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NEOPLASTIC DISEASE

Collision Tumour of Squamous Cell Carcinoma and Malignant Melanoma in the Oral Cavity of a Dog

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Summary

A 7-year-old, male cocker spaniel was presented with a gingival proliferative lesion in the rostral maxilla and enlargement of the regional lymph node. Morphological and immunohistochemical analysis revealed a collision tumour composed of two malignant populations, epithelial and melanocytic, with metastasis of the neoplastic melanocytes to the regional lymph node. The epithelial component consisted of trabeculae and islands of well-differentiated squamous epithelium immunoreactive to cytokeratins. The melanocytic component had a varying degree of pigmentation of polygonal and spindle-shaped cells, growing in nests or densely packed aggregates and immunolabelled with S100, melanoma-associated antigen (melan A), neuron-specific enolase and vimentin antibodies. Protein markers involved in tumorigenesis or cell proliferation (i.e. COX-2, p53, c-kit and Ki67), were overexpressed by the neoplastic cells. To the authors' knowledge, this is the first description of an oral collision tumour involving malignant melanoma and squamous cell carcinoma in the dog.

Keywords: dog; melanoma; oral mucosa; squamous cell carcinoma

Although melanocytes and keratinocytes normally exist in close association in the basal layer of the skin and the oral mucosa, the simultaneous malignant transformation of both populations is a rare event (Pool *et al.*, 1999). Squamous cell carcinoma (SCC), malignant melanoma (MM) and fibrosarcoma are the most common neoplasms in the oropharynx of dogs (Ramos-Vara *et al.*, 2000; Head *et al.*, 2002; Nemec *et al.*, 2012). The term 'collision tumour' indicates two co-existent, but independent, neoplasms with distinct morphology that may be adjacent to each other or intermingled (Pool *et al.*, 1999; Head *et al.*, 2002). A benign dermal melanocytoma–acanthoma, composed of two different mature cell populations, epithelial and melanocytic, was described in a German shepherd dog (Espinosa de los Monteros *et al.*, 2000).

A 7-year-old, male cocker spaniel was presented with a 2-week history of ptyalism and anorexia. A firm, 2.5 cm diameter, grey, ulcerated, nodular growth, with peripheral black discolouration, was located in the gingival mucosa. Enlargement of the mandibular lymph node was observed. Radiographical evaluation did not indicate bone or lung involvement. An incisional biopsy and aspiration cytology were obtained from the mass and lymph node, respectively. Cytological preparations from the lymph node showed polygonal or spindle-shaped cells, with variably pigmented and vacuolated cytoplasm and round-to-oval nuclei, containing fine chromatin and one or more distinct nucleoli. Cellular pleomorphism and mitotic figures were common (Fig. 1, inset). A preliminary cytological diagnosis of oral melanoma with lymph node metastasis was made and, in view of the poor prognosis and the deterioration of its general condition, the dog was humanely destroyed 2 weeks later. The owner declined post-mortem examination.

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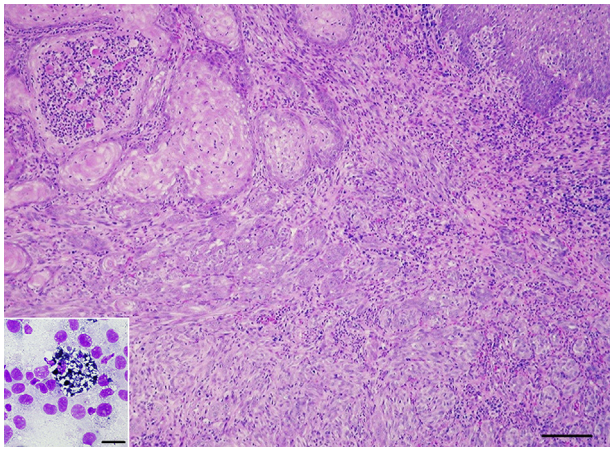

Fig. 1. Relationship between the epithelial component (top) and spindle-shaped or polygonal melanocytes (bottom) of the tumour. HE. Bar, 200 μm. Inset. Aspiration cytology from the mandibular lymph node showing pleomorphic cells with round and oval nuclei, prominent nucleoli and fine black granules. Larger black granules are present within the cytoplasm of a melanophage. Diff Quik stain. Bar, 50 μm.

The oral biopsy sample was fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections (4 μm) were stained with haematoxylin and eosin (HE). For immunohistochemistry (IHC) the avidin–biotin–peroxidase complex (ABC) method was used (Vector Corporation, Burlingame, California, USA). Sections were labelled with commercially available (Table 1) monoclonal antibodies (mAbs) against cytokeratins 5 and 8 (RCK-102), cytokeratins 8, 18 and 19 (NCL-5D3), neuron-specific enolase (NSE) and melanoma-associated antigen (melan A) (Dako, Carpinteria, California, USA). Polyclonal antibodies (pAbs) against pancytokeratins (AE1–AE3), vimentin and S100 protein were also employed (Dako). Proteins related to tumour progression (i.e. prognostic biomarkers), including cyclo-oxygenase (COX)-2, p53 and tyrosine kinase receptor (c-kit), proliferation marker (Ki67) and anti-major capsid protein of papillomavirus, were also used (Dako). Predigestion of tissue sections, for 5 min with 0.1% pronase or for 20 min with 10 mM citrate buffer, pH 6.0, at 98°C, was employed for tissues treated with mAbs (Sigma Chemical Co., St. Louis, Missouri, USA). After antigen retrieval, slides were covered with 10% normal rabbit (for mAbs) or swine (for pAbs) sera in phosphate buffered saline (PBS) for 30 min, followed by incubation with the primary antibodies for 18 h at 4°C. Slides were incubated with biotinylated swine anti-rabbit immunoglobulin diluted 1 in 200 in PBS and containing 1% normal swine serum (for pAbs) and with biotinylated rabbit anti-mouse immunoglobulin diluted 1 in 50 in PBS containing 1% normal rabbit serum (for mAbs) for 30 min and with ABC for 1 h, both at room temperature. Between each step, slides were washed three times for 10 min in PBS. 'Visualization' of antibody binding was achieved by adding 0.5% 3, 3′ diaminobenzidine (DAB) diluted 1 in 10 in 0.05 M Tris buffer containing H_2O_2 0.3% in distilled water for 2–3 min (Sigma). Tissues were counterstained with Mayer's haematoxylin. After dehydration, slides were mounted with a xylene-based solution (DPX; BDH Laboratory Supplies, Poole, UK). Two sections from the oral mucosa of dogs, which included normal mucosa, SCC, MM, gingival hyperplasia and viral papilloma, were used as positive controls. For negative controls, normal sera or unrelated mAbs replaced the primary antibodies.

Table 1
Summary of antibodies, procedures and immunohistochemical findings

Antibody	*Procedures*			*Immunolabelling*	
	Type	*Dilution*	*Antigen retrieval*	*Epithelial component*	*Melanocytic component*
RCK-102	Monoclonal	1 in 20	Pronase	C	–
NCL-5D3	Monoclonal	1 in 20	Pronase	C	–
AE1-AE3	Polyclonal	1 in 100	–	C	–
Vimentin	Polyclonal	1 in 160	–	–	C
S100 protein	Polyclonal	1 in 500	–	–	N/C
NSE	Monoclonal	1 in 1,000	Pronase	–	N/C
Melan A	Monoclonal	1 in 160	Pronase	–	C
COX-2	Monoclonal	1 in 20	HTAR	C	C
p53	Monoclonal	1 in 100	HTAR	N	N
c-kit	Monoclonal	1 in 250	HTAR	–	C
Ki67	Monoclonal	1 in150	HTAR	N	N
Papillomavirus	Monoclonal	1 in 100	Pronase	–	–

Pronase, 0.1% Protease E; HTAR, high-temperature antigen retrieval solution; C, cytoplasm; N, nucleus; N/C, nuclear and cytoplasmic.