



NOTE

Pathology

A cutaneous mixed tumor in a dog

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ABSTRACT. The atypical cutaneous tumor of a 9-year-old mixed breed female dog was examined. The tumor was well-demarcated and histologically composed of a trichoblastic area, tricholemmal area and apocrine glandular area. Neoplastic cells in trichoblastic area and tricholemmal area had PAS-positive granules in the cytoplasm and were positive for pan-cytokeratin, cytokeratin 5/6, 14 and 19 and p63. Neoplastic cells in trichoblastic area were also positive for cytokeratin 15 and CD34. Neoplastic cells in apocrine glandular area were positive for pan-cytokeratin and cytokeratin 7, 18 and 19. Myoepithelial cell proliferation with osteocartilaginous metaplasia was observed in this area. Since neoplastic cells showed multiphenotypic differentiation for hair follicles and apocrine glands, the present case was diagnosed as a cutaneous mixed tumor.

KEY WORDS: cutaneous mixed tumor, dog, hair follicle, multiphenotypic differentiation

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Adnexal tumors are common in dogs. In the World Health Organization (WHO) classification of tumors of domestic animals, adnexal tumors have been categorized into five fascicles [1]: follicular tumors, nailbed tumors, sebaceous and modified sebaceous gland tumors, apocrine and modified apocrine gland tumors, and eccrine gland tumors, based on their differentiation patterns. However, adnexal tumors rarely show multiphenotypic differentiation; the complexity of their histological appearance results in diagnostic difficulties [7, 8].

In human pathology, a cutaneous mixed tumor is an uncommon benign adnexal tumor formerly called “chondroid syringoma” [6]. The tumor is well-circumscribed and composed of a mixture of epithelial and stromal components with a wide spectrum of differential patterns. The epithelial component consists of solid, follicle and tubular structures mainly with apocrine and eccrine differentiation. The follicular differentiation of epithelial components also occurs in some cases [2, 3]. The stromal component shows chondroid, myxoid and fibrous differentiation. Local recurrence is uncommon, and the prognosis of the tumor is very good.

In the present study, we describe the morphological findings and immunohistochemical profiles of a canine case of an atypical adnexal tumor with follicular and apocrine differentiation similar to a human cutaneous mixed tumor.

A nine-year-old mixed breed female dog was brought to a local veterinary clinic for a cutaneous mass on the head. The mass was round, well-circumscribed and approximately 10 mm in diameter. One year later, the mass increased to approximately 20 mm in diameter with ulceration on the surface. Excision was performed, and the mass was submitted to the Japan Animal Referral Medical Center for a histological examination. An immunohistochemical examination was performed at the University of Tokyo.

The mass was fixed in 10% neutral-buffered formalin and embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS). Immunohistochemistry (IHC) was performed using the primary antibodies listed in Table 1. Some sections were pretreated for antigen retrieval as shown in Table 1. Sections were treated with 3% hydrogen peroxide (H₂O₂)-methanol at room temperature for 5 min and incubated in 10% skimmed milk Tris-buffered saline with 0.1% Tween 20 (TBST) at 37°C for 40 min to avoid non-specific reactions. Sections were incubated at 4°C overnight with primary antibodies. After being washed three times in TBST, the sections were incubated with Envision horseradish peroxidase (HRP) mouse or rabbit polymers (Dako-Japan, Kyoto, Japan) at 37°C for 30 min. Sections were washed again and visualized with 0.05% 3–3′diaminobenzidine (DAB) and 0.01% H₂O₂ in 0.05 M pH7.6 Tris-HCl buffer. Counterstaining was performed with Mayer’s hematoxylin.

The mass was located from the dermis to subcutaneous tissue and was well-demarcated. It was indistinctly separated into three areas based on morphological patterns (Fig. 1). In trichoblastic area, the peripheral area of the tumor, small basaloid epithelial

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Table 1. Primary antibodies and protocols for immunohistochemistry

Antibody	Clone	Dilution	Source	Antigen retrieval
Pan-Cytokeratin	AE1/AE3	1:200	Dako	-
Cytokeratin 5/6	D5/16 B4	1:50	Dako	pH9.0 Autoclave
Cytokeratin 7	OV-TL 12/30	1:100	Dako	Proteinase K
Cytokeratin 14	NCL-LL002	1:50	Leica	pH6.0 Autoclave
Cytokeratin 15	LHK15	1:50	Thermo	-
Cytokeratin 18	Ks18.04	ready to use	PROGEN	Proteinase K
Cytokeratin 19	b170	ready to use	Leica	Proteinase K
CD34	(polyclonal)	1:80	Santa Cruz	pH6.0 Autoclave
p63	BC4A4	1:100	Biocare Medical	pH6.0 Autoclave
α -SMA	1A4	1:100	Dako	-

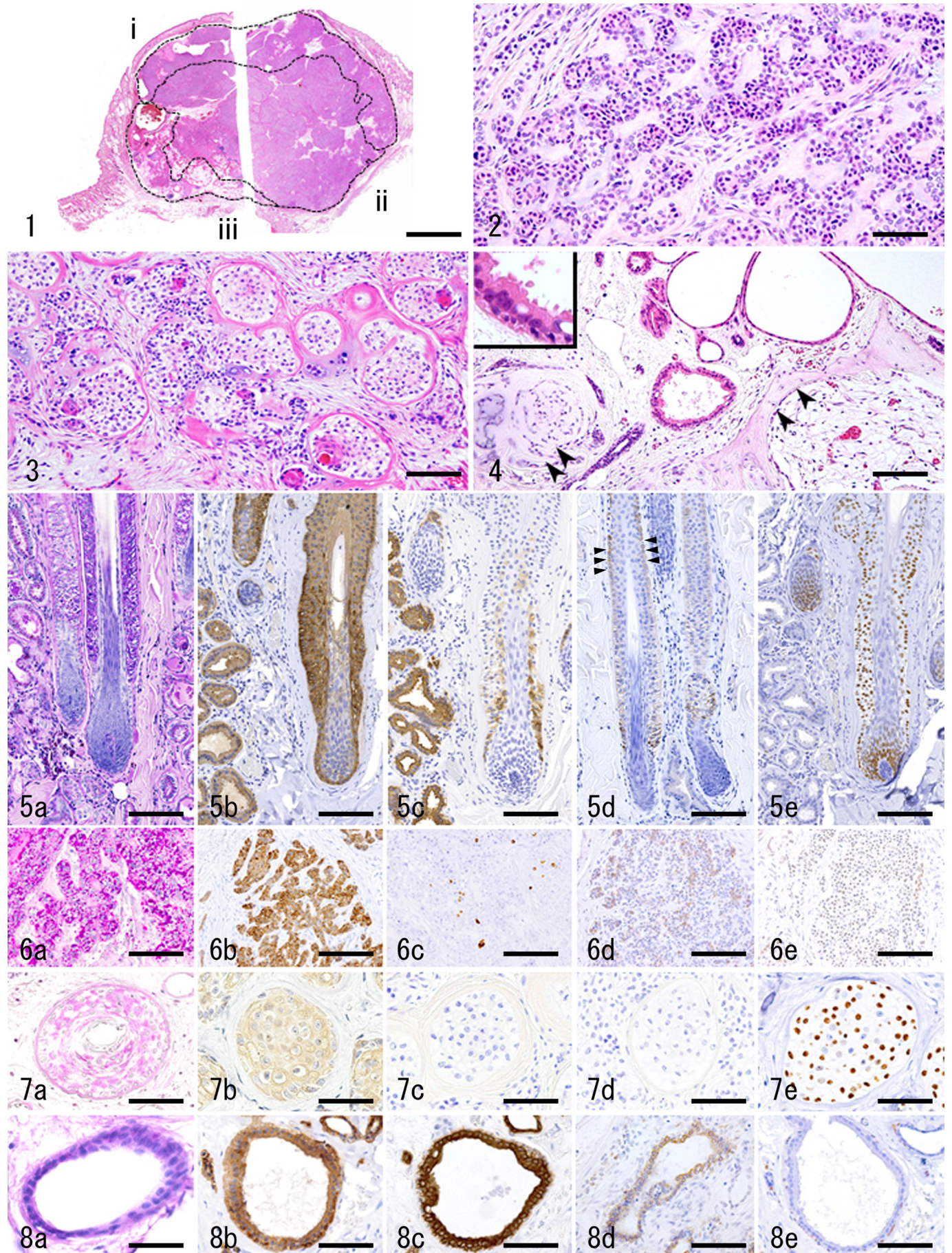
Table 2. Results of immunohistochemistry on neoplastic cells and normal cutaneous adnexal tissue

	Pan-CK	CK 5/6	CK14	CK7	CK18	CK15	CK19	CD34	p63	α -SMA
<i>Normal tissue</i>										
Dermal papilla	-	-	-	-	-	-	-	-	-	-
Hair matrix	-	-	-	-	-	-	-	-	+	-
Hair shaft medulla	+	+	-	-	-	-	-	-	-	-
Hair shaft cortex	+	-	-	-	-	-	-	-	-	-
Inner root sheath	-	-	-	-	-	-	-	-	-	-
Outer root sheath isthmus	++	++	++	-	+	+	+	+	++	-
Outer root sheath infundibulum	++	+	++	-	-	++ ^{a)}	-	+	+	-
Sebaceous gland	+	+	+	-	-	±	-	-	±	+
Apocrine gland glandular epithelium	+	-	-	+	+	+	+	-	-	-
Apocrine gland myoepithelium	+	+	+	-	+	-	-	-	+	+
<i>Neoplastic cells</i>										
Small round cell, trichoblastic area	++	++	+	-	-	+	+	+	++	-
Clear polygonal cell, tricholemmal area	+	+	+	-	-	-	±	+	+	-
Glandular epithelial cell, apocrine glandular area	++	-	-	+	+	+	+	-	-	-
Glandular myoepithelial cell, apocrine glandular area	++	+	+	-	+	-	-	-	+	+

CK, cytokeratin; -, negative; +, positive; ++, strongly positive. a); outermost layer only.

cells formed narrow branching cords with mucinous or fibrous stroma (medusoid pattern) (Fig. 2). Neoplastic cells had a clear cytoplasm and small round nuclei with a few mitotic figures. The cytoplasm was strongly positive for PAS. In tricholemmal area, the central area of the tumor, clear polygonal cells resembling an external root sheath formed island patterns surrounded by a thick fibrous capsule (Fig. 3). Neoplastic cells had a clear to pale cytoplasm with some PAS-positive glycogen granules and oval nuclei with a few pleomorphic and mitotic figures. The same granules were also present in normal external root sheaths. Neoplastic cells showed follicular keratinization within the follicle. In apocrine glandular area, the deep area of the tumor, polygonal cells formed a biphasic multitubular pattern with myoepithelial cell proliferation (Fig. 4). Neoplastic cells had an eosinophilic cytoplasm and oval nuclei. Myoepithelial cells showed osteocartilaginous metaplasia.

The results of IHC are summarized in Table 2. In the normal follicle, the outer root sheath was strongly positive for pan-cytokeratin (AE1/AE3), cytokeratin 5/6 and 14, and p63. In the deep area, the outer root sheath isthmus was positive for cytokeratin 18 and 19 and CD34. The outermost layer of the outer root sheath infundibulum at the insertion point of the arrector pili muscle is called the bulge area. This area was positive for cytokeratin 15. The apocrine gland glandular epithelium was positive for pan-cytokeratin and cytokeratin 7, 15, 18 and 19. Apocrine gland myoepithelial cells were positive for pan-cytokeratin, cytokeratin 5/6, 14 and 18, p63 and α -smooth muscle actin (α -SMA) (Figs. 5, S1). In trichoblastic area of the mass, neoplastic cells were positive for cytokeratin 14; and strongly positive for pan-cytokeratin, cytokeratin 5/6 and p63. Positive ratios of neoplastic cells for stem cell markers (cytokeratin 15, 19 and CD34) were as follows; cytokeratin 15: 28.5%, cytokeratin 19: 58.5% and CD34: 39.5% (Figs. 6, S2). In tricholemmal area, neoplastic cells were positive for pan-cytokeratin, cytokeratin 5/6 and 14 and p63. Positive ratios of neoplastic cells for the stem cell markers were as follows; cytokeratin 15: 0%, cytokeratin 19: 3.5% and CD34: 32.5% (Figs. 7, S3). The IHC characteristics of these two cell types were similar to those of the external root sheath. In apocrine glandular area, neoplastic glandular epithelial cells were positive for pan-cytokeratin and cytokeratin 7, 18 and 19 (Figs. 8, S4). Positive ratios of glandular epithelial cells for the stem cell markers were as follows; cytokeratin 15: 100%, cytokeratin 19: 100% and CD34: 0%. Neoplastic myoepithelial cells were positive for pan-cytokeratin, cytokeratin 5/6, 14 and 18, p63 and α -SMA; and negative for the stem cell markers. These immunohistochemical characteristics of neoplastic cells were similar to those of the apocrine gland.



Histological and IHC examinations revealed follicular differentiation (trichoblastic area and tricholemmal area) and apocrine differentiation in tumor tissue. Regarding the follicular component, trichoblastic primitive hair germ and external root sheath differentiation were predominant. These areas were continuous, and the borders between the different components were unclear; therefore, the tumor was more likely to be a follicular tumor with multiphenotypic differentiation than a collision tumor. In addition, osteocartilaginous metaplasia was observed within apocrine glandular area of tumor. Based on these results, the tumor was diagnosed as the cutaneous mixed tumor of the dog. In the WHO classification of tumors of domestic animals, there is no category for adnexal tumors with multiphenotypic differentiation [1]. In humans, these tumors are categorized as “cutaneous mixed tumors”, with tumors with the osteocartilaginous differentiation of myoepithelial cells being categorized as chondroid syringoma [6]. Adnexal tumors with multiphenotypic differentiation are rare in humans and animals, and there have been no reports of a cutaneous mixed tumor in a dog. Sharif *et al.* reported two canine cases of an adnexal tumor with an external root sheath and sebaceous gland differentiation as clear cell type trichoblastoma [8]. Clear cell type trichoblastoma is a recent variant with the proliferation of small epithelial cells with a vacuolated cytoplasm, and only a few cases have been reported in humans [9, 10]. In these reports, the origins of tumor cells were not elucidated; some cases showed external root sheath differentiation [10], whereas others displayed sebaceous gland differentiation [9]. Sebaceous gland differentiation was not observed in the present case; however, follicular differentiation and apocrine gland differentiation with osteocartilaginous metaplasia were detected. Kobayashi *et al.* previously demonstrated that canine hair stem cells in the bulge area have multipotency for follicular and adnexal differentiation similar to human hair stem cells and are positive for the hair stem cell markers, cytokeratin 15 and 19 [4, 5]. In the present case, trichoblastic cells in area 1 were positive for cytokeratin 15 and 19 and CD34, indicating the hair stem cell features of tumor cells. The present study describes a case of an atypical cutaneous tumor with multiphenotypic differentiation in a dog. The IHC results of the tumor and normal canine skin tissue provided will contribute to the identification of adnexal differentiation in a canine cutaneous mixed tumor.

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Fig. 1. Low magnification of the cutaneous mass. The mass is well-demarcated and separated into three areas: i) a trichoblastic area, ii) tricholemmal area and iii) apocrine glandular area. Hematoxylin and eosin (HE) stain. Bar=5 mm.

Fig. 2. Trichoblastic area of the mass. HE stain. Bar=100 μ m.

Fig. 3. Tricholemmal area of the mass. HE stain. Bar=100 μ m.

Fig. 4. Apocrine glandular area of the mass. HE stain. Bar=100 μ m. Insert: High magnification of neoplastic cells. Neoplastic cells showed apocrine secretion. Arrowhead: Osteocartilaginous metaplasia of myoepithelial cells.

Fig. 5. PAS stain and immunohistochemistry of a normal hair follicle. Bar=200 μ m. a: PAS stain. b: Pan-Cytokeratin (AE1/AE3). c: Cytokeratin 18. d: Cytokeratin 15. e: p63. Arrowhead: bulge area.

Fig. 6. PAS stain and immunohistochemistry of the trichoblastic area. Bar=200 μ m. a: PAS stain. b: Pan-Cytokeratin (AE1/AE3). c: Cytokeratin 18. d: Cytokeratin 15. e: p63.

Fig. 7. PAS stain and immunohistochemistry of the tricholemmal area. Bar=50 μ m. a: PAS stain. b: Pan-Cytokeratin (AE1/AE3). c: Cytokeratin 18. d: Cytokeratin 15. e: p63.

Fig. 8. PAS stain and immunohistochemistry of the apocrine glandular area. Bar=100 μ m. a: PAS stain. b: Pan-Cytokeratin (AE1/AE3). c: Cytokeratin 18. d: Cytokeratin 15. e: p63.