

Olfactory Neuroblastoma in a Horse

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ABSTRACT. An 11-year-old thoroughbred gelding was euthanatized because of right nasal cavity tumor. The tumor consisted of round to oval cells with a scanty cytoplasm and hyperchromatic nuclei. Homer-Wright rosettes and pseudorosettes, as well as microcysts were seen. Neoplastic cells were immunoreactive to vimentin, S-100 protein, and neuron-specific enolase, glial fibrillary acidic protein and microtubule-associated protein in varying degrees, indicating neurogenic nature. Based on these findings, this tumor was diagnosed as an olfactory neuroblastoma. Since this type is an uncommon tumor showing histological variety, the nature is discussed.

KEY WORDS: equine esthesioneuroblastoma, immunohistochemistry, sinonasal tumor.

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Olfactory neuroblastomas (esthesioneuroblastomas) are uncommon, malignant neoplasms, most of which arise in the esthemoturbinate region of the caudal nasal cavity. The tumors consist of islands and sheets of exclusively round-to-oval neuroblastic cells, and pseudorosette or microcyst formation is characteristic of this tumor [4, 15]. However, olfactory neuroblastomas show heterogeneous histology. In human cases, epithelial, endocrine or rhabdomyoblastic differentiation has been reported [9, 10]. Therefore, consistent immunohistochemical stainings have not yet been established, although positive reactions to neurogenic markers such as S-100 protein and neuron-specific enolase (NSE) are relatively identical [15, 16]. In veterinary medicine, only a few cases of olfactory neuroblastomas have been described in dogs, cats, cattle and monkeys [4, 6, 11, 15]. Rosette formation has been rarely seen in canine and bovine cases, whereas rosettes appear to be a more consistent finding in feline cases [4, 6, 15]. The cellular nature of olfactory neuroblastomas remains to be investigated. Two cases of olfactory neuroblastomas have been reported in horses [3, 8]. Because of heterogeneous histology, this report describes histopathological characteristics of a spontaneous equine olfactory neuroblastoma that we have recently encountered.

An 11-year-old thoroughbred gelding was referred to Veterinary Teaching Hospital of Osaka Prefecture University with a three-month history of lacrimation and exophthalmos of the right eye, as well as epistaxis from right nasal cavity. Endoscopic examination revealed a large nodule obstructing the right nasal cavity. During two months after presentation, the nodule quickly grew up with more marked nasal hemorrhage and proptosis. The nodule was regarded as a malignant neoplasia, and the horse was euthanatized at the owner's request. At necropsy, the nodule completely occupied the olfactory region of right nasal cavity, destroying the right-side nasal turbinates; the nodule extended up to 15 cm posterior to the external nose and invaded into the right-side frontal sinuses. No invasive growth into the cra-

nial cavity or metastasis into the regional lymph nodes and visceral organs was seen. The left nasal cavity was intact. The nodule was yellow in color with hemorrhagic and necrotic areas (Fig. 1).

The removed tumor was fixed in 10% neutral buffered formalin. The tissues were subsequently dehydrated and embedded in paraffin. Deparaffinized sections were stained with hematoxylin and eosin (HE), and by the azan-Mallory, periodic acid Schiff (PAS), and Watanabe's silver impregnation methods. Sections were also stained by the immunohistochemistry with avidin-biotin complex method (Dako, Copenhagen, Denmark). The primary antibodies used were polyclonal antibodies against S-100 protein (Dako; $\times 500$), myelin basic protein (MBP) (Dako; predilution), glial fibrillary acidic protein (GFAP) (Dako; $\times 1000$), chromogranin A (Dako; predilution), high-molecular weight neurofilament (NF) (Serotec, Oxford, UK; $\times 500$) and microtubule-associated protein (MAP) (Dako; $\times 100$), as well as monoclonal antibodies against NSE (Dako; predilution), synaptophysin (Chemicon International, Temecula, CA, U.S.A.; $\times 200$), Melan-A (Dako; $\times 200$), cytokeratin (clone MNF116; Dako; predilution), desmin (Dako; $\times 200$), α -smooth muscle actin (α -SMA) (Dako; $\times 100$) and vimentin (Dako; $\times 200$). After deparaffinization, microwave antigen retrieval for 10-20 min was performed for antibodies to NSE, MBP, chromogranin A, Melan-A, NF, cytokeratin and vimentin; sections for S-100 protein, GFAP, and α -SMA were pretreated with 0.1% trypsin for 15-30 min at 37°C. Then, sections were treated with 5% skimmed milk for 30 min, and incubated with each primary antibody for 14 hr at 4°C. A 1-hr incubation with biotinylated goat anti-mouse antibody or goat anti-rabbit antibody followed. Final incubation was carried out for 1 hr with an avidin-horseradish peroxidase complex, and positive reactions were visualized with 3,3'-diaminobenzidine. Sections were counterstained lightly with hematoxylin. Non-immunized mouse or rabbit serum in place of the primary antibody served as negative control. For electron microscopy, formalin-fixed samples, 1 mm cubes, were

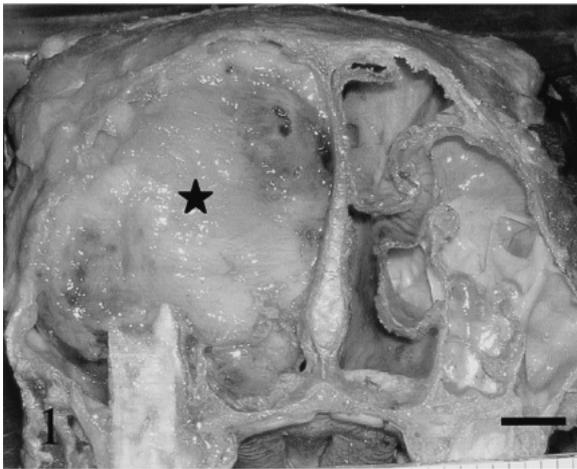


Fig. 1. Equine olfactory neuroblastoma; The cut surface of the tumor (asterisk) at the fourth molar tooth of the head. The tumor completely occupies olfactory region of the right nasal cavity. Bar=2 cm.

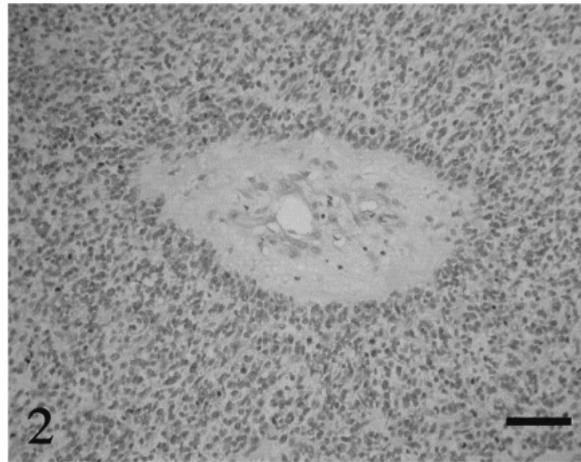


Fig. 2. The tumor consists of round to oval cells with scanty cytoplasm and hyperchromatic nucleus, and a rosette-like arrangement of tumor cells is present around the connective tissue surrounding blood vessel. HE. Bar=90 μ m.

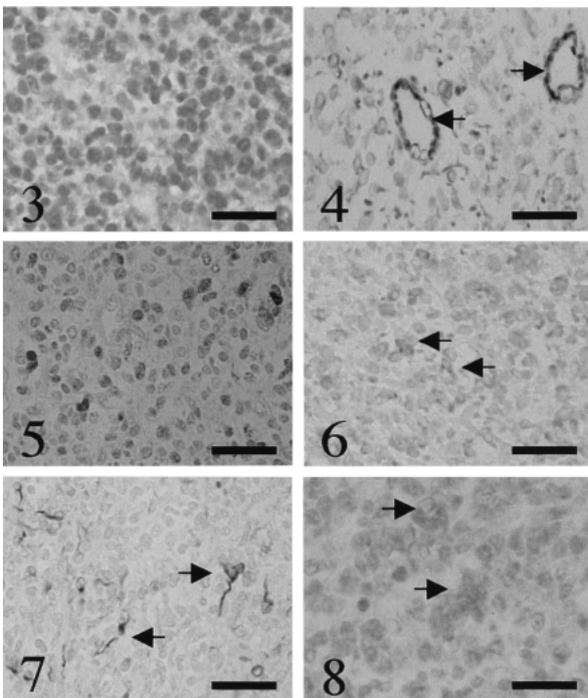


Fig. 3. A rosette formed by tumor cells gives an appearance of Homer-Wright rosette. HE. Bar = 40 μ m.

Fig. 4. Vimentin-positive cells and microcysts rimmed by neoplastic cells reacting to vimentin (arrows) are seen. Immunohistochemistry. Bar=50 μ m.

Fig. 5. Cytoplasm and nuclei of many neoplastic cells show a positive reaction to S-100 protein. Immunohistochemistry. Bar=50 μ m.

Fig. 6. Many neoplastic cells (arrows) react to neuron-specific enolase. Immunohistochemistry. Bar=50 μ m.

Fig. 7. Occasional neoplastic cells give a positive reaction to glial fibrillary acidic protein (arrows). Immunohistochemistry. Bar=50 μ m.

Fig. 8. Some neoplastic cells react to microtubule-associated protein (arrows). Immunohistochemistry. Bar=40 μ m.

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 microscope (H-7500, Hitachi, Tokyo, Japan).

The tumor was composed of a highly cellular, uniform cell population. The neoplastic cells were round to oval in shape, and showed a small amount of poorly defined cytoplasm with round and hyperchromatic nuclei (Figs. 2 and 3). Intercellular fibrillary background, demonstrable also by Watanabe's silver impregnation method, was present throughout tumor tissues, and a small amount of collagen fibers stained blue by azan-Mallory stain were seen among neoplastic cells. Rosette-like arrangements of the tumor cells were often seen around connective tissues surrounding blood vessels (Fig. 2) or around blood vessels, and Homer-Wright rosettes were occasionally seen in tumor tissues (Fig. 3). No external basement membrane around these rosettes was demonstrated by PAS reaction. Microcysts rimmed by neoplastic cells were seen mainly in the periphery of tumor tissues (Fig. 4). Numerous mitotic figures were seen. Electron microscopy revealed a few cytoplasmic microfilaments in neoplastic cells. Immunohistochemically, many neoplastic cells reacted to vimentin (Fig. 4), S-100 protein (Fig. 5), and NSE (Fig. 6), and occasional cells were positive for GFAP (Fig. 7) and MAP (Fig. 8). There were no neoplastic cells reacting to cytokeratin, desmin, α -SMA, NF, MBP, synaptophysin, chromogranin A or Melan-A.

In horses, malignant tumors arising in the nasal cavity are extremely rare; besides olfactory neuroblastomas, squamous cell carcinomas, adenocarcinomas, anaplastic carcinomas, and fibrosarcomas have been reported [4, 15]. Additionally, rhabdomyosarcomas, lymphosarcomas or malignant melanomas have been described in other domestic animals and humans as sinonasal malignant tumors [4, 5, 15]. Olfactory neuroblastomas needed to be differentiated

from aforementioned malignant tumors. Neoplastic rosette formation is a useful, but not an indispensable, diagnostic aid for olfactory neuroblastomas [4]. The presence of rosettes formed by neoplastic cells has not been described in carcinomas, lymphosarcomas, fibrosarcomas and rhabdomyosarcomas; no characteristic diagnostic pattern of NSE and S-100 protein could be also recognized in these tumors. The present tumor showed rosettes and immunoreactivities to S-100 protein and NSE, indicative of neurogenic origin. Although neuroectodermal tumors including melanomas may be positive for S-100 protein and NSE, Melan-A is specific for melanocytic tumors [1]. The present tumor cells did not react to Melan A. Ultrastructural findings such as microtubules, intermediate filaments or dense core secretory granules have been reported as a criterion for olfactory neuroblastomas [4], although not consistent findings. A few microfilaments were seen in the cytoplasm of the present tumor cells. Although dense core granules were not detected, some neoplastic cells immunohistochemically reacted to MAP. Based on anatomical location and histopathological findings, the present tumor was diagnosed as an olfactory neuroblastoma.

Neuroendocrine carcinomas are also a malignancy originating in the nasal cavity [4, 15]. Sinonasal neuroendocrine carcinomas have been reported exclusively in humans [14], and in domestic animals there is simply one report of a dog [4]. There are considerable overlaps of phenotypical characteristics, including rosette formation, immunoreactions to S-100 protein and NSE, and fine structures such as secretory granules, between these two tumors [4, 14]. Although neuroendocrine carcinomas would be expected to stain for chromogranin A and cytokeratin [4, 13, 14], positive cells to these markers have been described in human olfactory neuroblastomas [9, 10, 16]. There are difficulties in distinguishing olfactory neuroblastomas from neuroendocrine carcinomas [14, 15]. Therefore, some investigators have considered that these two tumors exhibit different manifestations of the same entity [9, 12, 15].

Olfactory neuroblastomas originate from the olfactory neuroepithelium, of which the texture consists of neurosensory cells extending their axons to the olfactory bulb, sustentacular cells, and basal cells [2]. The matured neurosensory cells in the olfactory mucosa express cytokeratin. The basal cells have capacity to differentiate into neurosensory cells and sustentacular cells [7]. Embryologically, the olfactory neuroepithelium develops from the olfactory placode [5, 13], and thus, olfactory neuroepithelial cells have highly pluripotential differentiation into neurogenic cells [7]. Neoplastic cells showing GFAP-immunoreactivity in the present tumor might be cells differentiating toward glial cells. In humans, olfactory neuroblastoma with epithelial and endocrine differentiation was transformed into ganglioneuroma after chemoradiotherapy [9]. Ganglion cell differentiation has been described in a canine olfactory neuroblastoma [4]. In human olfactory neuroblastomas, immunoreactivities to glial differentiation (GFAP), neuronal differentiation (synaptophysin, NF and

MAP), and endocrine cells (chromogranin A) and epithelial cells (cytokeratin) are diverse from case to case [10, 16]. Immunoreactivities to such markers should depend on different differentiation stages of neoplastic cells in olfactory neuroblastomas [9, 12, 14].

In 2005, Döpke *et al.* reported an equine olfactory neuroblastoma, which was the first equine case describing immunohistochemical findings [3]. In their case, besides immunoreactions to S-100 protein, NSE and GFAP, occasional neoplastic cells reacted to NF and synaptophysin. In the present tumor, no cells reacting to NF and synaptophysin were detected. The presence of intracytoplasmic neurosecretory granules was reported in their case, but not seen in our case. Pseudorosettes, which are seen in our case and in human cases [13], were lacking in their case. This might be due to different differential stages of neoplastic cells. Because of the absence of immunoreactivities to cytokeratin, NF and synaptophysin, the present tumor was regarded as a more poorly differentiated olfactory neuroblastoma.

Human olfactory neuroblastomas have a bimodal age distribution with an early peak from 11 to 20 years and a later peak between 51 and 60 years of age [5], and exhibit complex histology, ranging from neuroblastoma-like features to paraganglioma- or neuroendocrine carcinoma-like features [14]. Pathological investigations on further cases are required to better understand characteristics of olfactory neuroblastomas in domestic animals.

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