



NOTE

Pathology

Enteric peripheral neuroblastoma in a calf

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ABSTRACT. An 11-month-old female Japanese Black calf had showed chronic intestinal symptoms. A large mass surrounding the colon wall that was continuous with the colon submucosa was surgically removed. After recurrence and euthanasia, a large mass in the colon region and metastatic masses in the omentum, liver, and lung were revealed at necropsy. Pleomorphic small cells proliferated in the mass and muscular layer of the colon. The cells were positively stained with anti-doublecortin (DCX), PGP9.5, nestin, and neuron specific enolase (NSE). Thus, the diagnosis of peripheral neuroblastoma was made. This is the first report of enteric peripheral neuroblastoma in animals. Also, clear DCX staining signal suggested usefulness of DCX immunohistochemistry to differentiate the neuroblastoma from other small cell tumors in cattle.

KEY WORDS: cattle, doublecortin, neuronal marker, neuronal neoplasm, peripheral neuroblastoma

Neuroblastoma is an embryonal neuroectodermal neoplasm with limited neuronal differentiation that arises both in the central and peripheral nervous systems [12]. Peripheral neuroblastoma is a neuroblastoma occurring in the peripheral nervous system, and considered as a rare neoplasm in domestic animals. However, reports have indicated relatively common occurrence in dogs and cattle [9, 12], and one report has described a case of peripheral neuroblastoma in a pig [5]. This neoplasm is highly malignant with strong metastatic potential and is usually manifest in animals with premature birth or those that are stillborn [9]; in addition, benign and solitary peripheral neuroblastomas that localize in the adrenal medulla are found incidentally in adult cattle at slaughter [27].

Primary lesions of peripheral neuroblastomas are usually located in the adrenal medulla or sympathetic ganglia because they are derived from primitive neuroectodermal cells that migrate to these sites from the neural crest. Most reported cases of peripheral neuroblastoma had location in the renal-adrenal area or dorsal abdomen [2, 5, 6, 8, 13, 14]. Whereas, in rare cases in dog and cattle, peripheral neuroblastoma occurred in the head region presumably originated from the sympathetic ganglion [4, 23, 25]. Occurrence of peripheral neuroblastoma at any other site is rare. To our knowledge, there is only one case report of cutaneous neuroblastoma in a dog, in the literature [15]. Here, we report a case of ectopic abdominal peripheral neuroblastoma in a Japanese Black calf, without association with the adrenal gland, and application of anti-doublecortin (DCX) immunostaining on its tissue sample for differentiation.

An 11-month-old female Japanese Black calf presenting anorexia, fever, diarrhea, and black hard feces was brought to our clinic. A solid mass was detected by rectal palpation, and the presence of solid white mass of 20-cm size surrounding the colon was confirmed through exploratory laparotomy. Thus, the mass was incompletely removed with colon tissue inside the mass, and a part of the mass was fixed in 10% neutral-buffered formalin and sent to our laboratory for histopathological examination. Grossly, the cut surface of the mass had whitish, solid appearance, and was continuous with the submucosa of the colon in some areas (Fig. 1). Microscopically, in the mass and muscular layer of the colon, proliferation of small neoplastic cells in a diffuse pattern or with formation of nests separated by sparse fibrous stroma was observed (Figs. 2 and 3), and severe invasive lesions were also found in some area of the lamina propria of the colon. The neoplastic cells had scant cytoplasm and vague cell border. Some of them had fine fibrillar cytoplasmic processes. The neoplastic cells' nuclei were pleomorphic and hyperchromatic with multiple nucleoli. Mitotic figures and pyknotic figures were frequently observed.

The symptoms of the calf improved temporarily after surgery, but stomachache appeared again at 2 months after surgery. Palpation of the ventral aspect of the abdomen revealed the presence of a solid large mass, suggesting recurrence of the neoplasm.

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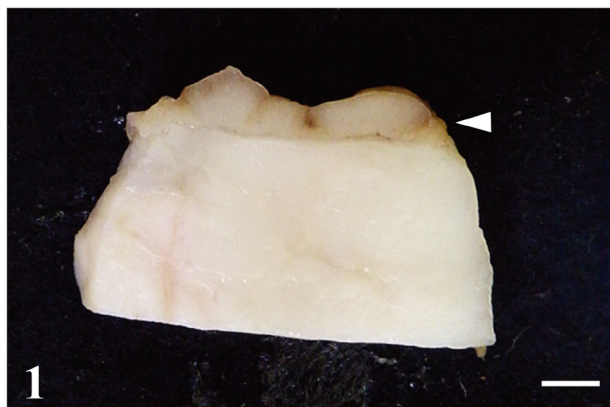


Fig. 1. Cut surface of a part of the biopsy sample. The mass is continuous with the submucosa of the colon. Arrowhead indicates the colon mucosa. Bar=5 mm.

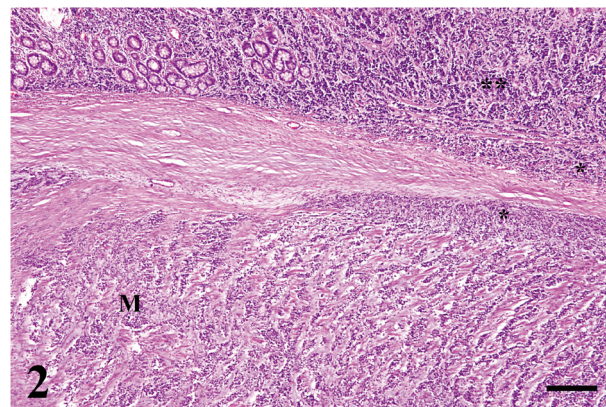


Fig. 2. Neoplastic lesion in the colon. In the colon wall, lesions are mainly located in the muscular layer (M). Severe invasive lesions are observed in the submucosa (*) and lamina propria (**). Bar=100 μ m.

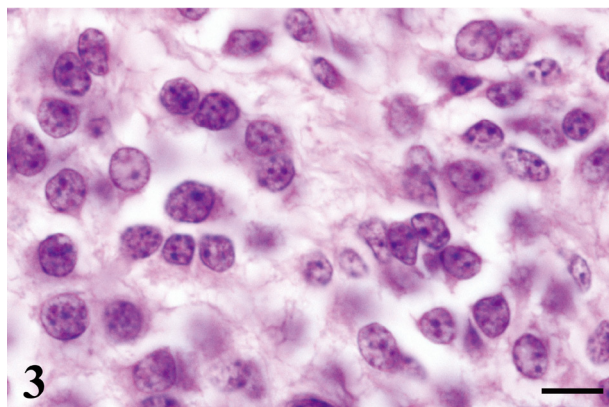


Fig. 3. Morphology of the neoplastic cells in the biopsy sample. The mass consists of highly pleomorphic small cells. The neoplastic cells have scant cytoplasm and vague cell border. Some of them have fine fibrillar cytoplasmic processes. Hematoxylin and Eosin (HE). Bar=10 μ m.



Fig. 4. Gross appearance of the mass in the colon. Formation of a large solid mass (arrow) in the colon that broadly encloses the colon wall is observed. Bar=4 cm.

The patient was euthanized due to poor prognosis and necropsy was performed in our laboratory.

At necropsy, a large solid mass with whitish appearance of approximately 25-cm diameter in the area of the colon at midabdominal level was observed. The colon and ileum were enclosed by the mass, and there was involvement of the mesentery (Fig. 4). No association of the mass with the adrenal glands or dorsal peritoneum. The cut surface of the mass revealed whitish, solid appearance with some fragile red to yellowish foci of necrosis. Multiple metastatic masses of 3 to 5-cm diameter were distributed in the omentum and liver, and a nodule with whitish appearance of approximately 5-mm diameter was present on the surface of the left posterior lung lobe. There was absence of neoplastic lesions in the other organs, including the adrenal glands and brain.

The masses in the area of the colon, liver, and omentum were encapsulated by fibrous tissue and consisted of neoplastic cells similar to those found in the biopsy sample. The nodule of the lung was also comprised of these cells. Multiple necrotic foci were also noticed.

Immunohistochemistry was performed both on the biopsy and necropsy samples using the following antibodies: mouse monoclonal antibodies against human cytokeratin (clone AE1/AE3, Dako, Carpinteria, CA, U.S.A.), pig vimentin (clone V9, Dako), human CD79a (clone HM57, Dako), human neurofilament (clone 2F11, Dako), cow synaptophysin (clone SY38, Dako), human NSE (clone BBS/NC/VI-H14, Dako), and rabbit polyclonal antibodies against human CD3 (Dako), cow PGP9.5 (Dako), cow S100 (Dako), cow GFAP (Dako), human chromogranin A (Nichirei Biosciences, Inc., Tokyo, Japan), human nestin (Immuno-Biological Laboratories, Fujioka, Japan), and human DCX (Sigma-Aldrich, St. Louis, MO, U.S.A.). Immunoreactivity was visualized with peroxidase-diaminobenzidine reaction.

In most tumor cells, strong positivity for DCX (Fig. 5a), S100 (Fig. 5b), PGP9.5 (Fig. 5c), vimentin (Fig. 5d), and nestin

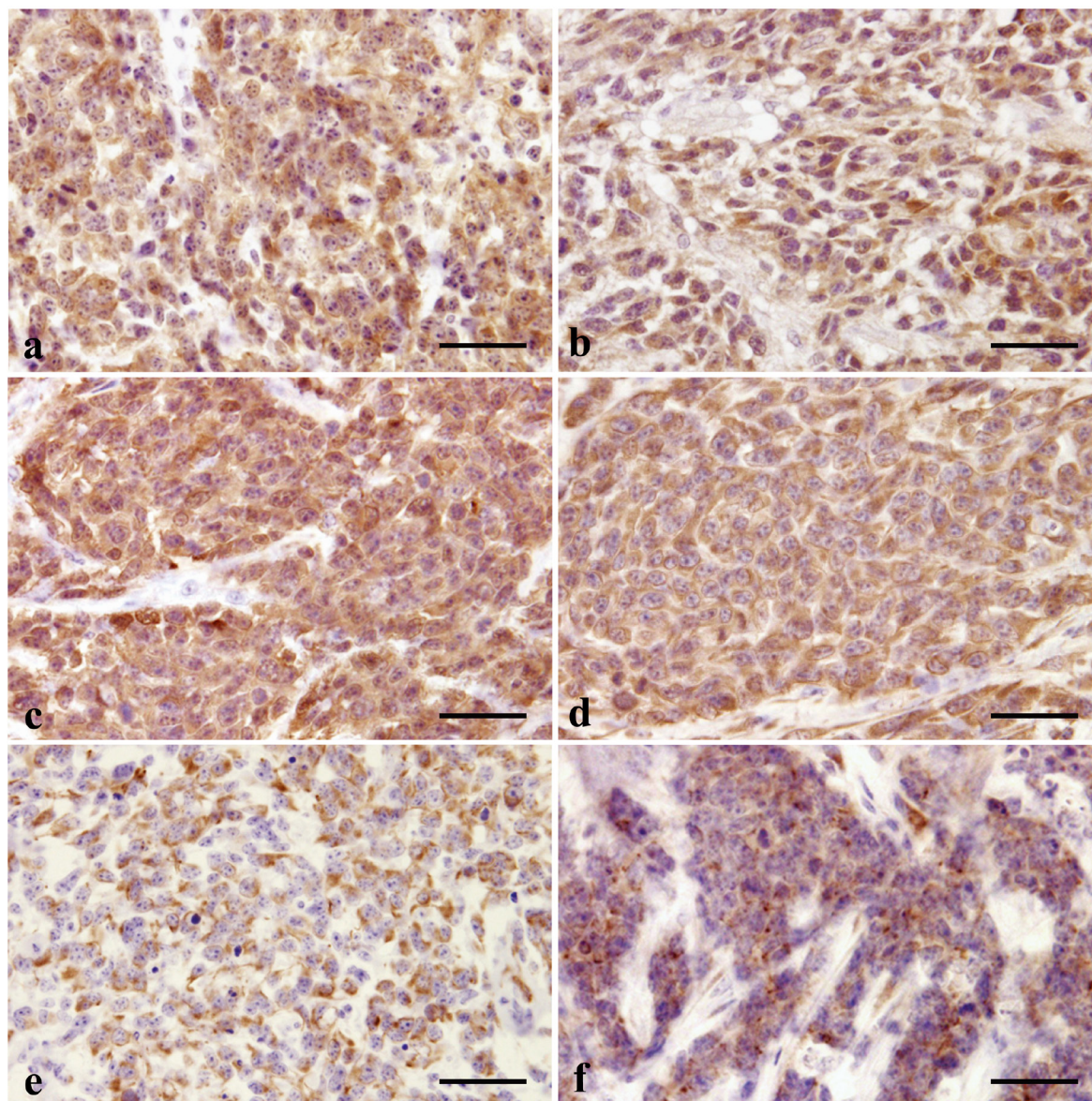


Fig. 5. Immunohistochemistry of the neoplastic cells. The neoplastic cells show positive staining for anti-doublecortin (DCX) (a), S100 (b), PGP9.5 (c), vimentin (d), nestin (e), and NSE (f). Bars=20 μ m.

(Fig. 5e), weak positivity for NSE (Fig. 5f), and negative findings for other markers were obtained. Immunoreactivity for PGP9.5, NSE, and DCX is considered to indicate that premature neuronal cells are the origin of the neoplastic cells, and that for nestin, the neuronal progenitor phenotype of the neoplastic cells. Diagnosis of peripheral neuroblastoma was made according to undifferentiated morphology, premature neuronal cell phenotype, and highly metastatic phenotype.

In our case, the tumor mass was located in the colon area and had no association with the adrenal glands or sympathetic ganglions, which suggests that the tumor primarily developed from the colon wall. Moreover, the enteric nervous system in the colon wall was considered as the tumor source, because neoplastic cells were mainly found in the muscular layer. However, histopathological evidence other than the lesions' location is lacking. Enteric neuroblastoma is extremely rare [11], despite the fact that the enteric ganglions are distributed throughout the gut, and cells of the enteric ganglions share origin of the neural crest with the cells of the sympathetic ganglion and adrenal medulla [24]. On the other hand, intestinal ganglioneuroma, a benign counterpart of neuroblastoma, is more common both in animals and humans [1, 16, 19–21]. To our knowledge, this is the first report of intestinal peripheral neuroblastoma in the field of veterinary medicine.

For the diagnosis of neuroblastoma, immunohistochemistry with neuronal cell marker molecules, NSE, neurofilament, and synaptophysin, is usually performed to confirm neuronal differentiation. However, the immunoreactivity varies among cases according to differentiation status [18, 22, 25]. In our case, DCX immunostaining was performed in addition to these conventional neuronal cell markers. DCX is a microtubule-associated protein which is expressed in immature migrating neuroblasts throughout the central nervous system and peripheral nervous system, but not in mature neurons or neural stem cells [7]. Thus, DCX has been

considered as a marker to detect neuroblasts by immunostaining technique in the field of neuroscience [3, 26]. DCX is expressed at high level in the neuroblastoma in humans, and DCX-targeting RT-PCR is useful to detect residual lesion in these cases [17, 28]. However, only one study used DCX immunohistochemistry for the diagnosis of neural tumors [10], and reported finding of high rate of DCX-positivity in cases of oligodendroglioma, cerebellar primitive neuroectodermal tumor (PNET), and neuroblastoma.

In our case, neuroblastoma cells showed clear positive immunoreactivity against DCX, despite negatively stained by neurofilament and synaptophysin. Considering variable immunoreactivity to mature neuronal cell markers in case of neuroblastoma, these results suggest that DCX has potential as a useful new marker to differentiate neuroblastoma from other small cell tumors such as lymphoma and undifferentiated carcinoma in cattle.

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