histogenesis of HB in humans [1, 18] and mice [2] have been investigated. Human HB is classified into several histologic types [22]. Small epithelial cell components in human HB had similarities to the putative human and rodent hepatic stem cells [18] while embryonal and fetal types of tumor cells showed a positive reaction for AFP [1]. As for the histogenesis of human HB, thus, small cell-embryonal-fetal cell line was suspected to be the most frequent course of differentiation [1, 18]. On the other hand, cells from murine HB lacked immunohistochemically demonstrable AFP [2, 9], and its histogenesis have not yet been established [9, 10]. Frith *et al.* [3] speculated that murine HB are probably either of a liver stem cell or ductule origin. Moreover, murine HB were almost invariably found within or adjacent to hepatocellular tumors [2, 3, 9]. Rosetts formation or ribbon arrangement of tumor cells were not always observed in murine HB [9]. Predominant cells of murine HB closely resembles the most primitive, small cell variant of human HB [2]. Therefore, there may be differences in histologic features depending on animal species or the degree of tumor differentiation. The histogenesis of canine HB were unknown because of scarcity of informations on hepatic stem cells of HB in a dog.

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REFERENCES

1. Abenoza, P., Manivel, J. C., Wick, M. R., Hagen, K., and Dehner, L. P. 1987. *Hum. Pathol.* 18: 1025–1035.
2. Diwan, B.A., Ward, J. M., and Rice, J. M. 1989. *Carcinogenesis* 10: 1345–1348.
3. Frith, C. H., Ward, J. M., and Turusov, V. S. 1994. pp. 223–270. *In: Pathology of Tumours in Laboratory Animals* (Turusov, V. and Mohr, U. eds.), IARC Scientific Publications, Lyon.
4. Haas, J. E., Muczynski, K. A., Krailo, M., Ablin, A., Land, V., Vietti, T. J., and Hammond, G. D. 1989. *Cancer* 64: 1082–1095.
5. Hirai, H., Nishi, S., Watabe, H., and Tsukada, Y. 1973. pp. 19–34. *In: Gann Monogr. Cancer Res.* 14. Japan Scientific Press, Tokyo.
6. Horie, A., Kotoo, Y., and Hayashi, I. 1979. *Cancer* 44: 2184–2193.
7. Hurlimann, J. and Gardiol, D. 1991. *Am. J. Surg. Pathol.* 15: 280–288.
8. Lack, E. E., Neave, C., and Vawter, G. F. 1982. *Am. J. Surg. Pathol.* 6: 693–705.
9. Nonoyama, T., Fullerton, F., and Reznik, G. 1988. *Vet. Pathol.* 25: 286–296.
10. Nonoyama, T., Reznik, G., Bucci, T. J., and Fullerton, F. 1986. *Vet. Pathol.* 23: 619–622.
11. Patnaik, A. K., Hurvitz, A. I., Lieberman, P. H., and Johnson, G. F. 1981. *Vet. Pathol.* 18: 427–438.
12. Patnaik, A. K., Hurvitz, A. I., Lieberman, P. H., and Johnson, G. F. 1981. *Vet. Pathol.* 18: 439–444.
13. Patnaik, A. K., Hurvitz, A. I., Lieberman, P. H., and Johnson, G. F. 1981. *Vet. Pathol.* 18: 445–453.
14. Patnaik, A. K. 1992. *Vet. Pathol.* 29: 405–415.
15. Philips, M. J., Poucell, S., Patterson, J., and Valencia, P. 1987. pp. 447–518. *In: The Liver - An Atlas and Text of Ultrastructural Pathology*, Raven Press, New York.
16. Prater, P. J., Patton, C. S., and Held, J. P. 1898. *JAVMA* 194: 383–385.
17. Rao, P. R., Sriraman, P. K., Naidu, N. R. G., Rao, D. S., and Rao, I. S. 1980. *Indian J. Anim. Sci.* 50: 762–764.
18. Ruck, P., Xiao, J-C., and Kaiserling, E. 1996. *Am. J. Pathol.* 148: 321–329.
19. Schmidt, D., Harms, D., and Lang, W. 1985. *Virchows Arch. A (Pathol. Anat.)* 407: 387–405.
20. Shiga, A., Shiota, K., and Nomura, Y. 1996. *J. Vet. Med. Sci.* 58: 909–914.
21. Shiotos, N., Virta, S., and Kessimian, N. 1991. *Am. J. Clin. Pathol.* 95: 172–175.
22. Stocker, J. T. and Ishak, K. G. 1987. pp. 127–136. *In: The Neoplasms of the Liver* (Okuda, K. and Ishak, K. G. eds.), Springer-Verlag, New York.
23. Trigo, F. J., Thompson, H., Breeze, R. G., and Nash, A. S. 1982. *J. Comp. Pathol.* 92: 21–39.
24. Usuda, H. and Tanoue, S. 1994. *J. Jpn. Vet. Med. Assoc.* 47: 43–46. (abstract in English).
25. Van Eyken, P., Sciot, R., Paterson, A., Callea, F., Kew, M. C., and Desmet, V. J. 1988. *Hum. Pathol.* 19: 562–568.
26. Yamada, T., Kakinori, M., Totsuka, K., Ashida, Y., Nishizono, K., Tsuchiya, R., and Kobayashi, K. 1995. *Vet. Immunol. Immunopathol.* 47: 25–33.
27. Yasoshima, H., Uematsu, K., Sakurai, K., Ueno, Y., Hori, K., Kanazawa, N., Tanaka, T., Yamanaka, N., and Okamoto, E. 1993. *Acta Pathol. Jpn.* 43: 783–789.