Case Report

A Rhabdomyosarcoma Arising in the Larynx of a Dog

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Abstract: A neoplastic nodular lesion, 2 × 3 cm in diameter, was found in the larynx of a 6-year-old spayed female dog. The tumor was ill-circumscribed, consisting histologically of large round cells with abundant cytoplasm interspersed with small round cells with less cytoplasm and occasional multinucleated cells (myotubes). Immunohistochemically, tumor cells were positive for myoglobin, desmin and vimentin in varying degrees, but negative for S-100 protein, GFAP or cytokeratin. Cytoplasmic myofilaments/myofibrils with a dense Z-line-like structure were seen, the fine structures of which were complemented by PTAH stain. Based on these findings, the tumor was diagnosed as a rhabdomyosarcoma, a very rare tumor in the larynx of dogs. (DOI: 10.1293/tox.24.179; *J Toxicol Pathol* 2011;24: 179–182)

Key words: dog, larynx, rhabdomyosarcoma

Tumors that arise in the larynx are very uncommon in dogs; the frequency accounts for 0.02% of all biopsy and necropsy specimens^{1,2}. Primary laryngeal tumors in dogs involve adenoma/adenocarcinoma, papilloma/squamous cell carcinoma, chondroma/chondrosarcoma, fibroma/fibrosarcoma, and rhabdomyoma/rhabdomyosarcoma^{1,2}. To our knowledge, the number of canine striated muscle-derived laryngeal tumors reported in English literature is eight^{1–7}; in Japan, there have been no reports on this tumor type. Because of the rarity, the pathological characteristics of striated muscle-derived laryngeal tumors should be investigated more. The information on various canine tumors would be useful for investigators in the toxicologic pathology field^{8,9}.

We encountered a laryngeal rhabdomyosarcoma in a 6-year-old spayed female mongrel dog (6.3 kg in body weight). The patient was presented to the animal teaching hospital of our university with 1.5-year history of difficult breathing, inspiratory stridor and loss of bark; these clinical signs were gradually exacerbated with time. A nodular lesion (2 × 3 cm in diameter) projecting from the epiglottis of the right larynx was found by the endoscopic examination (Fig. 1). The nodular lesion was not encapsulated and invad-

ed into surrounding tissues. The lesion was surgically removed as far as possible and examined histopathologically as a biopsy sample. The removed tissues were fixed in 10% neutral phosphate-buffered formalin, embedded in paraffin and cut into 4-μm sections. The sections were stained by hematoxylin and eosin (HE), Watanabe's silver impregnation and phosphotungstic acid-hematoxylin (PTAH) stainings. Immunohistochemical analyses were performed using mouse monoclonal antibodies against vimentin (clone V9, 1:400), desmin (clone D33, 1:200), α-smooth muscle actin (α-SMA, clone 1A4, 1:100), cytokeratin (clone AE1/AE3, 1:100) and Ki-67 (clone MIB-1, 1:100), as well as rabbit polyclonal antibodies to S-100 protein (1:500), glial fibrillary acidic protein (GFAP, 1:300) and myoglobin (1:200). These antibodies were purchased from Dako Japan Co., Ltd. (Tokyo, Japan). After antigen retrieval, sections were incubated with 3% H₂O₂ in phosphate-buffered saline (PBS) for 10 min to quench endogenous peroxidase and then with 5% skimmed milk in PBS for 30 min to inhibit nonspecific reaction. The sections were reacted with each primary antibody for 14 h at 4 °C, followed by horseradish peroxidase-conjugated secondary antibody for the monoclonal and polyclonal antibodies (Histofine Simple Stain MAX-PO, Nichirei, Tokyo, Japan) for 30 min. Positive reactions were visualized with 3, 3'-diaminobenzidine (DAB substrate kit, Vector Laboratories, Burlingame, CA, USA). Sections were counterstained lightly with hematoxylin. As negative controls, tissue sections were treated with mouse or rabbit normal IgG instead of the primary antibody. For electron microscopy, formalin-fixed samples, 1 mm cubes, were postfixed in

- Fig. 1.** Endoscopic finding. A nodular lesion (✱; 2 × 3 cm in diameter) projecting from the epiglottis (arrow) of the right larynx can be seen. Bar = 1 cm.
- Fig. 2.** Tumor consisting of neoplastic proliferation of large round cells with abundant eosinophilic cytoplasm and hyperchromatic nuclei and small round cells with less cytoplasm; these cells are arranged in a compact sheet. HE stain. Bar = 80 µm.
- Fig. 3.** Multinucleated cells with eosinophilic cytoplasm can be seen and are apparently myotubes. HE stain. Bar = 20 µm.
- Fig. 4.** Reticulin fibers surrounding individual cells or clusters consisting of several neoplastic cells are present. Watanabe's silver impregnation stain. Bar = 60 µm.
- Fig. 5.** Large round neoplastic cells with abundant cytoplasm have bizarre, granular cytoplasm stained blue by phosphotungstic acid-hematoxylin (PTAH) stain, whereas small round cells with less cytoplasm are not stained with PTAH (arrows). PTAH stain. Bar = 20 µm.
- Fig. 6.** Neoplastic cells reacting to Ki-67 can be seen, indicating potential proliferating activity. Immunohistochemistry, without nuclear stain. Bar = 40 µm.
- Fig. 7.** Neoplastic cells reacting variously to desmin are shown; however, small round cells with less cytoplasm are negative (arrows). Immunohistochemistry, counterstained with hematoxylin. Bar = 80 µm.
- Fig. 8.** Elongated neoplastic cells reacting to myoglobin can be seen (arrows). Immunohistochemistry, counterstained with hematoxylin. Bar = 60 µm.
- Fig. 9.** Vimentin immunoreaction can be seen mainly in small round neoplastic cells. Immunohistochemistry, counterstained with hematoxylin. Bar = 80 µm.
- Fig. 10.** Fine structure of a neoplastic cell having a number of mitochondria and filaments (✱) in cytoplasm. Cytoplasmic invagination into nuclei can be seen (small arrow). A basal lamina is present along the cell membrane (large arrows). Uranyl acetate and lead citrate stain. Bar = 2 µm.
- Fig. 11.** Fine structure of a neoplastic cell having filaments fragmented or organized in bundles with electron-dense plaque (apparently a Z-band). Uranyl acetate and lead citrate stain. Bar = 0.5 µm.

osmium tetroxide and embedded in epoxy resin. Ultrathin sections were double stained with uranyl acetate and lead citrate and examined in a transmission electron microscope (H-7500, Hitachi, Tokyo, Japan).

Histopathologically, the nodule lesion was composed of neoplastic proliferation of large round cells with abundant eosinophilic cytoplasm and hyperchromatic nuclei, and smaller round cells with scanty cytoplasm were interspersed throughout tumor tissue (Fig. 2); these neoplastic cells were arranged in a compact sheet with fine fibrous stroma. In the periphery of tumors with abundant collagen fibers, elongated, multinucleated cells with eosinophilic cytoplasm were occasionally seen (Fig. 3). Reticulin fibers, demonstrable by Watanabe's silver impregnation, surrounded individual cells or clusters consisting of several neoplastic cells in a part of the tumor (Fig. 4). Large round neoplastic cells with abundant cytoplasm and multinucleated elongated cells had bizarre, granular cytoplasm stained blue by PTAH stain (Fig. 5), but small round cells with less cytoplasm were not stained with PTAH. Mitotic figures were rare, but under a high power field (× 400), 5–10 cells showed a positive reaction for Ki-67 (Fig. 6), indicating potential proliferating activity.

Immunohistochemically, neoplastic cells reacted variously for desmin; however, there were occasional neoplastic cells negative to desmin (Fig. 7). While small round cells did not react to myoglobin, large round and elongated cells were positive for myoglobin (Fig. 8). Vimentin reaction was seen exclusively in small round cells (Fig. 9). There were no cells reacting to α -SMA, GFAP, S-100 protein or cytokeratin.

Electron microscopically, neoplastic cells had a number of mitochondria and filaments in cytoplasm (Fig. 10);

in some neoplastic cells, the filaments were fragmented or organized in bundles with electron-dense plaque (apparently Z-line; Fig. 11), indicating the presence of myofilaments/myofibrils. Rough endoplasmic reticula and glycogen granules were scattered in the cytoplasm. Cytoplasmic invagination into nuclei was seen occasionally (Fig. 10). Although desmosome-like structures were not found, a basal lamina along the cell membrane was present between neoplastic cells (Fig. 10). Cellular borders were distinct.

When the derivation of neoplastic cells is not identified, tumors consisting of large round cells with abundant eosinophilic cytoplasm arranged in a compact sheet may be diagnosed as oncocytomas based on the cellular morphology^{2,5}. Oncocytomas are considered to be of epithelial cell origin, and have been found in a variety of organs such as the thyroid glands, salivary glands, kidneys and liver; oncocytoma-consisting cells in these organs have finely granular and intensely eosinophilic cytoplasm and are packed with numerous mitochondria when examined under electron microscopy^{2,10–12}. Under light microscopical observation, the present tumor was characterized by large round cells with abundant cytoplasm interspersed with small round cells with less cytoplasm. Therefore, a differential diagnosis should be made for oncocytomas².

The negative reactions to cytokeratin and S-100 protein/GFAP excluded the possibility that the tumor was derived from epithelia and neurogenic cells, respectively. Desmin was found in tumors derived from both skeletal muscles and smooth muscles^{2,13}; however, α -SMA, which is expressed in smooth muscle cells and myofibroblasts^{14,15}, was not seen in the present neoplastic cells. The appearance of multinucleated elongated cells (apparently myotubes), immunopositive reactions for desmin and myoglobin and cy-

toplasmic myofilaments/myofibrils with a dense Z-line-like structure indicate that the present tumor was generated from striated muscles in the larynx. The bizarre, granular cytoplasm stained blue by the PTAH stain indicated the presence of irregularly-organized myofilaments/myofibrils in the cytoplasmic and complemented the positive reaction for myoglobin in neoplastic cells. Under the electron microscopy, the basal lamina is present constitutively in the stratified muscles². However, numerous mitochondria, which may be packed in the cytoplasm of oncocytes^{10,12}, were not seen in the present tumor. These findings clearly demonstrated that the present tumor arising in the larynx was derived from striated muscle; based on the anatomical localization, these muscles might be laryngeal or palatopharyngeal muscles.

To our knowledge, six rhabdomyomas and two rhabdomyosarcomas in the larynx have been reported in dogs^{1–7}; a histopathological difference between benign and malignant tumors is in that the latter consists of more poorly differentiated, pleomorphic cells. The pathological characteristics of the present tumor appeared to be similar to those of the latter. Canine rhabdomyomas reported in other organs such as the heart and tongue are well-circumscribed, being composed of relatively well-developed striated muscles^{14,15}; out of them, a cardiac rhabdomyoma was positive for myoglobin and desmin, but negative for vimentin¹⁴. Myoglobin is found in relatively well-developed skeletal muscles such as myotubes and large round cells with abundant cytoplasm in neoplastic tissues, whereas neoplastic cells reacting to desmin and vimentin may be less differentiated^{2,13,16}. Neoplastic cells of laryngeal rhabdomyosarcomas in humans were immunopositive for desmin and vimentin¹³. In the present tumor, neoplastic cells were positive for myoglobin, desmin and vimentin in varying degrees, and neoplastic cells negative for these markers were also present. These findings indicated that the present tumor involved pleomorphic neoplastic cells at different stages of development of skeletal muscles. In addition, the present tumor was ill-circumscribed, and according to later information from the veterinary surgeon, the tumor recurred after removal. Human rhabdomyomas are usually encapsulated or sharply demarcated and do not metastasize or recur⁵. Based on these findings, the present striated muscle-derived laryngeal tumor was considered to be malignant in behavior and cellular morphology.

The incidence of human laryngeal rhabdomyosarcomas is very rare, and the pathogenesis remains to be investigated^{13,16,17}. Three histological subtypes of human rhabdomyosarcomas have been identified: the embryonal, alveolar and pleomorphic types¹⁷. The present canine case may be regarded as a low-grade pleomorphic type of rhabdomyosarcoma because of involvement of various stages of less- or well-differentiated cells. Human rhabdomyosarcomas are the most frequent soft tissue tumor in pediatric patients¹⁷. The ages of the 8 canine cases of striated muscle-derived tumors ranged from 2 to 8 years, and there was no species or sex predilection. Cases should be accumulated to clarify the etiology, clinical behavior and histogenesis of laryngeal striated muscle-derived tumors in dogs.

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