A Canine Peripheral Nerve Sheath Tumor Including Peripheral Nerve Fibers

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ABSTRACT. Peripheral nerve sheath tumor was found in a 7-year-old male mongrel dog. The tumors were located in the right cheek subcutis and oral submucosa. Histologically, neoplastic cells were arranged in streaming bundles, occasionally interlacing bundles or whorls of elongated and spindle cells. Cellular atypia was poor and mitotic figures were rarely observed. Ultrastructurally, neoplastic cells had basement membrane, typical of Schwann cells. One bundle of normal peripheral nerve fibers and some myelinated axons were seen within the tumor tissues. Immunohistochemically, neoplastic cells reacted to vimentin, glial fibrillary acidic protein, S-100 protein and neuron specific enolase. In addition to the above immunoreactions, the included nerve fibers were positive for myelin basic protein and neurofilament protein. This paper also discusses immunohistochemical findings on differential diagnosis in comparison with those of canine hemangiopericytomas reported hitherto.—KEY WORDS: canine, peripheral nerve sheath tumor (PNST), schwannoma.
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The term of peripheral nerve sheath tumor (PNST) has been used for benign neoplasms such as schwannomas and neurofibromas. In the dog, these tumors develop most commonly in the cranial nerve, spinal root and brachial plexus [1, 2, 6, 9, 15]. Histologically, the PNST is characterized by sweeping fascicles of spindle-shaped cells. Thus, differential diagnosis should be made among spindle cell tumors such as canine hemangiopericytomas (CHPs) and PNSTs [2, 6, 11, 12, 18]. CHPs show "fingerprint" patterns or patterns consisting of wholes around blood vessels [10–12, 17, 18]. On the contrary, PNSTs exhibit histology referred to as Antoni A and B patterns; the former comprises compact spindle cells arranged in interlacing fascicles and palisaded patterns, whereas the latter is less cellular, consisting of spindle or oval cells arranged loosely and supported by edematous matrix [2, 3, 5–7, 15]. However, we often encounter spindle cell tumors that do not show such typical histology, making us difficult for diagnosis. For such diagnostically difficult cases, the involvement of peripheral nerve fibers may permit a diagnosis of PNST. A few authors have been discussed the spatial relationship between the PNST and peripheral nerve fibers in animals [5, 13]. Here, we report a canine PNST with apparent involving myelinated axons. In addition, detailed immunohistochemical examinations were conducted in comparison with those of CHPs that have been reported previously [11].

A 7-year-old male mongrel dog suffered from swelling in the right cheek and was referred to a private veterinary hospital on 23 February 1996. Subcutaneous tumor found in the right cheek was a walnut-sized, white and firm nodule. Besides it, another nodule, soybean-sized, was found in the oral submucosa of right palate. These nodules appeared circumscribed, and were surgically resected. These biopsy samples were presented for pathologic examinations. Thereafter, the tumor recurred one month after the initial presentation, and the dog died 6 months later, presumably due to malnutrition caused by insufficient food intake due to the recurred tumor. The recurrent tumor resection and necropsy were declined by the owner.

Two nodules removed at the first presentation were fixed in 10% buffered formalin and embedded in paraffin. Paraffin-embedded sections were routinely prepared, and stained with hematoxylin and eosin (HE), Azan Mallory for collagen fibers, reticulin silver impregnation for reticulin fibers, luxol fast blue (LFB), and Bodian stains. Immunohistochemical stainings were carried out with the labeled streptavidin-biotin peroxidase technique by the Kit (LSAB Kit, Dako, Carpinteria, CA, U.S.A.). The primary antibodies used were rabbit polyclonal antibodies for S-100 protein (Dako), glial fibrillary acidic protein (GFAP; Dako), myelin basic protein (MBP; Dako) and factor-VIII related antigens (F-VIII; Dako), and mouse monoclonal antibodies for cytokeratin (Dako), vimentin (Boehringer Mannheim, Germany), neuron-specific enolase (NSE; Dako), neurofilament protein (NF; Dako) and α-smooth muscle actin (SMA; Dako). For electron microscopy, formalin-fixed samples were cut into 1-mm cubes, postfixed in osmium tetroxide, and embedded in epoxy resin. Ultrathin sections were double stained with uranyl acetate and lead citrate, and examined in a transmission electron microscopy (Hitachi H-500, Japan).

Histologically, the subcutaneous tumor was encapsulated partially by a thin layer of connective tissues. Neoplastic cells were arranged in streaming bundles, occasionally interlacing bundles or whorls of elongated and spindle cells; their nuclei were oval, fusiform or wavy in shape, and hyperchromatic (Figs. 1 and 2). Cellular atypia was poor, and mitotic figures were rarely observed. Moderate amounts of collagen fibers were present among neoplastic cells, and reticulin fibers surrounded each neoplastic cells to varying degrees. The present tumor showed Antoni type B pattern in some parts, and Antoni type A was not seen. Hemorrhagic areas were occasionally seen throughout tumor tissues. Interestingly, one bundle of nerve fibers about 2 mm in width (Fig. 1), apparently pre-existing normal peripheral nerve fibers, and some nerve fibers including axon-like structures (Fig. 2) were seen within the tumor

Table 1. Immunohistochemical findings of the present PNST in comparison with those of schwannomas and canine hemangiopericytomas

Tumor		Vimentin	Keratin	S-100	GFAP	NSE	MBP	NF	SMA	F-VIII
PNST	(Neoplastic cells)	+	–	+	+	+	–	–	–	–
	(Included nerve fibers)	+	–	+	+	+	+	+	–	–
PNST: peripheral nerve sheath tumor, +: positive; –: negative.										
Schwannoma		+	–	+	–	NE	NE	NE	–	–
CHP		+	–	+/-	–	NE	NE	NE	+/-	–

Schwannomas (n=7) and CHP (canine hemangiopericytomas, n=45) reported by Perez *et al.* [11].
+: almost all cases are positive, –: almost all cases are negative, +/-: half cases are positive, but half cases negative, NE: not examined. S-100: S-100 protein, GFAP: glial fibrillary acidic protein, NSE: neuron specific enolase, MBP: myelin basic protein, NF: neurofilament protein, SMA: alpha-smooth muscle actin, F-VIII: factor VIII related antigens.

tissues; these structures were stained positively by LFB and Bodian stains. Similar histologic findings were observed in the oral nodule, except for the presence of axon-like structures.

Immunohistochemical findings were shown in Table 1. Neoplastic cells reacted intensely to vimentin and GFAP (Fig. 3), moderately to S-100 protein and NSE (Fig. 4), but were negative for cytokeratin, F-VIII, SMA, NF and MBP. Nerve fibers seen in the subcutaneous tumor were strongly positive for S-100 protein, GFAP, NF and MBP (Fig. 5), moderately for vimentin and NSE, but were negative for cytokeratin, SMA and F-VIII; together with staining results by LFB and Bodian stains, these immunostaining results indicated the presence of myelinated axons in tumor tissues. Ultrastructurally, neoplastic cells were elongated in configuration, with some cytoplasmic processes. Their nuclei showed marginally distributed chromatin with occasionally prominent nucleoli. Poorly developed rough-endoplasmic reticulum and a small number of mitochondria were seen in the cytoplasm, and characteristically, various amounts of cytoplasmic intermediate filaments were present in each of neoplastic cells. Neoplastic cells had prominent basement membrane (Fig. 6), but occasionally the membrane was discontinuous.

Schwannomas are benign tumors arising from peripheral nerve sheath (Schwann cells). Neurofibromas are used for peripheral nerve sheath tumors with fibroblastic neoplastic cells and collagen fibers [3, 4, 7]. In humans, the diagnosis of neurofibromas seems to be used in relation to von Recklinghausen's disease (Neurofibromatosis type 1) [2, 3, 15]. These tumors are involved in PNST. Their malignant types are termed malignant peripheral nerve sheath tumors (MPNSTs) [3, 5, 7, 15]. Based on anatomical location, immunopositive reactions for S-100 protein and GFAP, and basement membrane-like structures, the present tumor was considered to be a tumor derived from peripheral nerve sheath, presumably the trigeminal (maxillary) nerve. It was speculated that the oral tumor extended from the subcutaneous tumor through the maxillary foramen. The immunohistochemical and ultrastructural findings seen in the present case have been reported in human and animal PNSTs [3, 7, 8, 11, 13, 15, 16]. Furthermore, the presence of nerve fibers strongly supported the derivation of the present tumor; pre-existing peripheral nerve fibers might be involved in tumor tissues, or these myelinated axons might be extended from the pre-existing peripheral nerve fibers into tumor tissues, because of production of a nerve growth factor by neoplastic Schwann cells. Histologic findings such as poor cell atypism and very low grade of mitotic figures indicated benign PNST to the present tumor, although the present tumor recurred. Other authors have also reported that canine PNSTs recur at a high rate after surgical resection [1, 9]. This has been explained by incomplete resection of original tumors [1, 9].

Recently, detailed immunohistochemical findings of CHPs have been reported [11]. These findings are summarized in Table 1. Except for reaction to GFAP, immunohistochemical findings of the present tumor were similar to those of schwannomas reported by Perez *et al.* [11] (Table 1). However, immunoreactions for GFAP have been reported in some cases of PNSTs [16]. We further showed that neoplastic cells of PNSTs are positive moderately for NSE (Table 1) [8]. A major immunohistochemical difference between PNSTs and CHPs is appearance of SMA-positive cells in CHPs, although all cases of CHPs did not show SMA-immunoreactions [11]. Pericytes are considered to react to SMA [11]. However, we can not rule out a possibility that CHP cells could differentiate into myofibroblastic cells, because myofibroblasts are major immunopositive mesenchymal cells in connective tissues for SMA [14]. Interestingly, about half cases of CHPs were immunoreactive for S-100 protein [11] (Table 1). Thus, Perez *et al.* reported that S-100 protein-positive and SMA-negative CHPs could not be distinguished from schwannomas [11]. Our results showed that nervous markers such as NSE and GFAP, in addition to S-100 protein, might be useful in the differential diagnosis between canine PNSTs and CHPs. Further accumulation of PNSTs with definite evidences such as nerve fiber involvement is needed to distinguish from CHPs.

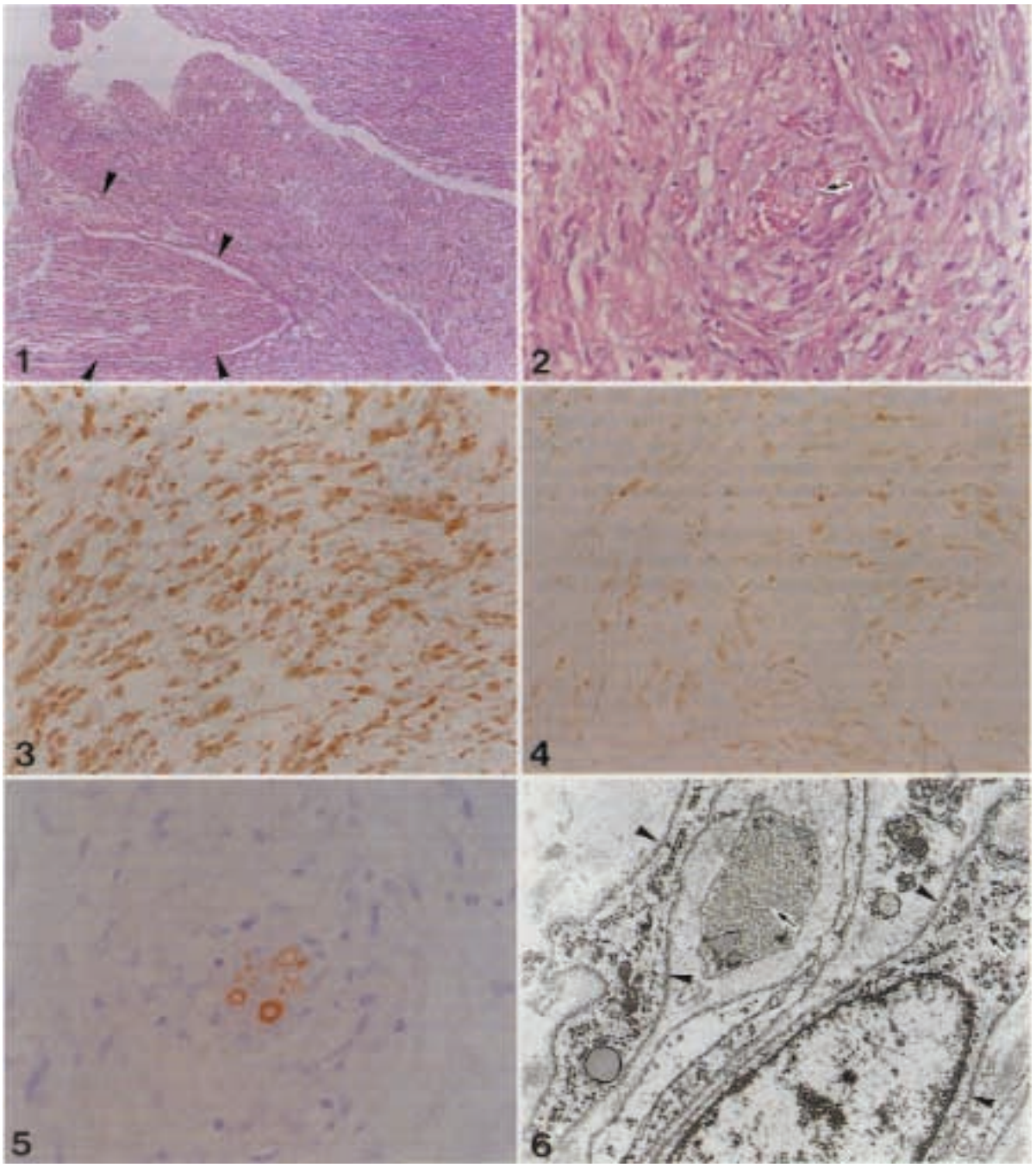

Fig. 1. Neoplastic cells are arranged in streaming bundles, and one bundle of normal peripheral nerve fibers (arrowheads) is seen within the subcutaneous tumor, peripheral nerve sheath tumor (PNST). HE. × 25.

Fig. 2. Small myelinated axons (arrow) are shown within the subcutaneous tumor. The nuclei of neoplastic cells are oval, fusiform or wavy in shape, and hyperchromatic. HE. × 150.

Fig. 3. Numerous neoplastic cells in the subcutaneous tumor are positive for glial fibrillary acidic protein. Immunohistochemistry. × 150.

Fig. 4. Positive reactions for neuron specific enolase are observed in the cytoplasm of moderate number of neoplastic cells in the oral tumor of PNST. Immunohistochemistry. × 150.

Fig. 5. The myelin of included axons in the subcutaneous tumor is positive for myelin basic protein, the same field as Fig. 2. Immunohistochemistry. × 300.

Fig. 6. Neoplastic cells with elongated and attenuated cytoplasmic processes, cytoplasmic intermediate filaments (arrows) and basement membrane (arrowheads) are indicated. Electron micrograph. × 9600.

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