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Malignant peripheral nerve sheath tumour with divergent epithelioid differentiation in a cat

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ABSTRACT

Divergent differentiation, mainly towards various subsets of mesenchymal cells, is encountered sporadically in human malignant peripheral nerve sheath tumours (MPNSTs) but this is the first report of epithelioid components within this neoplasm in a cat. An 8-year-old, spayed female Domestic Shorthaired cat was presented for surgical removal of a subcutaneous mass on the right flank. Morphological and immunohistochemical analysis revealed a malignant neoplasm with spindloid cells intermixed with an epithelioid component that had squamous differentiation. There was intense immunolabelling of vimentin, S100 protein, neuron-specific enolase, laminin and glial fibrillary acidic protein in the spindloid cell component and for cytokeratin (CK) AE1/AE3 and CK5/6 in the epithelial elements. Melanoma-associated antigen, desmin, α-smooth muscle actin, CD18, CD31, ionized calcium binding adapter molecule-1 and CK8/18 were not expressed, which helped differentiate the tumour from other feline spindloid cell neoplasms. These features are characteristic of divergent epithelioid differentiation of MPNST.

Malignant peripheral nerve sheath tumours (MPNSTs) are soft tissue sarcomas that have differentiation towards cells of the nerve sheath [1]. The histological characteristics of these tumours are heterogeneous because they are composed of spindloid cells of the peripheral nervous system (Schwann cells, perineurial cells and intraneural fibroblasts, or a combination thereof), which has resulted in a confusing classification and terminology of these neoplasms [2]. In humans, MPNSTs can have divergent differentiation, usually to mesenchymal elements, particularly taking the form of rhabdomyosarcoma. Such tumours are termed malignant triton tumours, referring to the studies of Masson who postulated that divergent myoid differentiation could occur in neoplastic neuroectodermal cells [3,4]. Epithelioid, melanotic, myogenic, cartilaginous and osteogenic differentiation have also been sporadically found in MPNSTs of domestic animals [5–7] and have been associated with a poor prognosis [8]. This report is the first description of epithelioid differentiation within a MPNST in a cat.

An 8-year-old, spayed female Domestic Shorthaired cat, otherwise in good condition, was presented with a solitary subcutaneous mass in the right flank. A firm, 3.2 cm in diameter, poorly defined growth was surgically excised. A tumour sample was fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin wax. Sections (4 μm) were cut and stained with haematoxylin and eosin (HE), periodic acid–Schiff (PAS) and Masson's trichrome. Mitotic figures were counted in 10 high-power fields (HPFs; 2.37 mm^2). For immunohistochemistry (IHC), the avidin–biotin–peroxidase complex (ABC) method was used (Vector Corporation, www.vectorcorp.com). Sections were labelled with commercially available antibodies for cytokeratins (CK) (CKAE1/AE3, CK5/6 and CK8/18), vimentin, S100 protein, neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), laminin, melanoma-associated antigen (Melan A), α-smooth muscle actin (α-SMA), desmin, CD31, ionized calcium binding adapter molecule-1 (Dako, www.agilent.com) and CD18 (Bio-Rad, www.bio-rad.com) (Table 1). After antigen retrieval (Table 1), slides were covered with 10% normal rabbit (for monoclonal primary antibodies) or swine (for polyclonal primary antibodies) serum in phosphate buffered saline (PBS) for 30 min, followed by incubation with the primary antibodies for 18 h at 4°C. A biotinylated rabbit anti-mouse or swine anti-rabbit IgG (Vector Laboratories, https://vectorlabs.com), diluted 1:200, was applied as secondary reagent for 30 min at room temperature. An ABC complex diluted 1:50 was applied as the third reagent. 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 (Sigma-Aldridge, www.sigmaaldridge.com) diluted 1:10 in 0.05 M Tris buffer containing H_2O_2 0.1% in distilled water for 2 min. Tissues were counterstained

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Table 1
Antibodies and procedures used for immunohistochemistry

Antibody	Clone	Antigen	Dilution	Antigen retrieval
CKAE1/AE3	CKAE1/AE3	Cytokeratin	1:200	Pronase
CK5/6	D5/16B4	Cytokeratin	1:150	Pronase
CK8/18	EP17/EP30	Cytokeratin	1:100	Pronase
Vimentin	V9	Vimentin	1:160	–
S100	Polyclonal	S100	1:500	–
NSE	BBS/NC/VI-H14	NSE	1:1000	Pronase
GFAP	Polyclonal	GFAP	1:100	Pronase
Laminin	Polyclonal	Laminin	1:150	HTAR
Melan A	A103	Melan A	1:100	HTAR
α-SMA	1A4	α-SMA	1:150	–
Desmin	D33	Desmin	1:200	HTAR
CD18	CA1.4E9	Integrin-β2	1:150	HTAR
CD31	JC70A	PECAM-1	1:500	HTAR
Iba-1	HL22	Iba-1	1:500	HTAR

Pronase: 5 min with 0.1% Protease E (Sigma).
HTAR: High-temperature antigen retrieval solution (10 min at 121°C in citrate buffer, pH 5).
NSE, neuron-specific enolase; GFAP, glial fibrillary acidic protein; Melan A, melanoma-associated antigen; α-SMA, α-smooth muscle actin; PECAM-1, platelet endothelial cell adhesion molecule-1; Iba-1, ionized calcium binding adapter molecule 1.

with Mayer's haematoxylin. After dehydration, slides were mounted with a xylene-based DPX solution. Sections treated with rabbit or swine normal serum, which replaced the primary antibodies, were included as negative controls. Internal positive tissue controls present in test tissue, as well as feline neoplasms, (including histiocytic sarcoma, schwannoma, haemangiosarcoma, amelanotic melanoma, leiomyosarcoma, rhabdomyosarcoma, fibrosarcoma, myxosarcoma, squamous cell carcinoma and osteosarcoma), run alongside test slides, served as positive controls to assess the performance of the primary antibodies. All primary antibodies included in the study showed cross-reactivity with feline normal and neoplastic tissues.

Histologically, the tumour consisted of neoplastic spindloid cells arranged in fascicles with various orientations or in concentric whorls around small vessels and nerves. There were multiple foci consisting of spindloid cells that formed a storiform pattern with areas of whorling and palisading (Antoni type A pattern) or, less commonly, cells set in a loose network of fibres (Antoni type B pattern). The cytoplasm was lightly acidophilic or had prominent vacuolation, with indistinct cell borders (Fig. 1A and B). Masson's trichrome did not stain any cytoplasmic component. The overall architecture was either diffuse or arranged in alternating hypocellular and densely packed cellular areas. Small foci of necrosis, haemorrhages and marked pleomorphism and mitotic activity were found throughout the neoplasm. The stroma contained moderate collagenous and myxoid deposits, with multifocal hyalinization.

The epithelial cell population comprised tubules, islands and trabeculae of cuboidal cells with foci of squamous differentiation. Islands were composed of amphophilic basal-like peripheral cells with central keratinocytes with abundant eosinophilic cytoplasm. Nuclei were large and vesicular with coarse or granular chromatin, and contained prominent single or multiple nucleoli. Bizarre nuclei and scattered multinucleated neoplastic cells were seen at the periphery of the neoplasm. PAS staining did not reveal any mucoid deposits in the lumen of the tubules or within the cytoplasm of neoplastic cells. The mitotic index was 34 and 28 for the spindloid and epithelioid cell components, respectively.

More than 80% of spindloid cells were immunolabelled for vimentin, GFAP, NSE, laminin and S100 (Fig. 2A and B). Intense cytoplasmic (vimentin, GFAP, NSE and laminin) or cytoplasmic and nuclear (S100) immunolabelling was detected in neoplastic cells. S100 and NSE immunolabelling accentuated the cytoplasmic processes of the spindloid cells. Absence of immunolabelling for Melan A, α-SMA, desmin, CD31 and CD18 allowed differentiation from other spindloid cell neoplasms frequently seen in cats that can mimic poorly differentiated MPNST, such as spindle cell amelanotic melanoma, leiomyosarcoma, rhabdomyosarcoma, haemangiosarcoma and histiocytic sarcoma, respectively. The cytoplasm and cell membranes of cells with an epithelial morphology were labelled with anti-CKAE1/AE3 and CK5/6 antibodies. CKAE1/AE3

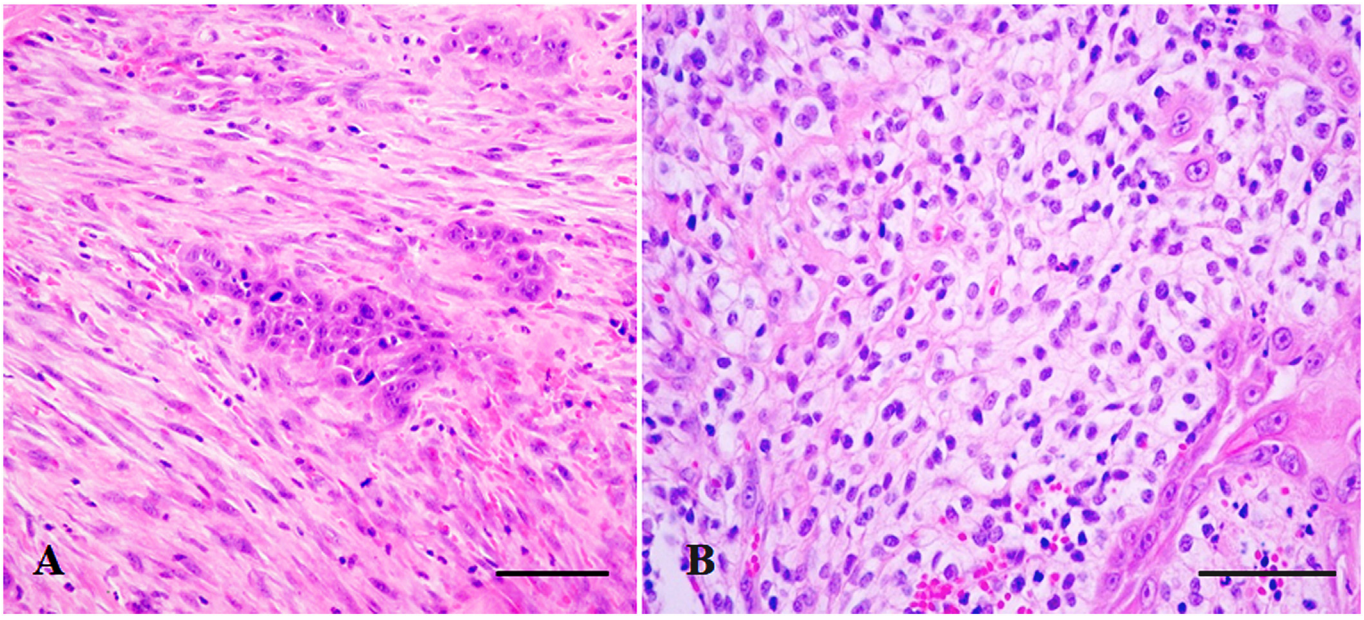

Fig. 1. Malignant peripheral nerve sheath tumour with epithelioid differentiation, right flank, cat. Neoplasm composed of fascicles of spindloid cells with elongated or ovoid nuclei and acidophilic (**A**) or vacuolated (**B**) cytoplasm. Neoplastic cells arranged in acinar (**A**) or tubular (**B**) patterns. Epithelioid component comprises cuboidal epithelial cells with ovoid vesicular nuclei and single prominent nucleoli. Moderate atypia and mitotic rate. Bars, 150 μm.

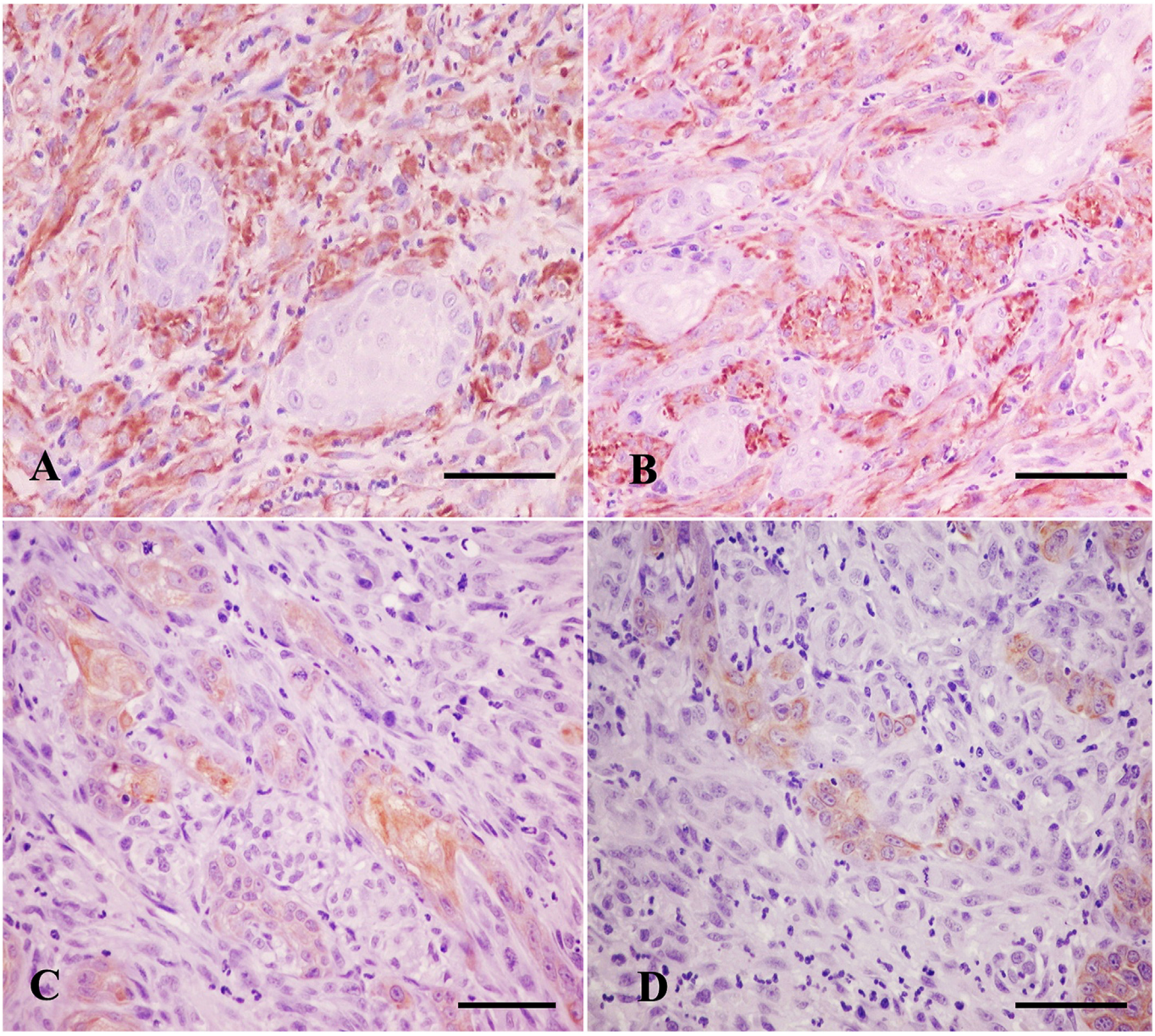

Fig. 2. Malignant peripheral nerve sheath tumour, right flank, cat. Immunolabelling of GFAP (**A**) and S100 protein (**B**) in spindloid cell component, with intermingled immunonegative epithelioid cells. Immunolabelling of neoplastic epithelial cells for CKAE1/AE3 (**C**) and CK5/6 (**D**). IHC. Bars, 150 μm.

immunolabelling was present in the more differentiated cells in the centre of the islands (Fig. 2C), while anti-CK5/6 antibody (Fig. 2D) reacted with basal cells of the islands and atypical scattered cells. Lack of immunoreaction with CK8/18, normally expressed in glandular cells, eliminated the possibility of anaplastic adenocarcinomas. Neoplastic, spindloid or epithelial cells characterized by both immunophenotypes were not observed.

Diagnosis of malignant spindloid cell tumours in cats and other domestic animals, using light microscopy alone, is challenging because these neoplasms share closely related histomorphological features and may arise from pluripotential mesenchymal cells capable of differentiating into various cellular subsets. These neoplasms include MPNST, fibrosarcoma, myxosarcoma, leiomyosarcoma, spindloid cell melanoma, histiocytic sarcoma and solid haemangiosarcoma. Immunohistochemical profiling may serve to determine the cellular origin [2], especially in poorly differentiated neoplasms. Intermediate filament proteins (vimentin, GFAP and laminin), S100 and NSE, although variably expressed in MPNSTs, are considered to be the most useful markers for distinguishing these tumours from other spindloid cell neoplasms in humans and animals [1–5,7]. Anti-GFAP antibody has been found to label normal Schwann cells and their tumours in humans, dogs, cats, calves and horses [1,2,9–11]; in a series of schwannomas in cats, 74% (44/59) were positive for this antigen [12]. In addition, the immunopositivity for CKAE1/AE3 and CK5/6, in conjunction with the lack of expression of CK8/18, serves to differentiate epithelial cells with squamous differentiation from carcinomas of glandular origin.

Divergent differentiation occurs in a subset of MPNSTs, but differentiation towards epithelial elements is unusual both in

humans and domestic animals [4,7]. The capacity of human MPNSTs to undergo divergent differentiation into sarcomatous or carcinomatous tissue is well known [13,14], but less than 1% of the tumours in 120 patients with MPNST had epithelial differentiation [15]. Published cases of epithelioid differentiation within MPNSTs in humans have been consistently encountered, with evidence of both rhabdomyosarcomatous and epithelial differentiation. In the present case, Masson's trichrome stain did not reveal any longitudinal striations, and myogenic antigens, such as α-SMA and desmin, were not found, which eliminates a concurrent skeletal muscle lineage. In addition, there was no evidence of PAS-positive cytoplasmic globules or mucoid material in extracellular spaces, which might have suggested epithelial cell differentiation into glandular elements [4,8].

While divergent differentiation, including carcinomatous, chondrosarcomatous and osteosarcomatous components within MPNSTs, has been documented in dogs [6,7], divergent differentiation of MPNST has only been described once in cats, with a rhabdomyosarcomatous component [16]. A large study of 59 PNSTs in cats [12] reported that 75% involved the skin, subcutis, skeletal muscle and/or mucous membranes, 27% were classified as malignant, and none had evidence of divergent differentiation.

Most MPNSTs resemble fibrosarcomas in overall architecture, but the cytoplasm of the spindloid cells of MPNSTs has subtle features of differentiation, such as lower staining intensity, a usually indistinct appearance and markedly irregular cellular contours. In addition, in poorly differentiated MPNSTs, the cellular arrangement into bundles, whorls or palisades is unusual or absent and the neoplastic cells are more densely packed [2,12,16]. The immunohistochemical profile of the spindloid cell component of the current tumour, which included positivity for vimentin, GFAP, NSE, laminin and S100, is consistent with MPNSTs of humans and animals [1,2,12,16,17]. Absence of immunolabelling for Melan A, α-SMA, desmin and CD31 allowed differentiation from other feline spindloid cell neoplasms.

Although the pathogenic basis is not known, it has been hypothesized that neural crest cells are capable of varied differentiation. Therefore, the epithelial cells might arise by metaplasia of neoplastic schwannian spindloid cells of MPNSTs [14,15] or possibly from autochthonous growth of a separate neural crest ectodermal cell line, which may have migrated along peripheral nerve fibres with the Schwann cells [18]. The potential of Schwann cells to undergo anaplasia and to form malignant epithelioid cell tumours has been sporadically documented in humans [19]. Although squamous differentiation has been occasionally described in MPNSTs with glandular elements in humans [8] and dogs [7], in the present case the epithelial component contained prominent squamous differentiation.

This novel case represents the first description in cats of divergent differentiation of MPNST towards an epithelial component with squamous differentiation, and increases the spectrum of soft tissue spindloid cell tumours in this species.

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Declaration of competing interests

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