J. Comp. Path. 2015, Vol. 152, 188–191

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NEOPLASTIC DISEASE

Neuroendocrine Carcinoma of the Mammary Gland in a Dog

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Summary

A 10-year-old female border collie was presented with a mass (2 cm diameter) in the fifth mammary gland. The mass was located in the subcutis and the cut surface was grey–white in colour. Microscopically, the mass was composed of tumour cells arranged in nests of various sizes separated by delicate fibrovascular stroma. The tumour cells had small, round hypochromatic nuclei and abundant cytoplasm. Metastases were observed in the inguinal lymph node. Immunohistochemically, most tumour cells expressed cytokeratin (CK) 20, chromogranin A, neuron-specific enolase, synaptophysin and oestrogen receptor-β, but not low molecular weight CK (CAM5.2), p63 and insulin. Ultrastructurally, the tumour cells contained a large number of electron-dense granules corresponding to neuroendocrine granules. Based on these findings, this case was diagnosed as a neuroendocrine carcinoma of the mammary gland.

Keywords: dog; mammary gland; neuroendocrine carcinoma

Mammary gland tumours are the most common neoplasms in female dogs and approximately 50% are malignant (Misdorp *et al.*, 1999; Sorenmo *et al.*, 2013). Mammary cancers are clinically significant in both veterinary and human medicine. According to the World Health Organization (WHO) classification, human breast carcinoma with neuroendocrine features is defined as a special type of invasive breast carcinoma and represents <1% of breast carcinomas. Carcinomas with neuroendocrine features are classified into the following three types, (1) neuroendocrine tumour, well differentiated, (2) neuroendocrine carcinoma, poorly differentiated/small-cell carcinoma and (3) invasive breast carcinoma with neuroendocrine differentiation, on the basis of evaluation of morphology, immunohistochemical expression of endocrine markers and proliferative activity (Bussolati and Badve, 2012).

In dogs, Goldschmidt *et al.* (2011) have recently proposed a new histological classification and grading system based on a modification of the WHO criteria. The canine malignant epithelial neoplasms include squamous cell, adenosquamous, mucinous, lipid-rich (secretory), spindle cell and inflammatory carcinomas (Goldschmidt *et al.*, 2011). However, carcinomas with neuroendocrine features have not been described. So far, neuroendocrine carcinomas arising primarily in the mammary gland have not been reported in domestic animals. To the best of our knowledge, this is the first report of a neuroendocrine carcinoma originating as a primary tumour in the mammary gland of a dog.

A 10-year-old female border collie was admitted to Namiki Animal Hospital, Chiba, Japan, with a mass (2 cm diameter) in the left fifth mammary gland. The mass and the inguinal lymph node were removed surgically. Apart from the mass, no other abnormalities were observed on physical examination, complete blood count or routine serum biochemical profile.

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0021-9975/$ - see front matter
http://dx.doi.org/10.1016/j.jcpa.2014.12.009

Detailed ultrasonographical and radiographical examinations detected no tumours or masses in the thoracic or abdominal cavities. Additional radiation and chemotherapy was not performed after the surgical excision. The dog died of malignant islet cell tumour and systemic metastases with hypoglycaemia and anaemia 19 months after excision of the mammary tumour. At necropsy examination, the tumour diagnosed at the time of biopsy collection was not observed in other organs, including the mammary glands.

The original mammary mass and inguinal lymph node were fixed in 10% neutral-buffered formalin and embedded in paraffin wax. Sections (3 μm) were stained with haematoxylin and eosin (HE), mucicarmine, periodic acid—Schiff (PAS) and Grimelius stains. Serial sections were subjected to immunohistochemistry (IHC) using a streptavidin—biotin complex method with primary mouse antibodies specific for cytokeratin (CK) AE1/AE3 (Dako, Glostrup, Denmark; 1 in 200 dilution), low molecular weight CK (CAM5.2; Becton Dickinson, Franklin Lakes, New Jersey, USA; prediluted), CK8 (Progen Biotechnik GmbH, Heidelberg, Germany; 1 in 50 dilution), CK20 (Dako; 1 in 50 dilution) and E-cadherin (Dako; 1 in 150 dilution), oestrogen receptor-β (OR-β, AbD Serotec, Oxford, UK; 1 in 50 dilution), neuron-specific enolase (NSE, Dako; 1 in 200 dilution), vimentin (Dako; 1 in 100 dilution), p63 (Neo-Markers Inc., Fremont, California, USA; 1 in 200 dilution) and Ki67 (Dako; 1 in 100 dilution)and primary rabbit antibodies specific for chromogranin A (Dako; 1 in 200 dilution), PGP9.5 (Dako; 1 in 200 dilution) and synaptophysin (Dako; 1 in 200 dilution) and guinea pig antibody specific for insulin (Biomeda Corp., Foster City, California, USA; 1 in 400 dilution). The antibodies used were validated by noting a positive reaction with their corresponding normal tissues and a negative reaction on replacement with normal mouse or rabbit immunoglobulins. The Ki67 index was assessed by counting both Ki67-positive and Ki67-negative tumour cells in 10 randomly selected fields at ×400 magnification and expressing the count as a percentage (Ki67-positive cells/total number of cells counted). For electron microscopical examination, small pieces of the formalin-fixed tumour tissue were refixed in 1% osmium tetroxide, followed by 0.2 M phosphate buffer, and then embedded in epoxy resin. Using an electron microscope, ultrathin sections were examined after staining with uranyl acetate and lead citrate.

The mass was located in the subcutis and the cut surface was grey—white in colour. Microscopically, the mass was composed of tumour cells arranged in nests of various sizes separated by delicate fibrovascular stroma. The tumour cells invaded the surrounding

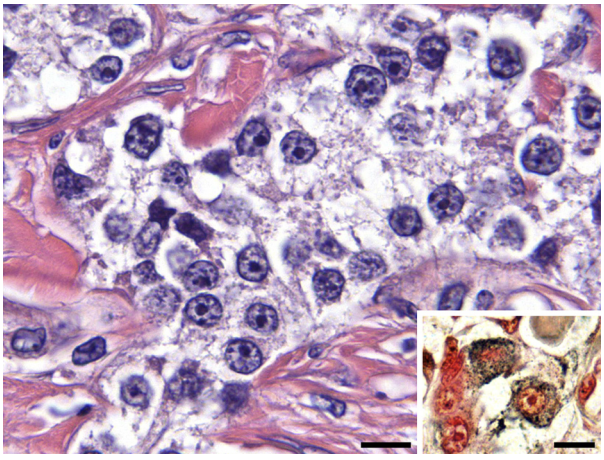

Fig. 1. Tumour cells arranged in nests separated by fibrovascular stroma. Cells have round nuclei and clear cytoplasm. HE. Bar, 10 μm. Inset: Grimelius stain. Bar, 10 μm.

ducts and in general had small, round hypochromatic nuclei containing large prominent nucleoli and abundant faintly eosinophilic cytoplasm (Fig. 1). The frequency of mitotic figures was 0—1 per high-power (×400) field. The tumour cells were positive for argyrophilic granules with Grimelius stain (Fig. 1 inset), but they were negative for mucicarmine and PAS stains. No rosette structures were observed. A metastatic lesion was observed in the inguinal lymph node (Fig. 2). Immunohistochemically, most tumour cells were positive for CK AE1/AE3, CK20, CK8, chromogranin A (Fig. 3), NSE, synaptophysin, E-cadherin, OR-β and PGP9.5, but they were negative for vimentin, CAM5.2, p63 and insulin. The Ki67 index of the tumour was low (2.1%). Normal luminal and ductal cells of the mammary glands expressed both CK20 and OR-β, but not chromogranin A. The immunohistochemical features of the metastatic

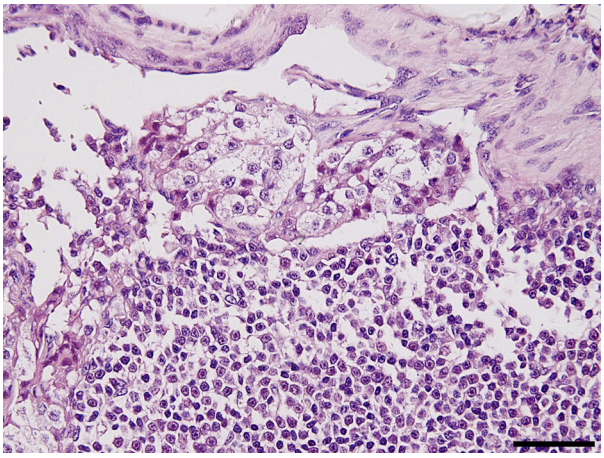

Fig. 2. Tumour cells in the sinus of the inguinal lymph node. HE. Bar, 50 μm.