Malignant Aortic Body Tumor in a Holstein Cow

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ABSTRACT. A malignant aortic body tumor was observed in a 5-year-old female Holstein cow. The neoplastic mass, of 22 × 17 × 15 cm in size, was located at the base of the left atrium, having irregular lobular structures. The tumor cells had slightly eosinophilic cytoplasm, and a round or oval nucleus. Metastasis was only present in the premediastinal lymph node. The tumor cells exhibited intense immunoreactivity for neuron-specific enolase (NSE) and synaptophysin, and were moderately positive for chromogranin A. Electron-microscopy revealed membrane-limited granules in the cytoplasm. The cultured cells were spindle in shape, and having projectional cytoplasm. They were intensely positive for NSE, synaptophysin, chromogranin A, and neurofilament (200 kD). Consequently, this case was diagnosed as a malignant aortic body tumor from the neuroecrodermal origin. — KEY WORDS: aortic body tumor, cultured cell, Holstein cow.

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Primary tumors of the heart are rare in either domestic animals and human beings. Rhabdomyoma, hemangioma, schwannoma, and mesothelioma have been described in the bovine heart [1, 10]. The tumors that arise from the aortic bulb are called as aortic body tumor, chemodectoma or heart base tumor [2, 4, 6, 8, 10], and fairly common in dogs [1, 2, 4, 7–9, 11], but rare in the other animals. There are only a few reports on aortic body tumor in cattle [2]. The present paper describes a case of bovine malignant aortic body tumor and the nature of cultured tumor cells, *in vitro*.

A 5-year-old female Holstein cow, weighing about 500 kg, was slaughtered. No clinical symptoms were noted in the physical examination. The neoplastic mass, of 22 × 17 × 15 cm in size, was located at the base of the left atrium. Premediastinal lymph node showed slight swelling. Tissue samples of the atrial tumor mass, lung, liver, spleen, kidney, premediastinal lymph node, and intestinal lymph node were fixed with 10% neutral buffered formalin, embedded in paraffin wax, and cut at 4 μm. Deparaffinized sections were stained with hematoxylin and eosin (HE), Azan, periodic acid-Schiff (PAS), phosphotungstic acid-hematoxylin (PTAH), Watanabe's silver impregnation for reticulin, and Grimelius' silver stain. Samples from the tumor mass were fixed with methanol-Carnoy's solution for immunohistochemical evaluation. Immunostaining was carried out using the avidin-biotin-peroxidase complex (ABC) method using a kit (Vectastain PK-400, Vector Laboratories, Burlingame, CA, U.S.A.). Following primary antibodies were used: rabbit sera against keratin (prediluted, Dako, Carpinteria, CA, U.S.A.), vimetin (1:100, Dako), desmin (prediluted, Dako), S-100 (1:400, Dako), α-smooth muscle actin (1:40, Dako), sarcomeric actin (1:20, Dako), myoglobin (prediluted, Dako), glial fibrillary acidic protein (GFAP, prediluted, Dako), neuron-specific enolase (NSE, prediluted, Dako), synaptopshysin (1:10, Dako), and chromogranin A (prediluted, Dako). Six different types of antibody against neurofilament were also employed. The source and working dilution of six antibodies were given in Table 1. The secondary antibodies were biotinylated goat antisera against rabbit or mouse immunoglobulins (1:200, Dako). The reaction products were visualized using 3,3'-diaminobenzidine (Sigma, St. Louis, MO, U.S.A.) counter-stained with Mayer's hematoxylin. Epoxy-resin-embedded sections were also made to allow ultrastructual examination. Ultrathin sections were stained with uranyl acetate and lead citrate, and observed under a transmission electron microscope (H-800, Hitachi, Tokyo, Japan), at 80 kV. The tumor tissue was minced, and digested with 4 mg/ml collagenase (232 U/mg, Wako, Tokyo, Japan) in Dulbecco's modified Eagle's medium (DMEM, Sigma) and Ham's nutrient mixture F-12 (Sigma) containing 10% fetal calf serum (FCS), 100 IU/ml penicillin, and 100 μg/ml streptomycin, for 6 hr at 37°C in a humidified atmosphere of 5% carbon dioxide in air. The digested tissue was filtered through nylon mesh cloth (80 μm), centrifuged at 1,000 rpm for 10 min and cultured according to Hiratsuka *et al.* [3]. Coverslips with cultured tumor cells were washed in PBS, fixed in cold acetone, and stored at −20°C for 30 min. The cells were incubated with one or other of the primary antibodies overnight at 4°C. The reacted antibodies were visualized by the ABC method.

At necropsy, the grayish-white mass was encapsulated with fibrous tissue. Cut surface were grayish-white to milk-white in color, and irregularly lobulated (Fig. 1). The tumor was divided into irregular lobules by connective tissue septa containing many capillaries. The tumor was composed of spindle-shaped and polyhedral cells, and the tumor cells had slightly eosinophilic cytoplasm. The nuclei were round-to-oval, and usually placed centrally in the cell. The chromatin pattern was finely granular, and mitotic figures were infrequent (Fig. 2). These cells also lacked cytoplasmic argyrophil granules. The neoplastic cells invaded the myocardium. Metastasis was only present in the premediastinal lymph node.

The results of immunohistochemistry of the tumor cells were summarized in Table 1. The tumor cells showed intense immunoreactivity for NSE, and synaptophysin. Some tumor cells were also moderately-positive for

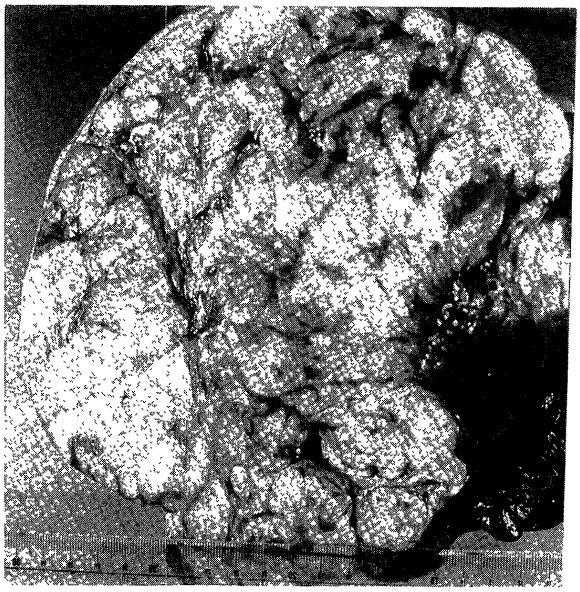

Fig. 1. The tumor mass revealing the base of the left atrium and encapsulating by connective tissue and the grayish-white cut surface was subdivided into irregular lobules.

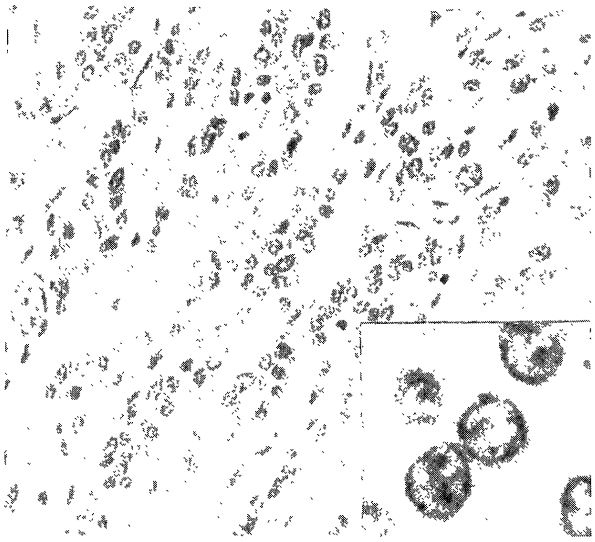

Fig. 2. Histological appearance of the proliferation tumor cells surrounding by connective tissue septa containing many capillaries. HE stain. × 200. Inset: The neclei were round-to-oval and the chromatin pattern was finely granular. HE stain. × 1,000.

chromogranin A. Tumor cells were negative for keratin, vimentin, desmin, S-100, α-smooth muscle actin, sarcomeric actin, myoglobin, and NF. Electron microscopy revealed dilated cisternae including electron-dense, membrane-limited secretory granules in the cytoplasm.

The cells proliferated in DME/F12 medium with 10% FCS, were passaged till 6 times. Under phase-contrast microscope, the cultured tumor cells were spindle in shape and some cells having cytoplasmic projection (Fig. 3). The

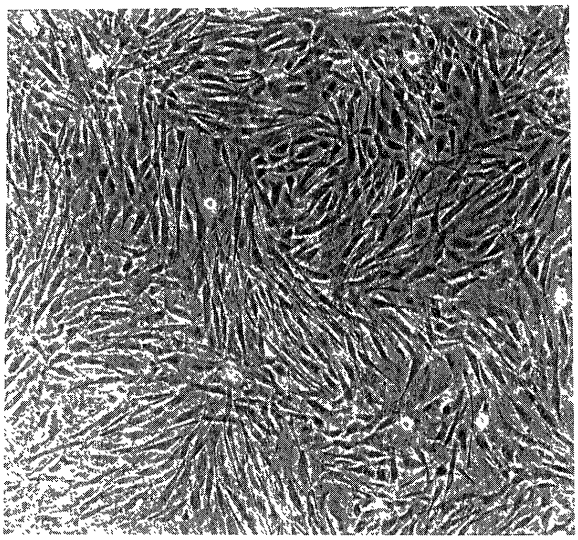

Fig. 3. The cultured tumor cells showing a subconfluent monlayer with long projectional cytoplasm. Phase-contrast. × 126.

immunohistochemical findings of these cultured cells after for 4 passages were summarized in Table 1. The cultured cells showed immunoreactivity for NSE, and synaptophysin, and were moderately-positive for chromogranin A. Moreover, the cells showed intense immunoreactivity for NF (200 kD) and vimentin, while the original tumor cells were not such reactivity.

From the findings described above, this bovine tumor was diagnosed as a malignant aortic body tumor. The immunoreactivity for NSE, synaptophsin, and chromoglanin A, and the presence of electron-dense-core membrane-bound granules strongly suggested that this tumor arose from a paraganglion in the parasympathetic nervous system [2, 12]. In general, the majority of bovine aortic body tumors are believed to be benign [12]. Azuma *et al.* [1] reported a case of bovine malignant aortic body tumor with metastasis to the premediastinal lymph node. Immunohistochemical features were not examined in that case, however the morphological features and the metastastic lesion were similar to those seen in the present case. Moreover, since the tumor cells in present case had only a few electron-dense-core granules, and a small number of cells showed moderate-to-slight immunoreactivity for chromogranin A, the present case might be an undifferentiated aortic body tumor. This made it possible to culture the tumor cells *in vitro*. Although many reports described the morphological characteristics of aortic body tumors *in vivo* [9], there is little information on the nature of this tumor, *in vitro*. In the present study, the cultured tumor cells showed different morphology and immunoreactivity *in vitro* compared with those of the original tumor. In particular, the additional expression of vimentin and NF in the cultured cells was quite unique, but was not a surprising phenomenon, since cultured cells have been known to exhibit vimentin in various degrees of differentiation [5]. Therefore the origin

Table 1. The results of immunohistochemistry staining for the original tumor and the cultured cell

Antibodies	Source	Dilution	Tumor	Cultured Cell
Keratin	Dako	Prediluted	–	–
Vimentin	Dako	1:100	–	++
Desmin	Dako	Prediluted	–	–
S-100	Dako	1:400	–	–
Sarcomeric actin	Dako	1:20	–	–
Smooth muscle actin	Dako	1:40	–	–
Myoglobin	Dako	Prediluted	–	–
h-NF (200 kDa)	Sigma	1:20	–	N.D.
h-NF (160 kDa)	BioMakor	1:200	–	N.D.
h-NF (68 kDa)	BioMakor	1:200	–	N.D.
b-NF (200 kDa)	TFR*	1:20	–	++
b-NF (158 kDa)	TFR	1:20	–	–
b-NF (68kDa)	TFR	1:20	–	–
GFAP	Dako	Prediluted	–	–
NSE	Dako	Prediluted	++	+
Synaptophysin	Dako	1:10	++	+
Chromogranin A	Dako	Prediluted	+	+

(++) Intensely positive; (+) Moderately positive; (-) Negative. h: Human, b: Bovine, *: Transformation Reseach Inc., N.D.: Not done.

of this cell type might not be mesencymal. Further attempts to culture these cells, and transplant them into nude or skid mice will confirm this point.

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