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NOTE

Pathology

Desmoplastic tricholemmoma in a dog

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ABSTRACT. A 10-year-old mixed breed dog was presented with a 0.8 cm diameter mass below the left eye region. The mass was surgically removed and processed for histopathological examination. Microscopically, tumor cells proliferated in small lobules, nests and cords, and the tumor parenchyma was separated by desmoplastic stroma. Majority of the tumor cells were periodic acid-Schiff (PAS)-positive, and the desmoplastic stroma was densely collagenous and mucinous. Immunohistochemical results showed that the tumor cells were diffusely positive for cytokeratin 15, cytokeratin 19 and CD 34, while cytokeratin 8 reactivity was limited to the tumor cells proliferating in cords. Few tumor cells were positive for nestin. Based on the histopathological findings, the tumor was diagnosed as desmoplastic tricholemmoma.

KEY WORDS: canine, desmoplastic tricholemmoma, hair follicle tumor

Tricholemmoma is an uncommon benign hair follicle tumor of pilar origin, characterized by a predominant outer root sheath differentiation, and rarely observed in domestic animals [1, 14]. Tumor cells of tricholemmoma are granularly positive for periodic acid-Schiff (PAS) due to the presence of abundant glycogen in the cytoplasm [14]. In veterinary literatures, tricholemmoma was first described as inferior tricholemmoma in 6 dogs by Diters and Goldschmidt [4]. In the latest WHO International Histological Classification of Tumors of Domestic Animal, tricholemmoma is classified into inferior and isthmic variants, depending on differentiation to the distinct two outer root sheath compartments [6]. In human, several reports on desmoplastic tricholemmoma had been published [3, 7, 12, 13], but this variant has never been reported in the canine counterpart. The present study describes histopathological and immunohistochemical findings of desmoplastic tricholemmoma in a dog.

A 10-year-old mixed breed female dog was brought to the Veterinary Medical Center, the University of Tokyo with a primary complaint of a facial mass, approximately 0.8 cm in diameter below the left eye region. The facial mass was surgically removed, fixed in 10% neutral buffered formalin and embedded in paraffin. Four-μm thick sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Masson's trichrome and Alcian blue (pH 2.5). Immunohistochemical staining was performed using the EnVision + System™ horseradish peroxidase (HRP)-labeled polymer and labeled streptavidin-biotin peroxidase method (Dako, Tokyo, Japan). The sections were then subjected to chromogen treatment with 3,3′-diaminobenzidine (DAB) and counterstained with Mayer's hematoxylin. Double immunofluorescence staining was performed using antibodies to pan-cytokeratin and vimentin. The sections were then reacted with Alexa 488-labeled anti-rabbit IgG (Thermo Fisher Scientific, Waltham, MA, U.S.A.) and Alexa 594-labeled anti-mouse IgG (Thermo Fisher Scientific), respectively. The nuclei were visualized and in the same time mounted with VECTASHIELD® Hard Set™ Mounting Medium with Dapi (Vector Laboratories, Burlingame, CA, U.S.A.). Primary antibodies and antigen retrieval methods used in the present study are summarized in Table 1. The antigen retrieval for nestin was according to that performed by Gerhards *et al.* [5].

Microscopically, the facial mass was located within the dermis without epidermal contiguity and well demarcated (Fig. 1a). Tumor cells in the lesion were arranged in small lobules, cords and nests (Fig. 1b). The small lobules were made up of few cells, which were surrounded by thick, eosinophilic hyaline mantles (Fig. 1b, inset). The lobular arrangement was gradually lost towards the central area of the tumor mass, where tumor cells were predominantly arranged in cords or nests with prominent desmoplastic stromal tissues (Fig. 1c and 1d). The tumor cells were ovoid to polygonal in shape, with a moderate amount of eosinophilic granular cytoplasm. Some cells were arranged in irregular cords often with an elongated, spindle appearance (Fig. 1d). The nucleus of tumor cells was round to oval in shape, centrally located, with coarse chromatin and less prominent nucleolus. Mitotic figures were 1 to 2 per high power field (×400). Multinucleated cells were scattered within the tumor parenchyma (Fig. 1c). Acantholysis

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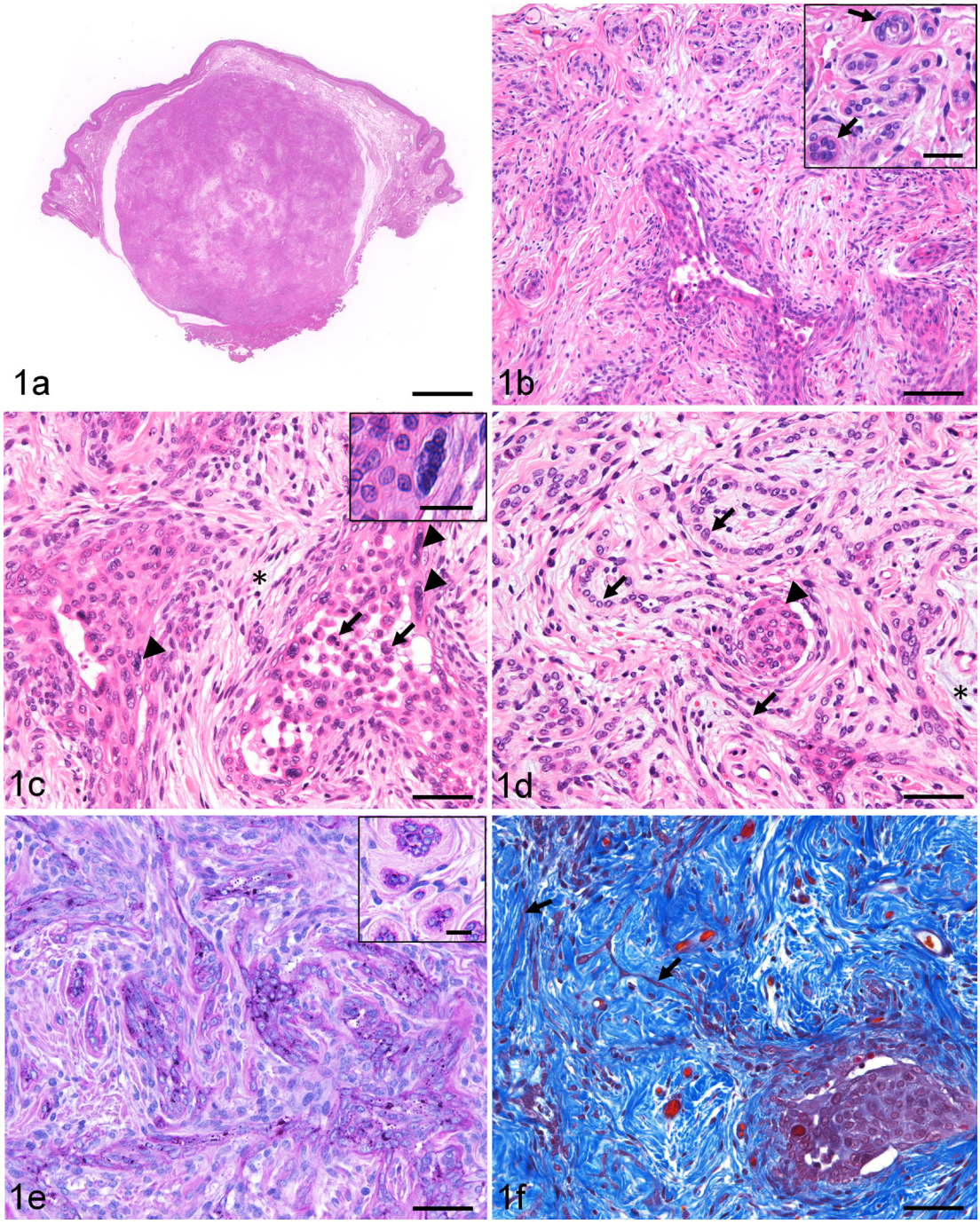

Fig. 1. Histopathological characteristics of the tumor. (a) The tumor is located at the dermis and well demarcated without epidermal contiguity. (HE). Bar=2,000 μm. (b) Tumor cells are arranged in small lobules, nests and cords surrounded by desmoplastic stroma. HE. Bar=100 μm. Inset shows the assemblies of few tumor cells forming small lobules surrounded by eosinophilic hyaline mantle (arrows). HE. Bar=30 μm. (c) Acantholysis of the tumor cells (arrows) and multinucleated cells (arrowheads) are observed at the center of the tumor nest. Mucin deposition (asterisk) is observed in the collagenous stroma. HE. Bar=50 μm. Inset represents the higher magnification of a multinucleated cell. HE. Bar=20 μm. (d) Polygonal- to spindle-shaped tumor cells are arranged in cords (arrows) or nests (arrowhead) embedded in a dense collagenous stroma with mucin deposition (asterisk). HE. Bar=50 μm. (e) Majority of the tumor cells are PAS-positive. HE. Bar=50 μm. Inset shows the assemblies of few PAS-positive tumor cells forming small lobules surrounded by PAS-negative hyaline mantle. Bar=20 μm. (f) The tumor stroma consists of massive collagen fibers. Irregular thin cords of spindle-shaped tumor cells (arrows) reside within the dense collagenous stroma. Masson's trichrome stain. Bar=50 μm.

was frequently observed at the center of the tumor cell nest (Fig. 1c). The tumor cells exhibited a low degree of anisokaryosis and anisocytosis. Majority of the tumor cells were PAS-positive, while the collagenous mantle surrounding the small tumor

Table 1. Primary antibodies and protocols for immunohistochemistry and immunofluorescence

Antibody to	Type	Dilution	Source	Antigen Retrieval
Cytokeratin	pAb	1:500	Dako, Tokyo, Japan	PIER (Proteinase K), room temperature, 30 min
CK 5/6	mAb (D5/16 B4)	1:100	Dako, Tokyo, Japan	HIER (Dako Target Retrieval Solution, pH 9.0), 121°C, 10 min
CK 8	mAb (Ks8.7)	RTU	Dako, Tokyo, Japan	HIER (Citrate buffer, pH 6.0), 121°C, 10 min
CK 14	mAb (NCL-LL002)	1:50	Leica Biosystems, Newcastle, U.K.	HIER (Citrate buffer, pH 6.0), 121°C, 10 min
CK 15	mAb (LHK 15)	1:100	Thermo Fisher Scientific, Waltham, MA, U.S.A.	HIER (Tris-EDTA, pH 9.0), 121°C, 10 min
CK 18	mAb (Ks18.04)	RTU	Progen Biotechnik, Frankfurt, Germany	PIER (Proteinase K), room temperature, 30 min
CK 19	mAb (b170)	RTU	Leica Biosystems, Newcastle, U.K.	PIER (Proteinase K), room temperature, 30 min
CD 34	pAb (C-18)	1:80	Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.	HIER (Citrate buffer, pH 6.0), 121°C, 10 min
Nestin	pAb (N1602)	1:200	IBL, Fujioka, Japan	HIER (Citrate buffer, pH 6.0), 90°C, 25 min; 15 min in absolute methanol
Vimentin	mAb (V9)	1:200	Dako, Tokyo, Japan	HIER (Citrate buffer, pH 6.0), 121°C, 10 min

CK, Cytokeratin; mAb, monoclonal antibody; pAb, polyclonal antibody; RTU, ready-to-use; HIER, Heat-induced epitope retrieval; PIER, Proteolytic-induced epitope retrieval.

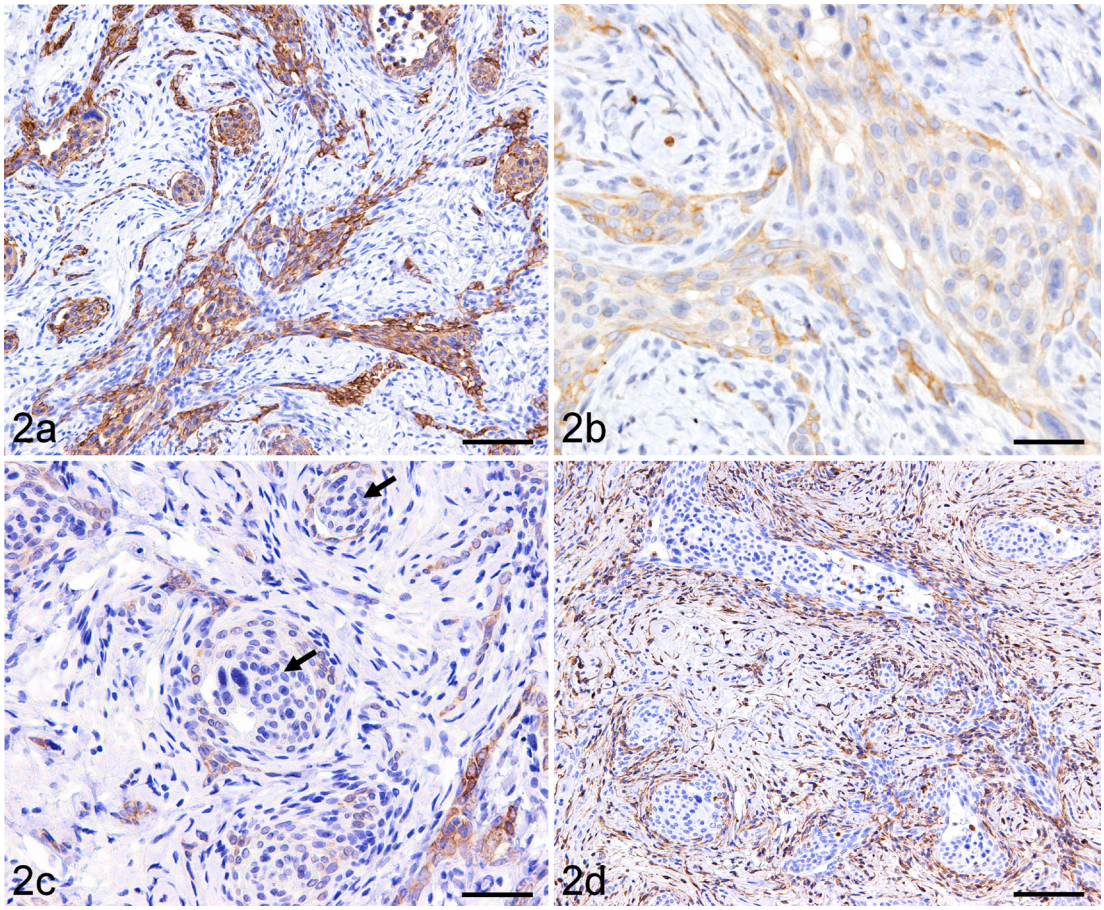

Fig. 2. Immunohistochemical characteristics of the tumor. (a) Tumor cells are strongly positive for cytokeratin 14. Bar=100 μm. (b) Tumor cells are weakly positive for CD34. Bar=40 μm. (c) Tumor cells in cords are positive for cytokeratin 8, while the cells in nests (arrows) are negative. Bar=50 μm. (d) Tumor stroma is strongly positive for vimentin. Bar=100 μm.

lobules was PAS-negative (Fig. 1e). In the tumor stroma, mucin deposition between the collagen fibers was frequently observed (Fig. 1c and 1d). The desmoplastic stroma was Masson's trichrome- (Fig. 1f) and Alcian blue (pH 2.5)-positive.

Immunohistochemically, tumor cells were strongly positive for pan-cytokeratin, cytokeratin 5/6 and cytokeratin 14 (Fig. 2a), and weakly positive for cytokeratin 15, cytokeratin 19 and CD34 (Fig. 2b). Only tumor cells proliferating in cords were positive for cytokeratin 8 (Fig. 2c). A small number of nestin-positive tumor cells were also observed. Tumor cells were negative for cytokeratin 18. The tumor stroma was strongly positive for vimentin (Fig. 2d). No co-expression of pan-cytokeratin and

vimentin was observed through double immunofluorescence stain (data not shown). Based on the histopathological and immunohistochemical findings, the facial mass was diagnosed as desmoplastic tricholemmoma.

The findings of the current case are in agreement with those in the previous reports of desmoplastic tricholemmoma in human [3, 7, 12, 13]. The diagnosis of desmoplastic tricholemmoma was made based on the differentiation toward the outer root sheath accompanied by an intense stromal reaction. The small lobules of PAS-positive tumor cells with the eosinophilic mantle structure resembled the inferior part of the outer root sheath. The mantle structures might be produced by the tumor cells. Acantholysis of tumor cells was observed within the tumor cell nest, indicating an on-going degenerative change in this area. The results of immunohistochemistry showed that tumor cells were positive for a basal cell marker (cytokeratin 14) and isthmus stem cell markers (cytokeratin 8, cytokeratin 15, CD 34 and nestin) [2, 5, 8, 9, 11, 15], and particularly, the tumor cells in cord structure were positive for cytokeratin 8. These immunohistochemical findings imply the heterogeneity of tumor cells. Cytokeratin 19 reactivity in the canine hair follicle is ambiguous, according to anti-cytokeratin 19 antibodies used [10, 15]. In the present study, tumor parenchyma as well as the inferior to isthmus parts of the outer root sheath in the anagen phase was positive for cytokeratin 19. The above findings suggest that the present canine tumor may be derived from isthmus stem cells and that the tumor cells differentiate toward the inferior segment of the outer root sheath.

Differential diagnoses for the present case had included desmoplastic basal cell carcinoma, desmoplastic trichoepithelioma and trichoblastoma with sclerosing stroma. Desmoplastic basal cell carcinoma was first ruled out based on the immunohistochemical results of the tumor cells. Desmoplastic trichoepithelioma was less likely, because we observed neither trichogenic differentiation nor differentiation into multiple hair follicle compartments within the tumor parenchyma. Trichoblastoma with sclerosing stroma was another probable diagnosis for the case. However, the tumor cells of the present case were more differentiated, because the majority of them were PAS-positive.

Hunt and his colleagues provided a comprehensive histopathological characterization of desmoplastic tricholemmoma in human [7]. In the report, the tumor was characterized by being well demarcated and composed of mainly PAS-positive neoplastic epithelial cells forming small lobules at the periphery of the lesion. The tumor lesion was gradually disrupted and replaced by the dense, desmoplastic stroma in which tumor cells were arranged in nest or cord. In addition, other human studies also highlighted dyskeratosis and individual cell necrosis as common histological findings of desmoplastic tricholemmoma [12, 13].

In the present case, co-expression of pan-cytokeratin and vimentin was never observed in the tumor lesion, which may exclude the possibility that the desmoplastic reaction is a result of the epithelial-mesenchymal transition [13], while the exact cause of the marked desmoplasia remained unknown.

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