J. Comp. Path. 2018, Vol. 162, 43–46

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NEOPLASTIC DISEASE

Evidence of Vasculogenic Mimicry in a Palpebral Melanocytoma in a Dog

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Summary

A 7-year-old neutered female Doberman pinscher was presented with a palpebral nodule on the haired eyelid of the left eye. The nodule was removed surgically. Microscopically, the nodule was consistent with eyelid melanocytoma. The tumour was characterized by the presence of numerous lacunar and slit-like spaces filled by erythrocytes and interspersed throughout the neoplastic melanocytes. Immunohistochemically, these spaces were lined by cells expressing PNL2 and factor VIII, but the cells were negative for CD31. These findings were consistent with neoplastic melanocytes without endothelial cell participation. This feature was interpreted as 'vasculogenic mimicry', a mechanism of tumour angiogenesis that is well-recognized in human melanomas, but has not yet been reported in melanomas in animals.

Keywords: dog; immunohistochemistry; melanocytoma; vasculogenic mimicry

Vasculogenic mimicry (VM) is a well-known feature of human melanoma (Maniotis *et al.*, 1999). VM has been defined as the de-novo generation of vascular channels by tumour cells and is not considered to be a strictly vasculogenic event because it does not result in formation of endothelial cell-lined vessels (Spiliopoulos *et al.*, 2015). Rather, these microcirculatory networks or capillary-like structures are comprised of extracellular matrix and are lined by neoplastic cells instead of endothelium (Spiliopoulos *et al.*, 2015). It is still not understood if these vascular structures are functional; however, these channels can be detected angiographically and red blood cells are recognizable within their lumina (Maniotis et al., 1999). Lining endothelial cells have not been identified by light microscopy, transmission electron microscopy or by the use of immunohistochemical markers (factor VIII-related antigen, CD31, CD34 and KDR [Flk-1]) (Maniotis *et al.*, 1999; Spiliopoulos *et al.*, 2015).

VM was first described in human melanoma by Maniotis *et al.* (1999) and has since been reported in different types of human tumours, including malignant mesothelioma (Pulford *et al.*, 2016) and cancers of the liver (Zhao *et al.*, 2015), pancreas (Guo *et al.*, 2014), stomach (Zang *et al.*, 2015), prostate (Wang *et al.*, 2016) and ovaries and breast (Hendrix *et al.*, 2003). The presence of VM in patients with malignant tumours (e.g. uveal and cutaneous melanomas) has been correlated with a worse prognosis and shorter survival than in patients with tumours without VM (Spiliopoulos *et al.*, 2015).

To date, in dogs, VM has been reported only in canine inflammatory breast cancer (Clemente *et al.*, 2010; Rasotto *et al.*, 2012) and has not been described previously in canine melanocytic tumours.

A 7-year-old neutered female Doberman pinscher was presented with a 5 mm, brown, palpebral nodule on the skin of the upper eyelid of the left eye. The

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0021-9975/$ - see front matter
https://doi.org/10.1016/j.jcpa.2018.06.003

nodule had been present for 1 year and was slowly enlarging. Complete ophthalmic examination revealed only mild bilateral epiphora. Pre-operative haematological and serum biochemical evaluations were within normal limits. The nodule was removed surgically, fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax. Sections were stained with haematoxylin and eosin (HE).

Microscopically, the dermis of the eyelid was expanded by a multilobular nodular neoplasm, which was moderately well demarcated and not encapsulated. Neoplastic cells were arranged in lobules and nests with multifocal areas of junctional activity. Neoplastic cells were epithelioid or, less commonly, spindle-shaped, with indistinct cell borders and a high nuclear to cytoplasmic ratio. The cytoplasm was moderate, granular and eosinophilic, occasionally vacuolated and filled multifocally by melanin pigment. Nuclei were oval, with marginated chromatin and a single prominent central nucleolus. Anisocytosis and anisokaryosis were moderate and there was an average of one mitotic figure in 10 high-power (×400) fields. Multifocally, throughout the neoplasm, numerous irregular slit-like or lacunar spaces were observed (Fig. 1). These spaces were filled by a moderate number of red blood cells, were supported by fine fibrous stroma and were lined by polygonal, epithelioid or spindle-shaped cells with moderate cytoplasm and a round to oval nucleus with a prominent nucleolus. The neoplasm was diagnosed as a mixed-type, sparsely pigmented dermal melanocytoma.

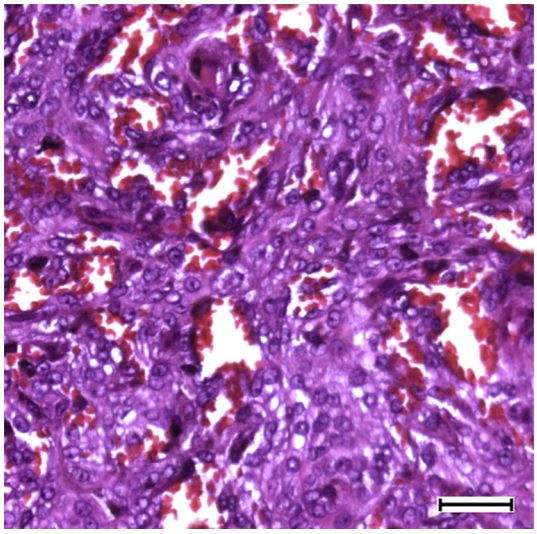

Fig. 1. The dermis is expanded by a multilobular melanocytoma admixed with numerous irregular slit-like or lacunar spaces filled by red blood cells. HE. Bar, 25 μm.

Periodic acid–Schiff (PAS) staining was performed to further characterize the lacunar spaces. There was moderate positive staining of the thin fibrous septa delimiting the blood-filled spaces.

Immunohistochemistry (IHC; ABC standard method) was performed to further characterize the cells lining the blood-filled lacunar spaces. The endothelial cell markers factor VIII (FVIII) and CD31 and the melanocytic marker PNL2 were specifically investigated. Serial sections were mounted on poly L-lysine coated slides (Menzel-Gläser, Braunschweig, Germany). PNL and FVIII labelling was performed manually by the standard ABC method. After heat-induced antigen retrieval in EDTA buffer (PNL2) or enzymatic-retrieval with pepsin (FVIII), slides were incubated with mouse monoclonal anti-PNL2 antibody (Monosan, Uden, Netherlands) at 1 in 25 dilution overnight at 4°C and rabbit polyclonal anti-factor VIII antibody (Dako, Carpinteria, California, USA), at 1 in 200 dilution overnight at 4°C, respectively. CD31 labelling was performed using a Ventana BenchMarck ULTRA immunostainer (Ventana Medical System, Roche, Oro Valley, Arizona, USA). The sections were subjected to antigen retrieval with Benchmark ULTRA CC1 (pH 8.4) at 95°C for 52 min and incubated with primary mouse monoclonal anti-CD31 (JC70A; Dako) at 1 in 20 dilution for 32 min at room temperature. 3,3′-diaminobenzidine (Roche) or 3-amino-9-ethylcarbazole (Vector Laboratories, Burlingame, California USA) were used as chromogens and sections were counterstained with Mayer's haematoxylin. Negative controls were prepared by replacing the respective primary antibody with normal rabbit or mouse serum (non-immune serum, Dako). The endothelium of blood vessels served as an internal positive control for FVIII and CD31.

The cells lining the red blood cell-filled spaces, as well as the neoplastic cells composing the melanocytoma, labelled strongly with antibody against PNL2 (Fig. 2), while they were negative for FVIII and CD31 (Figs. 3 and 4). Normal endothelium of pre-existing blood vessels within the same section was diffusely and intensely labelled for both FVIII and CD31.

Based on the histological and immunohistochemical results, the lacunar spaces were interpreted as areas of vasculogenic mimicry within an eyelid melanocytoma. Eight months after surgery, the dog was in good health with no indication of recurrence of the eyelid mass.

The term VM has been used to describe the ability of aggressive neoplastic cells to acquire an endothelial-like morphology and to form extracellular matrix (ECM)-rich vasculogenic-like networks