Olfactory Neuroepithelioma in a Dog: An Immunohistochemical and Electron Microscopic Study

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ABSTRACT. A case of olfactory neuroepithelioma was investigated electron microscopically and immunohistochemically. The tumor mass was found in the nasal cavities of a 10-year-old female dog, which showed epistaxis, nasal discharge and facial swelling. The tumor tissue consisted of tubular structure of cuboidal to columnar cells and compactly arranged nests of small cells surrounded by a fibrovascular stroma. Mitotic figures were frequently observed. Immunohistochemically, the tumor cells frequently showed positive for neuronal and epithelial features probably originating from the olfactory epithelium.

KEY WORDS: canine, neuroepithelioma, olfactory.

NOTE

Pathology

The olfactory neuroblastomas are uncommon, malignant neoplasms, most of which arise in the ethmoturbinate region of the caudal nasal cavity. It is unclear whether they arise from the olfactory neuroepithelial cells, remnants of neural crest cells, or local components of the dispersed neuroendocrine system. Thus not only the histological diagnosis but also the origin of the tumor is still a matter of controversy in humans as evidenced by the different terms used to describe the tumor [24]. These include olfactory neuroblastoma [6, 8, 18], olfactory esthesioneuroblastoma [4, 14], esthesioneurocytoma [2], esthesioneuroepithelioma [3], olfactory neuroepithelioma [21], intranasal neuroblastoma [19], and olfactory placode tumor [26]. Immunohistochemically, the tumor provided a variable number of cells positive for neurofilament protein, keratin, neuronspecific enolase and S-100 [23]. Based on these findings, Hassoun et al. [9] and Takahashi et al. [23] suggested that their cases consisted of cells differentiating in at least two distinct directions, neuronal and epithelial, and concluded that the tumors were of true olfactory epithelium origin, or more precisely, derived from the bipotential, undifferentiated basal cells of the epithelium and differed from olfactory neuroblastomas which are generally considered to be “APUD (amine precursor uptake and decarboxylation) tumors”. Based on these findings, the term of olfactory neuroepithelioma was added in the category of olfactory neuroblastoma as a new variant in the WHO classification of human tumors [20]. On the other hand, in the veterinary literature, the term olfactory neuroblastoma (esthesioneuroblastoma) is still used in the WHO classification [10] as well as in case reports [1, 7] of the tumor in domestic animals. Detailed immunohistochemical and electron microscopic study on these tumors in the veterinary literature is limited.

In this report, we describe histological, immunohistochemical and ultrastructural features of a spontaneous case of olfactory neuroepithelioma in a 10-year-old female dog.

A 10-year-old female mongrel dog, with a 2-month-history of epistaxis, nasal discharge and facial swelling was brought to the Veterinary Clinic in Tottori University. X-ray examination demonstrated an oval to round mass, 3 cm in diameter, in the left nasal cavity. The mass destroyed nasal turbinates extending into the right nasal cavity beyond the nasal septum. The animal was killed humanely because of poor prognosis. A complete necropsy was performed. Samples of the solid mass, reddish white in color, were taken and immersed in 10% neutral buffered formalin for histological examination. They were then dehydrated, embedded in paraffin wax, sectioned at 4 µm, and stained with haematoxylin and eosin (HE). The mass in the nasal cavity was tentatively diagnosed as an undifferentiated carcinoma. With the aid of labeled streptavidin-biotin kit (Dako, Glostrup, Denmark), selected sections from the tumor were assessed reactivity for cytokeratin, neurofilament protein (NFP), synaptophysin, and carnosine which are markers for the olfactory cells [17]. Polyclonal anti-carnosine antibody (generous gift from Dr. Sakai, Department of Anatomy, School of Medicine, Fujita Health University, Japan) was diluted at 1:2000. Monoclonal anti-cytokeratin antibody (Cytokeratin19, RCK108, Dako), anti-NFP antibody (2F11, Dako), and monoclonal anti-synaptophysin antibody (SY38, Dako) were diluted at 1:50. Deparaffinized sections were pretreated with 0.3% H2O2-methanol for 30 min at room temperature and normal goat serum for 30 min at 4°C. The sections were incubated with the primary antibodies overnight at 4°C. Immunoreactivity was detected with diaminobenzidine (DAB) as chromogen. The sections were counterstained with methyl green.

For ultrastructural study, formalin-fixed samples were diced at 1 mm3, washed in 0.1 M phosphate buffer (pH 7.4)
for three hours, and post-fixed in 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were cut, stained with lead citrate and uranyl acetate, and examined on an electron microscope (JEM-100CXII).

The tumor showed combination of tubular structure with occasional glandular or Flexner rosette-like patterns and thick, solid, medullary-like or fascicular cords of spindle cells. These cords were separated by thin connective tissue septa (Fig. 1). Occasional palisading pattern was also associated with the tumor. These tumor cells had eosinophilic cytoplasm with pale, round to oval nuclei. Mitotic figures were frequently observed. Some areas of haemorrhage, necrosis, and infiltration of inflammatory cells were found. Histological examination revealed invasive growth of the tumor to the right nasal cavity and metastasis of the tumor tissue to the subparotid lymph node, in which the same histological features as those of the original tumor tissue were observed. Columnar cells consisting of the tubular or acinar structure showed strong cytokeratin immunoreactivity (Fig. 2a), while small round or spindle cells occasionally showed immunoreactivity for both NFP (Fig. 2b) and synaptophysin (Fig. 2c). A small population of the columnar cells were positive for carnosine (Fig. 2d); positive findings were clearly shown in the normal olfactory cells in the intact olfactory epithelium of the dog. Ultrastructurally, the columnar type of the tumor cells was characterized by desmosome-like structure between the tumor cells. Neither dense-core granules nor neuritic structure were observed in the tumor cells (Fig. 3). A small number of microvilli-like structure were occasionally demonstrated in the luminal surface of the acinar structure. Compactly packed round to spindle cells also showed similar ultrastructural findings with rare structure of tight junction.

The olfactory epithelium is composed of three cell types: the neurosensory cells (olfactory cells) extending their axons to the olfactory bulb; the sustentacular cells being the supportive element; and the basal cells having a potential of differentiation to either neurosensory cells or sustentacular cells [6]. None of these cells normally contains neurosecretory granules. These olfactory structures are considered to be originated from the ectodermal placodes [13]. Ultrastructurally, reported olfactory neuroblastomas in both humans and animals frequently contained neurosecretory granules, leading researchers to believe that these tumors originated from regional neural crest tissue rather than from the olfactory epithelium [6, 7]. Based on the findings from

![Fig. 1. Light microscopy showing compactly packed round to spindle tumor cells with occasional glandular patterns. HE, × 380.](image1)

![Fig. 2. Tumor cells are immunoreactive for cytokeratin (a), neurofilament protein (b), synaptophysin (c) and carnosine (d). Immunohistochemistry, × 550.)](image2)

![Fig. 3. Electron microscopy showing desmosome-like structures (arrows) between the tumor cells. Note no signs of dense-cored secretory granules in the tumor cells. × 14,000.](image3)
immunohistochemistry and electron microscopy, Hassoun et al. [9] and Takahashi et al. [23] concluded that cases of the olfactory neuroblastomas contained a rare variant, olfactory neuroepithelioma/esthesioneuroepithelioma, which was a true neurosensorial tumor originating from the olfactory epithelium and differed from reported so-called olfactory neuroblastomas which were generally considered to be “APUD tumors”.

Histological examination of the present tumor arising in the nasal cavity of the 10-year-old dog demonstrated mixture of two cell types resembling bipolar neuron-like cells with occasional palisade formations and columnar cells with characteristic tubular or rosette-like arrangements, which were originally described by Berger et al. [3].

NFP, synaptophysin, keratin, and carnosine were detected immunohistochemically in a variable number of tumor cells in the present study. Immunohistochemical study on the human and rat olfactory epithelium demonstrated that sensory neurons were positive for NFP and sustentacular cells for keratin [22, 27]. Carnosine immunoreactivity was also demonstrated in the olfactory sensory neurons in the human and rat olfactory epithelium [16, 17]. Peripheral neuronal tumors such as neuroblasta, ganglioneuroblastoma, and ganglioneuroma, neoplastic neuroblasts and ganglion cells have been shown to be immunoreactive for NFP [5, 15]. On the other hand, keratin has been shown to be a useful marker for the epithelial nature of the tumor [12, 15]. These findings imply that the present tumor consisted of two distinct cell types, neuronal and epithelial.

Reported ultrastructural studies on so-called olfactory neuroblastoma frequently demonstrated dense-cored or membrane-bound secretory granules in the tumor cells [6, 25], while no dense-cored secretory granules were found in esthesioneuroepitheliomas [9, 11, 23]. In this study, tight junction being a character of the epithelial cells was observed but no sign of secretory granules.

Results from the immunohistochemical and ultrastructural studies suggest that the present tumor, diagnosed as olfactory neuroepithelioma, is derived from the bipotential, undifferentiated basal cells of the olfactory epithelium. Nasal tumors including undifferentiated carcinomas with mixed phenotypic expression should be carefully examined for the proper diagnosis.

REFERENCES


