NEOPLASTIC DISEASE

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Short Title: Pulmonary Basaloid Squamous Cell Carcinoma in a Dog

Pulmonary Basaloid Squamous Cell Carcinoma in a dog

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Graphical Abstract

A 9-year-old neutered male crossbred dog with a 4-week history of progressive vestibulocerebellar signs was presented for necropsy examination. Gross and histological examination led to the diagnosis of pulmonary basaloid squamous cell carcinoma with metastases in thoracic lymph nodes, the left kidney and cerebellum.

Summary

A 9-year-old neutered male crossbred dog with a 4-week history of progressive vestibulocerebellar signs was presented for necropsy examination. Gross examination revealed neoplastic growth in the lungs, thoracic lymph nodes, the left kidney and the cerebellum. Microscopically, the tumour consisted of an infiltrative, densely cellular, basaloid epithelial neoplastic growth with extensive areas of abrupt keratinization. Immunohistochemically, neoplastic cells expressed p63 and partially expressed cytokeratins 5/6. Based on these findings, the tumour was diagnosed as a primary pulmonary basaloid

squamous cell carcinoma (BSSC) with metastasis to regional lymph nodes, kidney and brain. As far as the authors are aware, this is the first description of BSCC in an animal species.

Keywords: squamous cell carcinoma; basaloid; lung; dog

Lung neoplasia is uncommon in domestic animals, apart from in dogs and cats. The incidence of canine lung neoplasia increases with age and the most common primary tumour is bronchioalveolar carcinoma. Adenocarcinomas, adenosquamous and squamous cell carcinomas (SCCs) are less common (Meuten, 2017).

Basaloid squamous cell carcinoma (BSCC) is a rare neoplasm of the lung, which was first described in man in 1992 (Brambilla *et al.*, 1992). In the current 2015 World Health Organization (WHO) tumour classification, BSCC is considered to be a subtype of SCC (Travis *et al.*, 2015). Squamous markers, such as p40 and p63, are expressed consistently in BSCC (Bishop *et al.*, 2012; Travis *et al.*, 2015). Other lung tumour subtypes of SCC include keratinizing squamous cell carcinoma, non-keratinizing squamous cell carcinoma and squamous cell carcinoma in situ (Travis *et al.*, 2015). Comprehensive descriptions of pulmonary BSCC appear to be lacking in animals. One case report describes a poorly differentiated, not further classified, pulmonary SCC in a dolphin with histological features potentially compatible with BSCC (Ewing and Mignucci-Giannoni, 2003).

A 9-year-old neutered male crossbred dog was submitted for post-mortem examination. The animal was presented 3 days before for neurological examination with a 4-week history of progressive vestibulocerebellar signs. Serum biochemistry revealed elevated alkaline phosphatase (412.0 U/l, reference range <130.0 U/l), total bilirubin (8.6 µmol/l, reference range 0.1-4.2 µmol/l) and creatine kinase (CK) (334.0 U/l, reference range 20.0–

225.0 U/l) and a low sodium:potassium ratio (27.4, reference range 28.8–40.0). Neurological

examination localized a lesion within the caudal cranial fossa affecting the

vestibulocerebellar structures. A degree of left lateralization of the lesion was considered

possible based on the observed proprioceptive deficits. At this point, the main differential

diagnoses included neoplasia or inflammatory disease. A magnetic resonance imaging (MRI)

examination of the brain revealed a rim-enhancing cerebellar mass measuring 1.1×1.3 cm

located in the centre of the cerebellum, with associated perilesional oedema. Mild central

compression of the brainstem and mild rostral transtentorial herniation with extension to the

foramen magnum was observed. Cerebrospinal fluid (CSF) analysis revealed moderate to

severe 'albuminocytological dissociation' (protein 2.40 g/l, reference range 0.14–0.30 g/l;

nucleated cell count 3.0×10^9/l, reference range 0–6×10^9/l).

Given the clinical findings, the presence of a neoplastic lesion was considered highly

likely, although an inflammatory lesion could not be excluded based on the diagnostic tests

performed. Medical management with glucocorticoids and possible chemotherapy was

proposed to the owners. However, the owners elected for humane destruction based on the

poor prognosis and quality of life, and gave consent for a full post-mortem examination.

On gross examination, the main lesions were observed in the lung, the left kidney and

the cerebellum. The lung contained multifocal, variably sized, 0.3–4.0 cm diameter, raised

spherical nodules which were pale tan in colour, often umbilicated, poorly demarcated, solid

and with a firm, often gritty consistency. The two largest masses measured up to $3.5 \times 3.0 \times$

4.0 cm and were located at the level of the bifurcation of the main bronchi and within the

mediastinal edge of the left caudal lobe (Fig. 1). The remaining lung tissue was diffusely

dark red, wet and heavy, compatible with congestion and oedema. Mediastinal,

tracheobronchial and bronchial lymph nodes were enlarged (up to 1×1.5 cm) and expanded

by similar tissue as described in the lungs, compatible with tumour metastasis. The left

kidney contained a focal wedge-shaped, $0.6 \times 0.8 \times 0.5$ cm, pale tan, solid mass within the cortex, compatible with tumour metastasis. On coronal serial sections of the brain, at the level of cerebellum and brainstem, a focal, irregularly-shaped, poorly-demarcated, infiltrative, non-encapsulated, pale tan and firm mass was located centrally in the white matter of the cerebellum between the fourth ventricle and the cerebellar vermis (Fig. 2). Samples of all the main organs and lesions were fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax.

Microscopically, the lung nodules corresponded to non-encapsulated, infiltrative, densely cellular epithelial proliferations with extensive areas of abrupt keratinization (Fig. 3). The cells were mostly closely packed in a lobular or cribriform pattern, appeared basaloid and were supported by dense fibrous stroma. Proliferating, interpreted as neoplastic, cells often lined extensive lakes of keratin, and were round to polygonal, with round, hyperchromatic nuclei and mostly scant cytoplasm. Nuclear palisading was frequent, compatible with a basaloid cellular differentiation. Distinct intercellular bridges were not observed. Mitotic activity was moderate (0–6 mitoses per $\times 400$ high power field). Within the cellular mass there were multifocal extensive areas of necrosis, some admixed with abundant neutrophils and often with foci of dystrophic calcification. Multifocal intrapulmonary micrometastases were present.

In the cerebellum, the mass consisted of a focally extensive, highly infiltrative epithelial mass, interpreted as neoplastic with a histological appearance comparable with the neoplastic nodules described in the lungs. A large central area of coagulative necrosis was present. There was marked spongiosis and gliosis in the parenchyma surrounding the tumour. The lesions within the thoracic lymph nodes and the kidney corresponded to a cellular growth with histological features as described for the lungs and brain; these were interpreted as tumour metastases.

Immunohistochemically, neoplastic cells expressed p63 (Ventana Medical Systems, Inc., Tucson, Arizona, USA); anti-p63 [4A4] mouse monoclonal antibody; prediluted) and had partial expression of cytokeratin (CK) 5/6 (Ventana Medical Systems, Inc.; anti-cytokeratin 5/6 [D5/16B4] mouse monoclonal; prediluted). For both antibodies, human tonsil was used as positive control tissue and tissue sections were heat pretreated in cell conditioning solution 1 (Roche Diagnostics GmbH, Mannheim, Germany) at 95°C for 64 min. The labelling for p63 was diffuse, strong and nuclear (Fig. 4), while the CK 5/6 labelling was strong and cytoplasmic with a variable percentage of positive cells within the tumour (ranging from <5% to >80%).

Based on these findings, a final diagnosis of BSCC of the lung with metastases to thoracic lymph nodes, left kidney and cerebellum was made.

Pulmonary BSCC are rare tumours, which are defined in man as a histological subtype of SCC, either keratinizing or non-keratinizing, with a basaloid component of >50% of the tumour (Travis *et al.*, 2015). In the new WHO lung tumour classification (Travis *et al.*, 2015), former basaloid carcinomas were moved from the category of large cell carcinoma to become a subtype of SCC. This reclassification was based on the recognition that the former basaloid carcinomas express squamous markers, such as p40 or p63. Furthermore, genetic analyses of these tumours revealed a specific genetic profile, which is different from other, non-basaloid, SCCs (Brambilla *et al.*, 2014). For these reasons, the subtyping of SCC was modified to consist of keratinizing, non-keratinizing and basaloid subtypes. Tumours are classified as BSCC if the basaloid component is >50% of the tumour, regardless of the presence of any keratinization. In the present case, the basaloid component was almost diffuse and large lakes of abrupt keratinization were present, consistent with BSCC. Features of progressive keratinization, including intercellular bridges were not evident in the samples

examined. BSCC is a well-recognized tumour type in men in other non-respiratory organs such as head and neck, oesophagus and anal canal (Moro-Sibilot *et al.*, 2008).

Human pulmonary BSCC is a highly malignant tumour with a poor prognosis and a high rate of metastasis (Cakir *et al.*, 2007; Moro-Sibilot *et al.*, 2008; Wang *et al.*, 2011). BSCC usually occurs in elderly men (Cakir *et al.*, 2007). Interestingly, the dog in this case was also male and over 9 years old. The present canine case also showed widespread tumour metastasis involving thoracic organs, the brain and one kidney. The prognosis of human BSCC is worse than for non-basaloid SCC (Brambilla *et al.*, 2014). A distinction between these two tumour entities is therefore justified and important.

Immunohistochemical analysis of lung tumours is often needed for definitive characterization. Sensitive markers of squamous differentiation include cytokeratins 5/6 and p63 which are recommended for use in cases of suspected BSCC (Serrano *et al.*, 2008). P63 is a member of the p53 gene family and is expressed constitutively by a variety of epithelial stem cells, including squamous epithelia, urothelium, bronchial epithelium and the myoepithelial layers of breast, prostate and submucosal glands (Di Como *et al.*, 2002; Wang *et al.*, 2002; Yang *et al.*, 2002; Emanuel *et al.*, 2005). In normal lung tissue, intense nuclear p63 expression is seen in bronchial reserve cells, but not ciliated cells, alveolar epithelial cells or non-epithelial cells (Kaufmann *et al.*, 2001; Di Como *et al.*, 2002; Wang *et al.*, 2002). In lung tumours, strong and consistent nuclear p63 expression is observed in SCCs, including BSCCs and poorly differentiated carcinomas (Kaufmann *et al.*, 2001; Wang *et al.*, 2002; Crapanzano *et al.*, 2011; Pelosi *et al.*, 2011). Small-cell carcinomas are described as p63 negative, while bronchioloalveolar carcinomas and adenocarcinomas show variable expression. The present canine case had strong and diffuse nuclear expression of p63. CK 5 and 6 are related proteins, often detected with the CK5/6 antibody. In normal adult tissues, the basal and myoepithelial cells of simple epithelia generally express CK5, while CK6 is

detected in normal non-cornifying stratified squamous epithelia (Jerome Marson *et al.*, 2004); CK5 is present in all stratified squamous epithelia and is a more sensitive and specific marker than p63 (Brunnstrom *et al.*, 2013). In lung tumours, strong and diffuse membranous CK5/6 labelling can be observed in squamous pulmonary carcinomas and carcinomas with a squamous component (Jerome Marson *et al.*, 2004; Pelosi *et al.*, 2011; Brunnstrom *et al.*, 2013). All other primary pulmonary carcinomas fail to express significant CK5⁄6. The present canine case showed distinct and strong cytoplasmic CK5/6 labelling with a variable percentage of positive cells within the tumour, ranging from <5% to >80%. This irregular labelling pattern may potentially correspond to variable cellular differentiation within the tumour. Indeed, CK5/6 labelling intensity has been shown to correlate negatively with the degree of differentiation (Jerome Marson *et al.*, 2004).

This report indicates that BSCC can occur in dogs and that it can present with widespread metastasis and a poor prognosis, mimicking the human form of BSCC. Studies including a higher number of canine cases would be needed to effectively compare both human and canine BSCCs. To the authors' knowledge this is the first report of a pulmonary BSCC in the peer-reviewed veterinary literature.

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Figure Legends

Fig. 1. Lung with BSCC, ventral view. Note the multiple, poorly demarcated, pale tan,

slightly raised neoplastic nodules in the lung parenchyma. Remaining lung tissue is markedly

congested and oedematous. Bar, 1 cm.
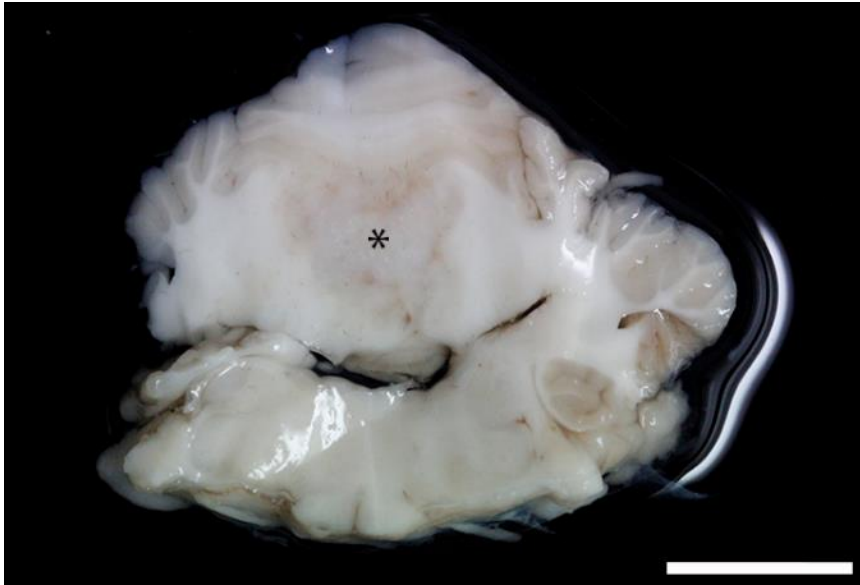
Fig. 2. Cerebellum and brainstem with metastatic pulmonary BSCC, coronal section. Note the focal, poorly-demarcated, pale mass (*) within the white cerebellar matter. Bar, 1 cm.
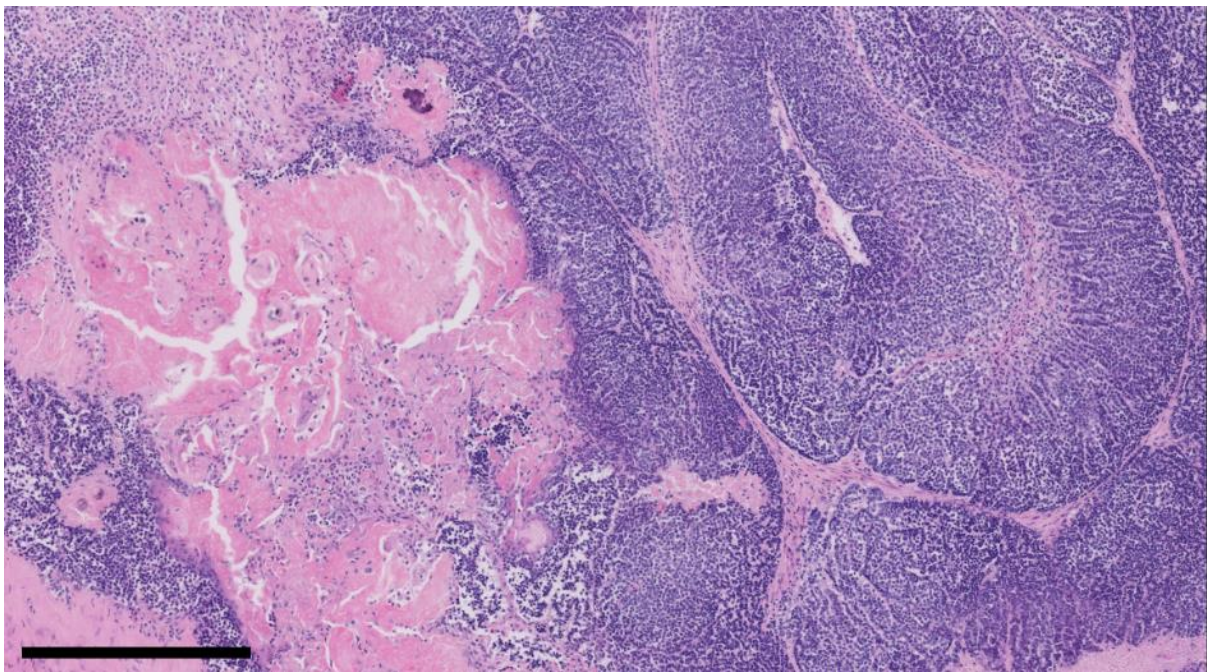

Fig. 3. Pulmonary BSCC. The tumour consists of a densely cellular basaloid epithelial neoplastic proliferation with extensive areas of abrupt keratinization. Haematoxylin and eosin. Bar, 400 μm.
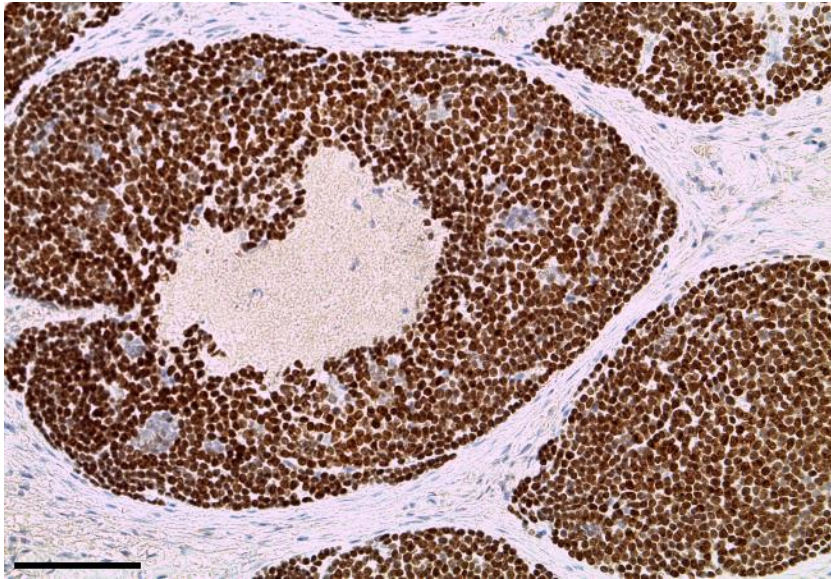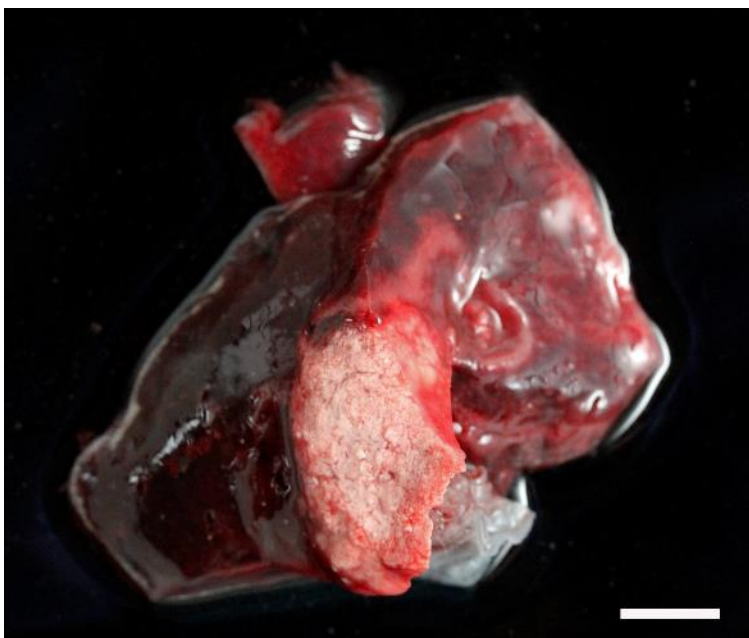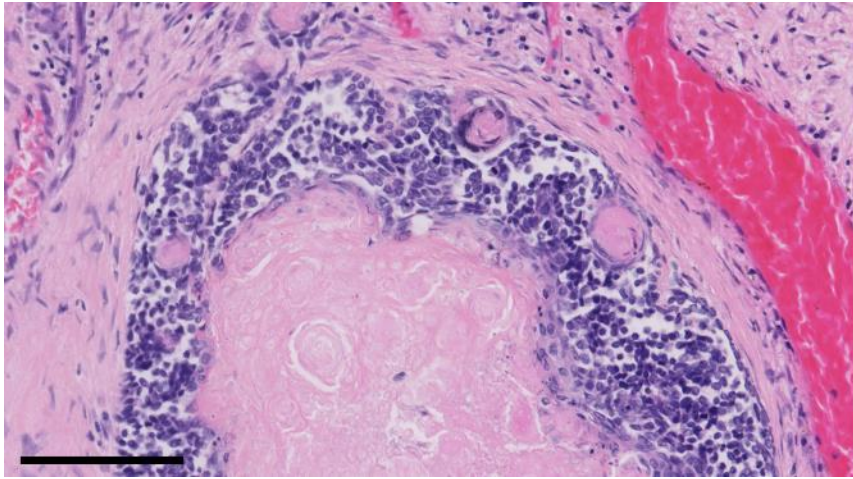
Fig. 4. Pulmonary BSCC. Neoplastic cells show strong and diffuse nuclear expression of p63.

Immunohistochemistry. Bar, 100 μm.

Supplementary Figure Legends

Supplementary Fig. 1. Lung with a cut section of BSCC. Remaining lung tissue is markedly

congested and oedematous. Bar, 1 cm.

Supplementary Fig. 2. Pulmonary BSCC with extensive areas of abrupt keratinization.

Haematoxylin and eosin. Bar, 100 μm.