Peripheral Neuroblastoma in a Young Labrador Retriever

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NOTE

Peripheral neuroblastoma is a neoplasm arising from the adrenal gland, sympathetic ganglia, and resting neural crest cells [11]. Although this neoplasm is rather common malignant tumor in childhood, there are few reports concerning peripheral neuroblastoma in dogs [1, 4, 7, 8, 12]. Histopathologically, neuroblastomas are mainly composed of small round cells and should be discriminated from other “small round cell tumors”, such as rhabdomyosarcoma, lymphoma, primitive neuroectodermal tumors (PNET) and Ewing sarcoma [11, 14]. Detection of some immunohistochemical markers is efficient to rule out differential diagnoses and employed routinely in humans [2, 3, 6, 9–11, 13–15]. The present paper describes morphological and immunohistochemical features of peripheral neuroblastoma in a young Labrador Retriever.

A 2-year-old male Labrador Retriever was presented to a private veterinary practitioner for depression of the right temporal region. The dog developed pitiuita and hematchezia 6 to 7 weeks later. The owner noticed no ocular movement of the right eye. The dog further exhibited generalized seizure, suffered from coma and died. Complete necropsy was performed on the following day. At necropsy, the dog showed emaciation and the right temporal and masseter muscles markedly reduced in volume. Two neoplastic masses, approximately 3 and 4 cm in diameter, respectively, were found in the right mandibulopharyngeal area. The masses were suspended from ventral and medial area of tympanic bulla with the cord-like tissue (Fig. 1). The cut surface of these masses was white to tan in color. Another neoplastic mass, approximately 2 cm in diameter, was found under the right facial nerve and an additional intranasal mass, approximately 1 cm in diameter, were observed. In the intracranial space, two neoplastic masses, approximately 2 and 1 cm in diameter, existed at the base of brain. One was at the right hemisphere, involving some right cranial nerves, and compressed the cerebrum, midbrain, cerebellum and pons (Fig. 2). The other was at caudal area of the optic chiasm and replaced pituitary gland. Three white masses, less than 1 cm in diameter, were also found on the dura mater of the left brain. Grossly, apparent invasion of the tumor mass into the cranial bone was not observed. No marked lesions were found in the other visceral organs, except for pulmonary edema with haemorrhage and duodenal erosion.

For routine histopathology, tissue samples were fixed in 10% formalin. Paraffin-embedded sections of 4–6 µm thick were stained with hematoxylin and eosin (HE). Immunostaining was performed using the Envision Polymer reagent (DAKO Japan, Kyoto, Japan). The primary antibodies used were rabbit antibodies against glial fibrillary acidic protein (GFAP, prediluted, DAKO Japan), CD3 (1:20, DAKO Japan), synaptophysin (1:20, DAKO Japan), S-100 (prediluted, DAKO Japan), tyrosine hydroxylase (TH, 1:100, Protos biotech corporation, New York, N.Y., U.S.A.), chromogranin A (prediluted, DAKO Japan) and adrenocorticotropic hormone (ACTH, prediluted, DAKO Japan) and mouse monoclonal antibodies against neuron specific enolase (NSE, prediluted, Dako Japan), neurofilament protein (NFP, prediluted, DAKO Japan), vimentin (prediluted, DAKO Japan) and NB84a (1:20, BioGenesis Ltd., Poole, England). The reaction products were visualized using the peroxidase and 3,3'-diaminobenzidine system.

Histologically, each neoplastic mass was divided into multiple lobules by fibrovascular septa and mainly composed of solid proliferation of tumor cells with multifocal neurophil-like stroma (Fig. 3). Most of the neoplastic cells were small and round to spindle in shape, and had scanty cytoplasm and hyperchromatic nucleus. Some middle- to large-sized neoplastic cells with eosinophilic and polygonal to stellate cytoplasm and nuclei containing coarse to
clumped chromatin, were scattered within neoplastic foci (Fig. 4). Apparent Homer-Wright type rosette formation was not found. Mitotic figures were less frequently detected in most neoplastic masses. In the right mandibulopharyngeal masses, multiple necrotic foci were distributed in the central areas of neoplastic lobules, and haemorrhage was prominent. Degenerative swollen ganglion cells remained in the former mass. Only the mandibulopharyngeal mass contained abundant collagen, hyalinization of connective tissue and mineral deposits (Fig. 5). In the mass replacing pituitary gland, acidophilic cells of pituitary gland were scattered. Metastatic lesions were found in the pulmonary hilar lymph node, lung and adrenal gland. The hilar lymph node was completely occupied by the proliferation of the neoplastic cells. Multiple metastatic foci with severe congestion and hemorrhage were observed in the lung. Small number of neoplastic cells infiltrated to the adrenal medulla. Morphological features of these metastatic lesions were similar to those in the right mandibulopharyngeal masses. Most neoplastic cells exhibited moderate to intense immunoactivity to vimentin, synaptophysin, chromogranin A, and TH (Fig. 6). Scattered cytoplasmic immunoreactivity for NSE and NFP was observed. Neuropil-like stroma was positive for vimentin, synaptophysin (Fig. 8), chromogranin A and TH. No significant immunoreactivity to GFAP, CD3, ACTH, S-100 and NB84a was found. Other than neoplastic changes, there were severe atrophy of right temporal and masseter muscles, multifocal leukomalacia with infiltration of gitter cells in left cerebrum and chronic gastroenteritis.

Based on morphologic and immunohistochemical features, the present neoplasm was diagnosed as a peripheral neuroblastoma. Morphologic features of this neoplasm are similar to those of poorly differentiated neuroblastomas in human. It has been well known that these human neoplasms were composed of small round cells, scattered larger angulated cells and neuropil-like stroma separated by fibrovascular septa [1]. Although the primary site is difficult to discern in a rapidly spreading malignant neuroblastoma [1], we suppose that the present canine tumor may have arisen from the sympathetic ganglion, presumably cranial cervical ganglion. In human cases, the anatomic distribution of neuroblastomas [7, 8] were negative for S-100. A monodifferentiated neuroblastomas in human [15] and canine peripheral neuroblastomas diagnosed by these immunohistochemical markers. Louden et al. [7] have shown positive immunoreactivity of canine neuroblastoma cells to NSE and Matsumiya et al. [8] have done that to NFP, NSE, synaptophysin and chromogranin A. TH is one of catecholamine-synthesizing enzymes and its expression was reported in several human neuroendocrine cells and derived neoplasms [2, 6]. The present neoplastic cells were positive for vimentin and negative for S-100. In general, vimentin expression indicates mesenchymal or undifferentiated nature of tumor cells and S-100 expression suggests Schwann cell differentiation in neuroblastomas. Undifferentiated and poorly differentiated neuroblastomas in human [15] and canine neuroblastomas [7, 8] were negative for S-100. A mono-

Fig. 1. Masses in the right mandibulophryngeal area (arrows), suspended from the ventral and medial area of tympanic bulla with cord-like tissue (arrowhead).
Fig. 2. Transverse cut sections of the brain. The mass (arrows) compressed the cerebrum, midbrain, cerebellum andpons.
Fig. 3. Solid proliferation of neoplastic cells with multifocal neuropil-like stroma (arrows). The lesion is divided into lobules by fibrovascular septa. HE. Bar=165 µm.
Fig. 4. Tumor cells are small and round to spindle in shape with hyperchromatic nuclei. Angulated tumor cells (arrows) with larger nuclei are scattered. HE. Bar=30 µm.
Fig. 5. Mineral deposits and hyalinized connective tissue in the right mandibulopharyngeal mass. HE. Bar=165 µm.
Fig. 6. Positive immunoreactivity to chromogranin A of neoplastic cells and a degenerative ganglion cell (arrow) in the right mandibulopharyngeal mass. Envision Polymer method. Bar=30 µm.
Fig. 7. Positive immunoreactivity to TH of neoplastic cells. Envision Polymer method. Bar=30 µm.
Fig. 8. Positive immunoreactivity to synaptophysin of neuropil-like stroma (asterisks) and neoplastic cells. Envision Polymer method. Bar=40 µm.
clonal antibody, NB84a, was produced using a human neuroblastoma as a source of antigen [9, 13]. The present canine neoplastic cells were negative for NB84a, probably due to less antigen cross-reactivity of the two species.

REFERENCES