Immunohistochemical characterisation of a canine case of granular cell type trichoblastoma: a case report

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ABSTRACT: Herein we describe a case of granular cell type trichoblastoma in a dog. A nine-year-old, intact male Scottish terrier presented with a solitary, exophytic and non-ulcerated nodule on the shoulder. Histopathologically, the nodule showed a ribbon-like structure, which was composed of branching cords of epithelial cells surrounded by fibrous stroma. In addition, islands of large clear cells were also noted. The clear cells showed eccentric nuclei and abundant cytoplasmic vacuoles, which were strongly positive for periodic acid-Schiff staining. Cytoplasmic immunostaining for cytokeratin (CK) 14 and nuclear immunostaining for p63 were observed in the neoplastic cells. In contrast, CK18 staining was scant. Furthermore, the hair bulge stem cell marker CK15 showed strong cytoplasmic staining. Based on these histopathological and immunohistochemical findings, the tumour was diagnosed as a granular cell type trichoblastoma, which is rarely reported in dogs.

Keywords: dog; trichoblastoma; immunohistochemistry; cytokeratin

List of abbreviations

BCC = Basal cell carcinoma, CK = cytokeratin; H&E = haematoxylin and eosin; PAS = periodic acid-Schiff

Trichoblastoma is a benign cutaneous tumour, which is known to originate from the hair germ. It usually presents as a solitary, firm, alopecic nodule that is dome-shaped or polypoid (Gross et al. 2005). Histologically, canine trichoblastoma is divided into four types: ribbon, trabecular, granular and spindle cell. Most cases are a mixture of these patterns with a predominance of ribbon epithelial aggregates surrounded by fibrous stroma (Gross et al. 2005; Campos et al. 2014). The neoplastic epithelial cells have scant, pale eosinophilic cytoplasm with uniform, ovoid and euchromatic nuclei. Mitotic figures are usually infrequent (Gross et al. 2005). On immunohistochemical examination, canine and feline trichoblastomas show diffuse staining with the polyclonal reagent 34bE12 (cytokeratins; CKs 1, 5, 10, 11) and relatively strong patchy staining with CK14 (Gross et al. 2005; Sakuma et al. 2010; Campos et al. 2014). In addition, p63, which is used as a basaloid cell marker, is also expressed in trichoblastomas (Saraiva et al. 2008, Mineshige et al. 2014). Although trichoblastomas share these immunohistochemical characteristics with basal cell carcinomas (BCC), trichoblastomas can be differentiated from BCC by the absence of contiguity with the epidermis and relatively low malignancy (Gross et al. 2005).

Granular cell type trichoblastomas are a rare variant of trichoblastoma in dogs (Gross et al. 2005; Sharif and Reinacher 2006). Histopathological findings are similar to those of ribbon-type trichoblastoma, but some or all of the lobules are composed of cells with abundant, finely granular or vacuolated cytoplasm that stains strongly with periodic acid-Schiff (PAS). The nuclei of the granular cells are small, angular, and eccentric (Gross et al. 2005; Sharif and Reinacher 2006). We here described a case of a granular cell type trichoblastoma in a dog. The definitive diagnosis was made by histopathological and immunohistochemical examinations using CK14, CK18, p63, and CK15 antisera.

Case description

A nine-year-old, intact male Scottish terrier was brought to a local veterinary clinic presenting with

Table1. Antibodies used in the present case

Antigen/Antibody	Clone	Source	Working dilution
Cytokeratin 14	LL002	Abcam	1 : 100
p63	SC8431	Santa Cruz	1 : 100
Cytokeratin 18	C-04	Abcam	1 : 100
Cytokeratin 15	LHK15	Thermo	1 : 50

a solitary nodule on the shoulder. The nodule was initially detected five months previously after which it had increased in size to 3 × 5 × 1.5 cm. The nodule was exophytic and non-ulcerated with a lobular white cut surface. At initial presentation, no metastases were evident on chest radiographs or transabdominal ultrasonography. Complete blood count and serum biochemistry values were within normal ranges.

The nodule was surgically removed and then subjected to histopathological analysis. Tissue specimens were fixed in 10% neutral buffered formalin, sectioned in 4 mm increments and stained with PAS and haematoxylin and eosin (H&E). Immunohistochemical analysis was performed using the antibodies listed in Table 1. Antigen-antibody complexes were detected using the avidin-biotin procedure and then colourised with 3-amino-9-ethylcarbazole with counterstaining using Mayer's haematoxylin. On histopathological analysis, the mass was composed of well-demarcated lobules and did not have contiguity with the epidermis. The lobules showed a ribbon-like structure characterised by branching cords of epithelial cells surrounded by fibrous stroma (Figure 1A). The epithelial cells had scant eosinophilic cytoplasm and the round to ovoid nuclei had one to two small nucleoli (Figure 1B). In addition, a small group of large cells with abundant cytoplasmic vacuoles and eccentric nuclei was also noted (Figure 1C and 1D). Clear cells were arranged in islands surrounded by an eosinophilic sheath (Figure 1C). Mitotic figures were rarely seen; zero to two per high power field (× 400 magnification) (Figure 1B and 1D). In addition, the cytoplasm of the clear cells stained strongly positive for PAS (Figure 2A and 2B).

In order to obtain a definitive diagnosis, immunohistochemical examination was performed using antibodies for CK14, p63, CK18, and CK15. Strong positive expression for CK14 was observed in both epithelial cells and clear cells (Figure 3A). In addition, nuclear staining for p63 was also noted in both epithe-

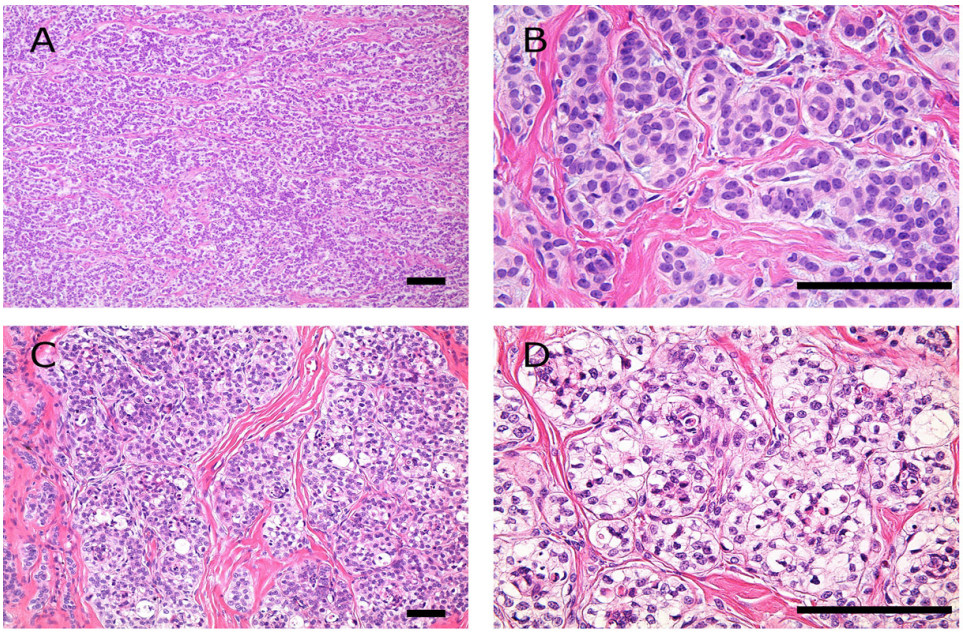

Figure 1. Haematoxylin and eosin (H&E) staining. The lobules have a ribbon-like structure, which is composed of branching cords of epithelial cells surrounded by fibrous stroma (**A**). The epithelial cells had scant eosinophilic cytoplasm and round to ovoid nuclei with one to two small nucleoli (**B**). Islands of clear large cells surrounded by an eosinophilic sheath were also noted (**C**). The clear cells had abundant cytoplasmic vacuoles and the nuclei were eccentric (**D**). Mitotic figures were rarely seen; zero to two per high power field (B and D). Bars indicate 100 μm

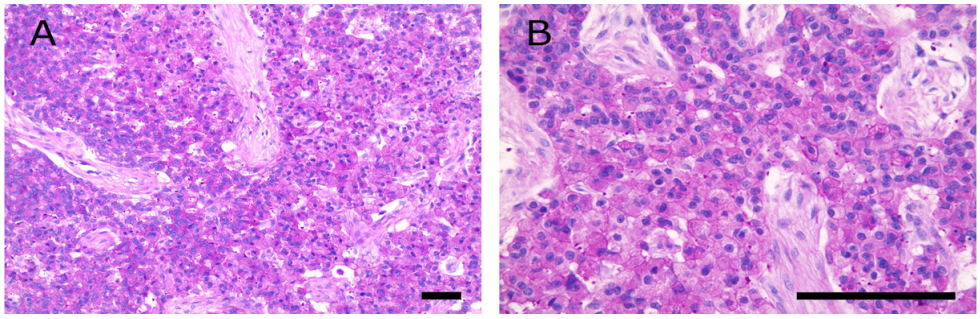

Figure 2. The neoplastic cells showed strong periodic acid-Schiff (PAS)-positive cytoplasmic granules

lial cells and clear cells (Figure 3B). Weak CK18 staining was also observed in the present case (Figure 3C). Furthermore, the CK15 hair bulge stem cell marker showed strong cytoplasmic immunostaining in both epithelial cells and clear cells (Figure 3D). Based upon those histopathological and immunohistochemical findings, the present case was diagnosed as a granular cell type trichoblastoma.

DISCUSSION AND CONCLUSIONS

Cutaneous tumours with clear or vacuolated cytoplasm are uncommon in dogs. Clear cell BCC, sebaceous carcinoma, clear cell adnexal carcinoma, tricholemmoma and a variant of trichoblastoma are all included in the differential diagnosis (Gross et al. 2005; Schulman et al. 2005; Sharif and Reinacher 2006). Trichoblastomas are thought to originate from hair germ cells, and so tumour cells are positive for CK8, but negative for CK18, which is only expressed in sweat glands. Conversely, clear cell adnexal carcinomas show positive staining for both CK8 and CK18 (Kato et al. 2007; Yasuno et al. 2009; Sakuma et al. 2010). Thus, the weak expression of CK18 in the present case excluded the possibility of clear cell adnexal carcinoma. In addition, strong expression of p63 also excluded the possibility of sebaceous carcinoma, which rarely stains positive for p63 (Saraiva et al. 2008). The strong expression of both CK14 and p63 in the present case indicates that the neoplastic cells originated from basaloid cells of either the epidermis or hair follicle. However, the ribbon-like architecture and absence of epidermal contiguity support a diag-

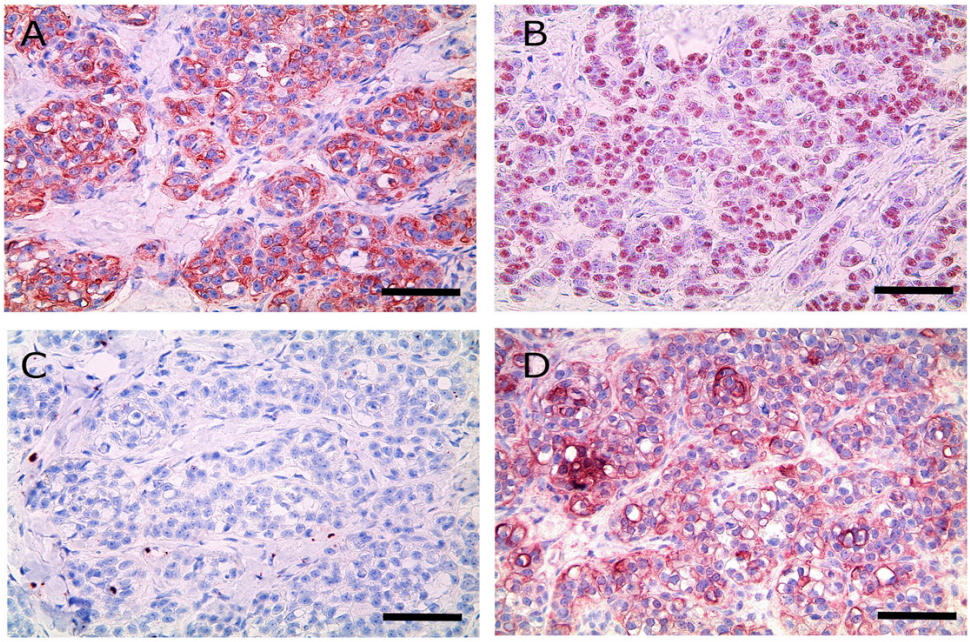

Figure 3. Immunostaining for CK14, p63, CK18, and CK15. Strong cytoplasmic staining for CK14 (**A**) and nuclear staining for p63 (**B**) were observed. The cells stained weakly for CK18 (**C**). A large amount of cytoplasmic staining for CK15 was noted (**D**). Bars indicate 100 μm

nosis of hair follicle tumour, either a tricholemmoma or trichoblastoma, rather than BCC.

Inferior tricholemmoma, which is a benign neoplasm of the outer root sheath of the hair follicle, is also characterised by PAS-positive clear cells analogous to outer root sheath cells of the hair follicle. However, inferior tricholemmoma has more islands and trabeculae than the cords and nests observed in trichoblastoma (Gross et al. 2005). In addition, the nuclei of tricholemmoma are not peripherally displaced as was observed here. Thus, based upon these histopathological findings, the tumour was diagnosed as a trichoblastoma with clear cells. The clear cells showed PAS positivity and thus the present case was diagnosed as a granular cell type trichoblastoma, which is rarely reported in dogs (Gross TL et al. 2005; Sharif and Reinacher 2006).

CK15, which is used as a reliable marker for follicular stem cells in humans and dogs, was strongly expressed in the tumour. Immunohistochemical studies using three putative stem cell markers, CK15, CK17, and CK19, suggest that human trichoblastomas are of follicular stem cell origin (Kurzen et al. 2001). Similar to the findings in humans, a recent immunohistochemical study of stem cell markers in hair follicle and epidermal tumours in dogs showed that trichoblastoma had the highest number of CK15-positive cells (Brachelente et al. 2013). Thus, findings from previous studies and in our case support the notion that stem cells might be involved in the pathogenesis of trichoblastoma and CK15 might be a useful marker for diagnosis.

In this report, we characterised a nodule as a granular cell type trichoblastoma based on clinical and histological analysis. This tumour type is rarely reported in dogs. Tumour cells showed strong positive expression of CK14, p63 and CK15, immunohistochemical findings which provide additional information pertinent to the diagnosis of trichoblastoma.

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