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Olfactory Neuroblastoma in Dogs and Cats – a Histological and Immunohistochemical Analysis

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Summary

Olfactory neuroblastoma (ONB) was identified in 13 dogs and nine cats. The tumours were subjected to microscopical examination and were graded using a human pathological grading system. In the canine and feline tumours there was more necrosis and higher mitotic activity (mitotic index and Ki67 labelling index) than reported in human ONB. Rosettes were a common feature of feline ONBs. A significant correlation was observed between the histological grade and the Ki67 labelling index. The histopathological diagnosis of ONB was confirmed immunohistochemically by demonstration of the neuronal marker neuron-specific enolase (NSE). Two other neuron-specific antibodies specific for microtubule-associated protein-2 (MAP-2) and neuronal nuclei antigen (NeuN) were evaluated. MAP-2 expression proved to have higher specificity than labelling for NSE. NeuN expression was less sensitive and of limited practical value.

Keywords: cat; dog; immunohistochemistry; olfactory neuroblastoma

Introduction

Olfactory neuroblastoma (ONB), also known as esthesioneuroblastoma, is a rare malignant neuroectodermal tumour. This tumour is thought to be derived from the olfactory neuroepithelium and arises within the upper nasal cavity at the level of the cribriform plate. ONB was first described by Berger and Luc (1924) as 'esthesioneuroepitheliome olfactif' and has been reported previously in dogs, cats, horses and a cow (Anderson and Cordy, 1981; Cox and Powers, 1989; Schrenzel *et al.*, 1990; Hara *et al.*, 2002; Döpke *et al.*, 2005; Kitagawa *et al.*, 2006; Yamate *et al.*, 2006; Ueno *et al.*, 2007).

Despite the morphological heterogeneity of ONB, some specific and reliable histological, ultrastructural and immunohistochemical diagnostic criteria have been established in man (Mills *et al.*, 2000; Wenig *et al.*, 2005). Histological features of well-differentiated ONBs include growth in circumscribed lobules, separated by a richly vascularized fibrous stroma, or less commonly, a diffuse growth pattern. Pseudorosettes and true rosettes can also be found. The neoplastic cells are surrounded by a neurofibrillary matrix and have sparse cytoplasm and round to oval nuclei with inconspicuous nucleoli. Nuclear pleomorphism, high mitotic activity and necrosis are infrequently observed. Ultrastructural characteristics include dense core neurosecretory granules measuring 50–250 nm in diameter and neurite-like cell processes with neurofilaments and neurotubules. Immunohistochemically, neuron-specific enolase (NSE), a neuronal marker can be detected (Mills *et al.*, 2000; Wenig *et al.*, 2005). Synaptophysin, neurofilament protein, class III beta-tubulin and microtubule-associated protein-2 (MAP-2) are less frequently detected. Chromogranin,

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glial fibrillary acidic protein (GFAP) and cytokeratin may be present; however, epithelial membrane antigen (EMA) and carcinoembryonic antigen (CEA) are consistently negative (Mills *et al*., 2000; Wenig *et al*., 2005).

In man, a histological system of classification for ONB was established to provide a more accurate basis for prognosis (Hyams, 1988). The classification takes into account the grade of cellular differentiation and the mitotic index (MI) and includes four grades. Microscopically, lower grade tumours (grades I and II) have been reported to have a better 5-year survival than higher-grade tumours (grades III and IV) (Mills *et al*., 2000; Wenig *et al*., 2005). Reports on the application of this classification system for ONB in animals are limited (Hara *et al*., 2002; Ueno *et al*., 2007).

Diagnosis of ONB is also made difficult by the similarities between this tumour and sinonasal undifferentiated carcinoma (SNUC), malignant melanoma and neuroendocrine carcinoma (NEC) (Koestner *et al*., 1999). To assist with differential diagnosis, NSE expression is used to support the diagnosis of ONB, despite the low specificity of this immunohistochemical labelling. Other potential markers may include the neuron-specific markers MAP-2 and NeuN (neuronal nuclei antigen), which are reliable markers of neuronal and neuroendocrine tumours in man (Wolf *et al*., 1996; Blümcke *et al*., 2001, 2004; Englund *et al*., 2005; Hasselblatt *et al*., 2006; Preusser *et al*., 2006). In human pathology, the use of NeuN provides clearly defined nuclear labelling, making this marker suitable for quantitative analysis of neoplastic tissue (Wolf *et al*., 1996). NeuN expression has not been examined in animal or human ONBs, but MAP-2 expression has been reported in human and one equine ONB (Liu *et al*., 2003; Wenig *et al*., 2005; Yamate *et al*., 2006).

The aim of the present study was to evaluate 13 canine and nine feline ONBs, using the Hyams' histological classification and a range of immunohistochemical markers including NSE, NeuN and MAP-2. The proliferative activity of the tumours was also estimated by use of the MI and determination of the Ki67 labelling index (LI).

Materials and Methods

Tumour Samples

The 13 canine and nine feline ONBs were retrieved from the archive of the Institute of Pathology, Ludwig-Maximilians University, Munich and the Specialty Practice for Veterinary Pathology, Munich (Table 1). Samples had been obtained between 1984 and 2008. All specimens had been fixed in 10% neutral buffered formalin, embedded in paraffin wax, sectioned (5 μm) and stained with haematoxylin and eosin (HE).

Immunohistochemistry

Immunohistochemistry (IHC) was performed as described by Blümcke *et al*. (2001) and Preusser *et al*. (2006). Sections were dewaxed and subjected to antigen retrieval by incubation in citrate buffer (pH 6.0) and heating in a microwave oven (5 min at 800 W and 20 min at 400 W). For Ki67 IHC, sections were treated additionally with 0.1% trypsin (37°C for 8 min) before antigen retrieval in citrate buffer. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide. Non-specific binding was blocked by use of goat serum (diluted 1 in 20; Novocastra Laboratories, Newcastle, UK) for 30 min. Incubation with primary antibodies was performed overnight at 4°C with the following reagents: mouse anti-Ki67 (clone MIB1; Dako, Glostrup, Denmark; diluted 1 in 200), mouse anti-NeuN (clone A60; Chemicon R International, Billerica, Massachusetts, USA; diluted 1 in 500), mouse anti-MAP-2 (clone AP18; Kamiya Biomedical Company, Seattle, Washington, USA; diluted 1 in 100) and rabbit anti-NSE (polyclonal antibody; Biomol GmbH, Hamburg, Germany; diluted 1 in 500). Sections were subsequently washed in phosphate buffered saline (PBS; pH 7.6, 0.01 M). Secondary biotinylated goat anti-mouse and anti-rabbit antibodies (Vector Laboratories, Burlingame, California, USA) were diluted 1 in 200 and incubated with the sections for 30 min at room temperature. Sections were then washed again in PBS. Bound antibodies were 'visualized' using 3,3′-diaminobenzidine (DAB; Kem-En-Tec Diagnostics, Taarstrup, Denmark) as the chromogen. All sections were counterstained with Mayer's haematoxylin (AppliChem GmbH, Darmstadt, Germany) and mounted under xylene-based mounting solution (Paul Marienfeld, Lauda-Koenigshofen, Germany).

Evaluation of Sections

The Hyams' histological system of classification was initially applied to all tumours (Table 2). MAP-2, NeuN and NSE expression were evaluated descriptively. Immunoreactivity for MAP-2 was additionally analyzed semiquantitatively. The proportion of labelled cells was scored as: negative (−), <1%; mild (+), 1–33%; moderate (++), 33–66%; high (+++), >66%. The MI was determined as the number of mitotic figures within 10 high power fields (×400). To evaluate the LI for Ki67, the number of moderately and strongly labelled cells among 1,000 cells in randomized high power fields (×400) was counted. Quantification was accomplished by digital capture of images using a ZEISS Axioplan R microscope (Carl Zeiss GmbH, Jena, Germany) and ImageJ Version 1.41 software (http://rsbweb.nih.gov/ij/).