Peripheral Neuroblastoma and Primitive Neuroectodermal Tumor in Japanese Black Cattle

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**ABSTRACT.** Peripheral neuroblastoma was found in a 1-year-old, male, Japanese black cattle (Case 1) and primitive neuroectodermal tumor (PNET) in a 7-year-old, female, Japanese black cattle (Case 2). In Case 1, neoplastic tissue was replaced the right cranial vault and nasopharynx. A large, soft mass approximately 18 cm in diameter was also observed in the right mandibulopharyngeal area. In Case 2, a neoplastic mass of about 15 cm in diameter was found in the mandibulopharyngeal area. Histopathologically, massive necrosis showing a pseudopalisade arrangement was frequently observed in Case 1. On the contrary, Homer & Wright rosette formations of tumor cells were prominent in Case 2. Immunohistochemically, the proliferating cells in Case 1 were positive for vimentin, S-100, and neurofilament (NF) and those in Case 2 showed intense immunoreactivity for NF and neuron specific enolase, but were negative for vimentin and S-100. The different degrees of differentiation of the neoplastic cells originating from the neuroectoderm, might be reflected in their different morphological and immunohistochemical features. — **KEY WORDS:** bovine, peripheral neuroblastoma, primitive neuroectodermal tumor (PNET).

Neuroectodermal tumors, neoplasms derived from primitive neuronal cells, in animals are rigorously classified [5], into neuroblastomas, ganglioneuroblastomas, and ganglioneuromas according to the degree of differentiation of the neoplastic cells [3, 6, 15]. In general, neuroblastomas including medulloblastomas in the central nervous system, are very rare in domestic animals [6]. In the peripheral nervous system, few cases of neuroblastomas in the adrenal medulla, sympathetic ganglia, or nasal cavity (aesthesioneuroblastomas) have been reported in several animal species [1, 3, 4, 6, 11, 14]. Cordy [3] reported that peripheral neuroblastomas analogous to human \textit{in situ} neuroblastomas, were often found in adult slaughter cattle and malignant neuroblastomas were seen in premature or stillborn calves and in dogs of all ages [6].

Recently, very primitive tumors with a potential to differentiate into both neuronal and glial cells have been called primitive neuroectodermal tumors (PNETs) in the medical field [8–10, 15–17]. The term PNETs has been applied to poorly differentiated tumors both in the central and peripheral nervous system, but the term has not been well-generalized to tumors of domestic animals [17, 18]. Although the PNET was originally coined to designate unclassifiable tumors consisting of undifferentiated neuroectodermal cells with variable neuro-glial differentiation, recently the term is sometimes applied to conceptually to all embryonal neuroectodermal tumors including medulloepitheliomas, medulloblastomas or neuroblastomas [8–10]. However, several pathologists [8–10] proposed that the diagnosis of PNET should be applicable only in instances wherein no differentiation was detected by morphological examinations, or in instances wherein various differentiation was observed and the lesions did not fit neatly into a recognized diagnostic category.

Immunohistochemical examination of neuroblastomas or PNETs has been attempted for several human cases [7, 12, 13, 15, 19]. In general, human neuroblastomas show immunoreactivity for neurofilament (NF), and some exhibit positive reactions for S-100, neuron specific enolase (NSE), chromogranin A, or synaptophysin [15, 19] although the immunoreactivity varied among cases. In addition, tumor cells with squamous differentiation are sometimes positive for keratin or cytokeratin [15]. In animals, similar investigation have been done in a few cases [11] and there are few informations concerning the immunohistochemical natures of animal neuroblastomas or PNETs [18].

The present paper describes morphological and immunohistochemical natures of two bovine tumors supposed to be originated from the neuroectoderm and discusses their differences. The histopathological designations of these tumors are applied according to the recent human WHO classification of nerve tumors discussed by Okazaki [9].

**Case history:**

**Case 1:** The animal was a Japanese Black bull, born on April 4, 1994 in Miyazaki, Japan. He showed neurological signs with abnormal movement of the right eye in January 1995. From these neurological symptoms, the animal was presumed to have an infectious disorder of the nervous system and was treated with several antibiotics. The clinical sign did not improve and the disease continued to progress. On April 4, 1995, he exhibited astasia, exophthalmos of the right eye, fracture around the right horn, and severe swelling of the right side of the face and head. X-ray examinations of the head revealed a neoplastic mass replacing the right cranial vault or nasopharynx, but not the nasal cavity. The bull was euthanized on the next day and an autopsy was performed immediately in our laboratory.

**Case 2:** This animal was a 7-year-old Japanese Black cow. The cow showed severe dyspnea and was clinically...
Histopathology:

Histological procedures:

For routine histopathology, tissue samples were fixed with 10% neutral buffered formalin. The samples from Case 1 were also fixed with methanol-Carnoy's solution for immunohistochemical examination and with 2% glutaraldehyde for electron microscopy. Paraffin sections were stained with hematoxylin and eosin (HE) and Watanabe's silver impregnation. For electron microscopy, glutaraldehyde-fixed samples from Case 1 were post-fixed with 2% osmium tetroxide and embedded in epoxy-resin. Immunostaining was performed using a kit employing the avidin-biotin peroxide complex (ABC) method (Vectastain PK-4000, Vector Laboratories, Burlingame, CA, U.S.A.). Before sections from Case 2 were immunostained, hydrated autoclave pretreatment was performed. The primary antibodies used were monoclonal antibodies against bovine NF-200 (1:20, Transformation Research Inc., Framingham, MA, U.S.A.), bovine NF-145 (1:20, Transformation Research Inc.), bovine NF-68 (1:20, Transformation Research Inc.), synaptophysin (1:10, Boehringer Mannheim, Mannheim, Germany), and vimentin (1:10, Boehringer Mannheim), as well as rabbit antisera against S-100 (1:200, Dako, Carpinteria, CA), glial fibrillary acidic protein (GFAP, prediluted, Dako), and NSE (prediluted, Dako). The secondary antisera were biotinylated goat antisera against mouse immunoglobulins (1:200, Dako) and rabbit immunoglobulins (1:200, Dako).

Gross findings:

Case 1: The neoplastic mass in the right cranial vault, nasopharynx, and mandibulopharyngeal area consisted of proliferating small tumor cells and severe necrosis with hemorrhage. These tumor cells were arranged in lobules and broad sheets or ribbon-like structures. Severe necrotic foci surrounded by pseudopalisade arrangements of neoplastic cells or their debris were apparent (Fig. 3). Most of the tumor cells were small and round with eosinophilic cytoplasm and atypical round nuclei containing abundant chromatin (Fig. 4). Granular deposits of calcium were frequently seen in the necrotic foci. Mitotic neoplastic cells were very common. Stromal connective tissue was not abundant, whereas reticulum fibers surrounding packs of neoplastic cells were prominent by Watanabe's silver staining. The 5th and 7th cranial nerves had almost been replaced by a proliferation of neoplastic cells, although the neoplastic tissues had not invaded the brain. In the lungs and heart, metastatic lesions with or without necrotic foci were seen. The morphological characteristics of these neoplastic cells were almost identical to those in the right cranial vault, nasopharynx, and mandibulopharyngeal area. In the other visceral organs, there were no significant lesions. Electron microscopy showed that the neoplastic cells appeared to have intermediate filaments and a few cytoplasmic organelles such as mitochondria and rough endoplasmic reticulum. There were no evidence suggesting some specific differentiation of the tumor cells.

Case 2: The neoplastic mass in the mandibulopharyngeal area consisted of a solid proliferation of small cuboidal to round cells sometimes forming cord-like structures (Fig. 5). The tumor cells were characterized by clear cytoplasm and round hypochromatic nuclei with distinct nucleoli. These tumor cells were sometimes arranged in tubular or adenoid structures called as Homer & Wright rosette (Fig. 6). In the stromal area, abundant reticulum fibers surrounding packs of these neoplastic cells were observed. Small necrotic foci were sometimes observed, but pseudopalisade cellular arrangement was not seen.

Immunohistochemistry:

Case 1: The neoplastic cells exhibited intense immunoreactivity for antibodies against vimentin (Fig. 7a), S-100, and bovine NF-200 (Fig. 7b). Almost all tumor cells commonly showed intense positive reaction for vimentin, S-100, and NF, while a subset of cells lacked NF-immunoreactivity. There was no significant immunoreactivity of tumor cells for the other antibodies.

Case 2: The tumor cells showed intense immunoreactivity for bovine NF-200 (Fig. 8a) and NSE (Fig. 8b). Both NF- and NSE-immunostaining recognized intensely the cytoplasm of neoplastic cells and stromal area within rosettes. Although the tumor cells were negative for S-100 and vimentin, small population of S-100- and vimentin-positive cells thought to be Schwann cells, were observed in the periphery of small round neoplastic cells forming Homer & Wright rosettes.

Since the cranial vault and nasopharynx were severely involved with tumor in Case 1, there is a possibility that neoplastic cells were derived from olfactory sensory cells called as olfactory neuroblastoma [1, 4, 14, 17]. However,
the distribution of the tumor mass in Case 1 is quite different. The primary site is difficult to discern in a rapidly spreading malignant neuroblastoma [3], but we believe that the present tumors may have arisen from the ganglion or remaining neural crest cells in the peripheral nerve, and not from olfactory sensory cells.

The histological characteristics in Case 1, such as severe necrosis with pseudopalisade cellular arrangement and metastases show the malignancy of the tumor. Although a pseudopalisade structure is one of the characteristic lesions of glioblastomas and other malignant glial tumors including malignant schwannoma [15–17], any significant evidences of specific differentiation to glial cell were not found in case 1 and the tumor was supposed to be undifferentiated or very primitive tumors originating from the neuroectderm. In contrast, Homer & Wright rosette formations which are thought to be one of the characteristic lesions of neuroblastomas [15] were found in Case 2. Thus, Case 2 was diagnosed as a typical neuroblastoma.

The positive immunoreactivity of the neoplastic cells in both cases for NF and the negative reaction for GFAP suggest that these tumors have an potential to differentiate for neuronal cell. The tumor cells in Case 1 were also strongly positive for S-100 and vimentin, but a positive reaction for vimentin was uncommon for neuroblastomas [2, 11–13, 15]. Oppdal et al. [12] reported the immunohistochemical features of human neuroblastomas and related normal tissues. In their report, neuroblastomas were always negative for vimentin. In addition, the positive reaction for both vimentin and S-100 protein has commonly been found in glioblastomas, undifferentiated astrocytomas, and malignant schwannoma [15]. Omi et al. [11] examined 5 cases of bovine nervous tissue tumors and showed that ganglioneuroblastoma and anaplastic ganglioglioma were strongly positive for vimentin and S-100, whereas a peripheral neuroblastoma was negative for both. However, Winkle et al. [18] recently described the immunohistochemical natures of central PNET in dogs and cats and demonstrated that all seven PNETs were positive for vimentin as those in human cases. Thus, the positive reaction for vimentin may be common feature for the PNETs. From these previous data together with our histopathological and immunohistochemical findings, we considered that the tumor in Case 1 should be diagnosed as
PNETs with a potential of neuronal differentiation. In contrast, the neoplastic cells in Case 2 showed intense immunoreactivity for both NSE and NF. Since many human and animal neuroblastomas have shown immunoreactivity for low or intermediate molecular mass NF and some have been positive for S-100 and NSE [10-12, 15, 19], the immunohistochemical natures of Case 2 are almost in conformity with those in neuroblastomas. In Case 2 small spindle-shaped cells positive for both S-100 and vimentin in the periphery of rosettes might be non-neoplastic stromal Schwann cells as described in human peripheral neuroblastomas [15].

In conclusion, the present paper describes the morphological and immunohistochemical features of bovine peripheral neuroblastoma and PNET, and suggests a combination of immunostaining for NF, vimentin, S-100, and NSE would be useful to know the degree of differentiation of the tumors with potential to differentiate for neuronal cells.

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REFERENCES


