



## NEOPLASTIC DISEASE

# Salivary Gland Epithelial–Myoepithelial Carcinoma with High-Grade Transformation in a Dog

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## Summary

An 8-year-old male neutered standard dachshund was presented with a slowly growing mass in the left submandibular salivary gland. Histopathological examination revealed a tumour that was composed of bilayered duct-like structures with an inner layer of ductal cells and an outer layer of clear cells. Both inner and outer cells in the greater part of the tumour exhibited low to moderate atypia and low mitotic activity. However, a focal area towards the periphery showed enhanced cellular atypia and mitotic activity in tumour cells. Immunohistochemically, the outer layer of clear cells expressed myoepithelial markers, while the inner layer cells were positive for a luminal epithelial marker. No local recurrence or lymph node or distant metastasis was observed 18 months following surgery. Based on the morphology and immunohistochemical findings, a final diagnosis of epithelial–myoepithelial carcinoma with high-grade transformation was made.

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Primary salivary gland tumours are uncommon in dogs, with an overall incidence of 0.17% (Carberry *et al.*, 1988). There is no breed or sex predilection, but these tumours occur mostly in older dogs (Hammer *et al.*, 2001). Human salivary gland tumours are also uncommon, with an incidence of 0.4–13.5 cases per 100,000 people (Cheuk and Chan, 2007). Salivary glands are composed of ductal cells and basal/myoepithelial cells and several histological types of biphasic tumours can arise from the salivary glands. Biphasic tumours account for most human salivary gland tumours (Cheuk and Chan, 2007). According to the World Health Organization (WHO) classification of human salivary tumours (Barnes *et al.*, 2005), there are two major types of

biphasic tumours: pleomorphic adenoma and epithelial–myoepithelial carcinoma (EMC).

Pleomorphic adenoma, formerly called ‘mixed tumour’, is the most common tumour of the human salivary gland (Barnes *et al.*, 2005). Histologically, this tumour is characterized by a diverse morphology resulting from an admixture of epithelial and myoepithelial cells, with variable amounts of stroma that may comprise of fibrous, mucinous, myxochondroid, cartilaginous or even osseous tissue. The most important hallmark of their histology is the presence of a ‘melting pattern’, as the cellular periphery of the myoepithelial portion gradually blends into the surrounding stroma (Cheuk and Chan, 2007). Malignant transformation of pre-existing pleomorphic adenoma has been noted occasionally and is termed ‘carcinoma in pleomorphic adenoma’. This

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phenomenon has been associated with prolonged tumour duration, tumour recurrence, advanced age and tumour size (Cheuk and Chan, 2007; Kato *et al.*, 2008).

EMC is an uncommon tumour, accounting for approximately 1% of human primary salivary gland tumours (Barnes *et al.*, 2005). It has low-grade malignant potential with the capacity to recur locally with a low metastatic rate. Histologically, it is characterized by duct-like structures composed of an inner layer of luminal epithelial cells and an outer layer of clear cells corresponding to myoepithelial cells. EMCs lack any mucinous or myxochondroid stroma and the myoepithelial component is sharply delineated from the stroma (Ellis and Auclair, 2008).

In contrast to human patients, biphasic tumours account for a low percentage of tumours in canine salivary glands. In the WHO classification of salivary gland tumours of domestic animals, biphasic tumours include only pleomorphic adenoma and carcinoma in pleomorphic adenoma (Head *et al.*, 2003). A canine biphasic tumour arising from the submandibular gland, histologically similar to human EMC, is described in the present report.

An 8-year-old male neutered standard dachshund was presented to the Veterinary Medical Teaching Hospital of Nippon Veterinary and Life Science University with an unmovable, firm mass located in the subcutis of the left mandibular area. The mass was first found by the owner 3 months prior to presentation and had gradually enlarged. The dog was otherwise healthy and did not show any other clinical signs associated with the mass.

A computed tomography (CT) scan revealed a focal, apparently-circumscribed mass measuring  $4.0 \times 3.5 \times 3.5$  cm in the left submandibular salivary gland, exhibiting heterogeneous density with scattered calcification (Fig. 1). Total excision of the left submandibular salivary gland with the tumour mass was performed. Grossly, the resected tumour measured  $4.0 \times 3.5 \times 3.5$  cm and was firm and well-circumscribed, with a white–yellow multinodular cut surface. Neither radiation nor chemotherapy was performed after the surgery. The dog was alive with no evidence of local recurrence or lymph node or distant metastasis at 18 months after surgery.

A tissue sample from the tumour was immediately fixed in 10% neutral buffered formalin. The material was processed routinely and embedded in paraffin wax. Sections (4  $\mu$ m) were stained with haematoxylin and eosin (HE), periodic acid–Schiff (PAS) and alcian blue at pH 2.5 (AB). Serial sections were subjected to immunohistochemistry (IHC) using the labelled streptavidin–biotin (LsAB) method with the following mouse monoclonal antibodies: pan-

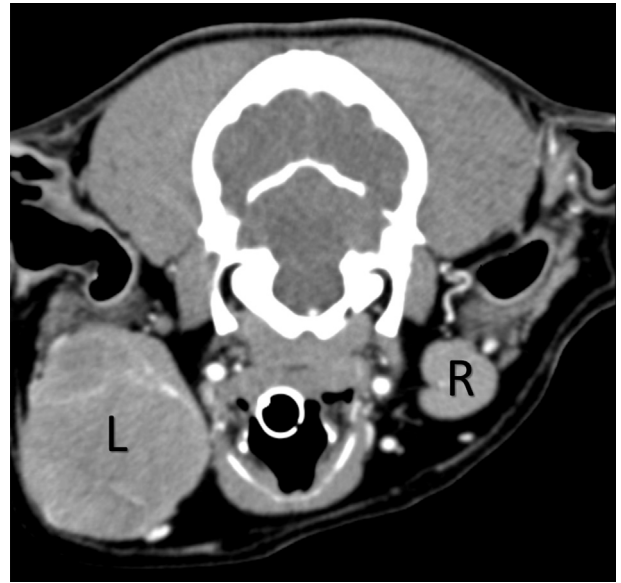


Fig. 1. Computed tomographic cross-section of submandibular salivary glands. The left submandibular gland (L) is much larger than the right, normal gland (R).

cytokeratin (pan-CK, clone AE1/AE3, 1 in 200 dilution; Dako, Glostrup, Denmark), cytokeratin 8 (CK8, clone Ks8.7, 1 in 10 dilution; Progen, Heidelberg, Germany), cytokeratin 14 (CK14, clone LL002, 1 in 50 dilution; BioGenex, San Ramon, California, USA), p63 (clone 4A4, 1 in 150 dilution; Neomarkers, Fremont, California, USA),  $\alpha$ -smooth muscle actin (SMA, clone 1A4, 1 in 400 dilution; Dako) and Ki67 (clone MIB-1, 1 in 100 dilution; Dako). For antigen retrieval, the sections were pre-treated at 121°C for 10 min in citrate buffer (pH 6.0).

Double enzyme IHC for p63/CK8, Ki67/CK8 and Ki67/SMA was performed as described by Yoshimura *et al.* (2014). Briefly, sections were incubated with either anti-p63 or anti-Ki67 antibody. After washing, the sections were incubated with Histofine Simple Stain MAX-PO (Nichirei, Osaka, Japan) for 30 min, followed by ‘visualization’ using 3, 3’ diaminobenzidine chromogen. Next, the slides were heated in a microwave oven in citrate buffer (pH 6.0) for 10 min at 95–100°C and incubated overnight with either anti-CK8 or anti-SMA antibody (4°C). After washing, the sections were incubated with Histofine Simple Stain AP (Nichirei) for 30 min and labelling was then ‘visualized’ with new fuchsin and counterstained with haematoxylin.

On histological examination, the tumour was found to be encapsulated by thick fibrous tissue. Normal salivary parenchyma was displaced to the periphery of the tumour, but some small normal acini were located within the tumour tissue. The tumour showed a multilobular pattern with delicate

connective tissue bands separating tumour nests of various sizes (Fig. 2). In the greater part of the tumour, the tumour nests consisted of bilayered structures lined by inner luminal epithelial cells and outer clear cells (Fig. 3). The inner layer cells were cuboidal with round nuclei and moderate amounts of eosinophilic cytoplasm, while the outer cells were large and polygonal with abundant clear cytoplasm. The degree of atypia of both cell types was low to moderate. On average, there were two mitotic figures per 10 high-power fields (HPFs;  $\times 400$ ). No necrosis or haemorrhage was observed. In contrast, in a focal area at the periphery of the tumour, which comprised approximately 20% of the whole tumour, the bilayered arrangement of tumour cells was less obvious (Fig. 4). The tumour cells appeared to form single-layered ducts with solid and sheet-like growth patterns and had greater cytological atypia and higher mitotic rate (six mitoses per 10 HPFs). Focal areas of necrosis were observed. Lymphatic or blood vessel invasion was absent. The tumour was negative for PAS and AB staining and contained no myxoid, hyalinized or chondroid stroma.

Immunohistochemically, the inner ductal cells of the bilayered structures expressed pan-CK and CK8, which is consistent with a luminal epithelial phenotype. Immunoreactivity for SMA (Fig. 3), p63 and cytokeratin 14, consistent with a myoepithelial phenotype, was detected in the outer clear cells. In the high-grade area of the tumour periphery, double IHC revealed that the ductular and solid structures were composed of two cell populations: CK8-positive luminal epithelial cells and p63-positive basal/myoepithelial cells (Fig. 4), although the proliferation appeared monomorphic in HE-stained tissue sections.

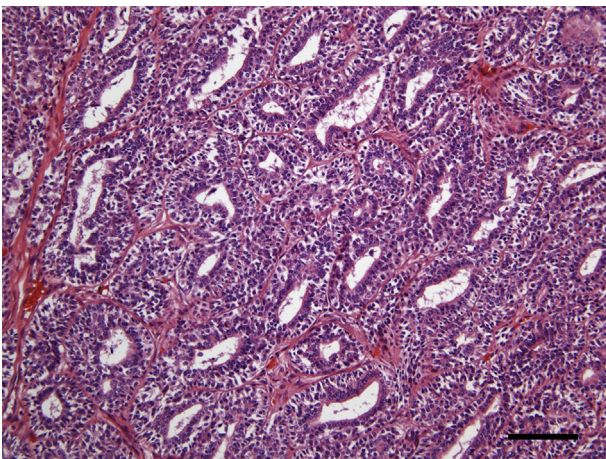


Fig. 2. Submandibular salivary gland tumour showing a multilobular pattern with delicate connective tissue bands separating tumour cell nests of various sizes. HE. Bar, 100  $\mu$ m.

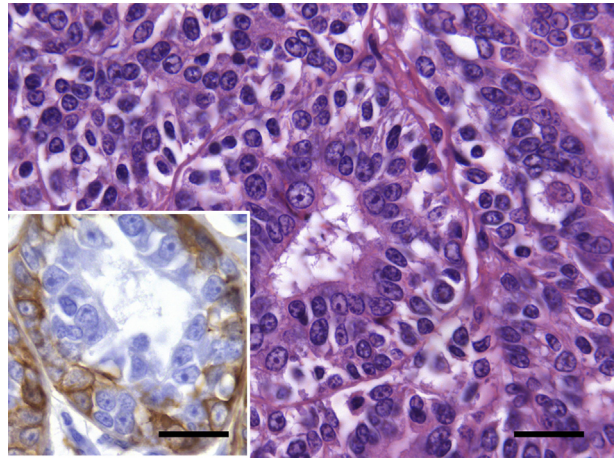


Fig. 3. Tumour nests consisting of bilayered structures lined by inner luminal epithelial cells and outer clear cells. Inset: outer cells of the bilayered structures express SMA. IHC. Bar, 20  $\mu$ m.

Using the Ki67/CK8 and Ki67/SMA double-labelled sections, the Ki67 indices of CK8-positive and SMA-positive tumour cells in the greater area of the tumour were 3.65% and 7.95%, respectively. In contrast, in the high-grade area at the periphery of the tumour, the Ki67 indices of CK8-positive and SMA-positive tumour cells were 8.8% and 14.25%, respectively.

The tumour in the present case was composed of biphasic glandular structures with inner ductal cells and outer clear cells, features indicating histological similarity to human EMC. The histological

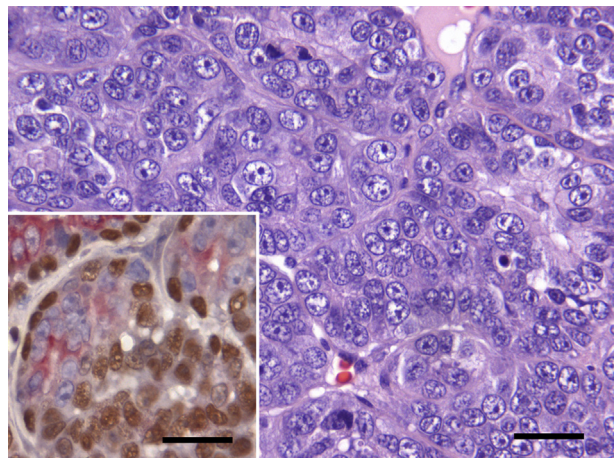


Fig. 4. High-grade component in a focal area of the tumour periphery. Tumour cells showing anisokaryosis and prominent nucleoli are arranged in solid nests. Inset: double enzyme IHC reveals that bilayered structures are composed of an inner layer of CK8-positive cells (red) surrounded by one or more layers of p63-positive cells (brown). IHC. Bar, 20  $\mu$ m.

appearance was inconsistent with that of pleomorphic adenoma, which is characterized by an architectural pleomorphism comprising epithelial, myoepithelial and mesenchymal elements (Barnes *et al.*, 2005). In particular, the present case lacked the ‘melting pattern’ that is a striking feature of pleomorphic adenoma (Cheuk and Chan, 2007). Basal cell adenocarcinoma and adenoid cystic carcinoma are also described as biphasic tumours in the WHO classification of human salivary gland tumours (Barnes *et al.*, 2005) and basal cell adenocarcinoma was reported in the salivary glands of two dogs (Sozmen *et al.*, 2003). However, the outer layers of basal cell adenocarcinoma and adenoid cystic carcinoma are lined with basal cells, but not clear cells.

The greater part of the tumour in the present case exhibited low-grade histology, similar to that of conventional human EMC. However, a focal area exhibiting active growth of tumour cells with progressive cellular atypia was observed at the periphery. In recent years, EMC with coexisting areas of high-grade carcinoma (termed ‘dedifferentiated EMC’ or ‘EMC with high-grade transformation’) has been reported in human salivary glands (Roy *et al.*, 2010; Nagao, 2013). Therefore, in the present case, the tumour was diagnosed as EMC with high-grade transformation arising in the submandibular salivary gland. However, some differences in component cell types were observed between the present case and human cases reported previously. The high-grade component of most human cases lacks the features of dual cell structures and exhibits monomorphic growth of either luminal epithelial or myoepithelial cell type (Nagao, 2013). In contrast, immunohistochemical analyses demonstrated that dual cell phenotypes remained in the high-grade area of the tumour periphery in the present case, despite its monomorphic features of solid appearance and sheet-like growth of tumour cells. According to a case series of human EMCs with a high-grade carcinoma component (Alos *et al.*, 1999), the Ki67 index was increased in carcinoma cells (12–30%) of the high-grade component compared with the luminal epithelial and myoepithelial cells (0.3–0.4% and 2–3%, respectively) in areas characteristic of conventional EMC. In the present case, the Ki67 indices of both luminal epithelial and myoepithelial components in the high-grade area were higher than those in the main area, suggesting simultaneous proliferation of the two components.

The low-grade malignancy of EMC in man is well-established and contrasts with their well-differentiated histological appearance, as their metastatic potential has been established by sufficient catamnestic studies (Ellis and Auclair, 2008). Furthermore, the

prognosis of EMC with high-grade transformation is considered to be uniformly worse than that of typical EMC (Roy *et al.*, 2010). In the current canine case, no evidence of local recurrence or metastasis was detected 18 months after surgery. Further studies are needed to establish the prognosis of tumours of this type in dogs.

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