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NOTE

Pathology

Pleomorphic adenoma of the labial gland, characterized by reticular pattern of myoepithelial cells in a dog

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ABSTRACT. An 11-year-old female golden retriever dog had a mass at the right corner of the upper lip, which gradually increased in size and protruded into the oral cavity. The mass was removed surgically. The cut surface of the mass was smooth, whitish and solid, and covered by the oral mucosal membrane. Histopathologically, the mass consisted mainly of reticular pattern of short spindle cells that stained positively for cytokeratin AE1/AE3, α-smooth muscle actin and p63, suggestive of a myoepithelial cell phenotype. Between the neoplastic cords, there was myxoid or edematous connective tissue. Additionally, neoplastic cells with luminal epithelial and basal cell phenotypes were arranged in ducts and small islands, respectively. Based on the diverse histological and immunohistochemical features, the tumor was diagnosed as pleomorphic adenoma of the labial gland. To our knowledge, the reticular proliferation pattern of myoepithelial cells has not been described in salivary gland tumors of domestic animals.

KEY WORDS: labial gland, myoepithelial cells, pleomorphic adenoma, salivary gland tumors

Salivary gland tumors are rare in animals and are classified into 6 benign and 8 malignant epithelial tumors according to their histology [6]. In dogs and cats, carcinomas are more common than adenomas, and major salivary glands are more frequently affected than minor salivary glands. In humans, pleomorphic adenoma is the most common salivary gland tumor, which is composed of a wide variety of cell types including epithelial, myoepithelial, and mesenchymal or stromal cells [1]. In contrast, mixed salivary gland tumors are rare in animals. In dogs, a few cases of pleomorphic adenomas and malignant mixed tumors in the parotid and mandibular glands have been reported [6, 11, 12]. Here, we describe a case of canine pleomorphic adenoma of minor salivary gland showing characteristic histological pattern of myoepithelial proliferation.

An 11-year-old female golden retriever dog was presented with a mass at the right corner of the upper lip. The mass gradually increased in size and protruded into the oral cavity. The mass was surgically excised. Recurrence of the tumor had not been seen for 21 months after surgery. Grossly, the mass was 4 × 2.5 × 2.5 cm in size, and the cut surface was smooth, whitish and solid (Supplementary Fig. 1). The tissues were fixed in 10% neutral-buffered formalin, routinely processed and embedded in paraffin. Histologic sections cut at 5 μm were stained with hematoxylin and eosin (HE) and alcian blue (pH 2.5 and 1.0). Immunohistochemical analyses were performed using monoclonal antibodies against pan-cytokeratin (clone AE1/AE3, 1:1,000, Dako, Glostrup, Denmark), cytokeratin 19 (CK19; clone b170, 1:100, Leica, Nußloch, German), α-smooth muscle actin (α-SMA; clone 1A4, 1:1,000, Dako), p63 (clone 4A4, 1:500, Novus Biologicals, Littleton, Colorado), proliferating cell nuclear antigen (PCNA; clone PC10, 1:10,000, Dako) and vimentin (clone V9; ready to use, Dako). After dewax, sections for p63, PCNA and vimentin were pretreated by microwave for 20 min in 0.01 M citrate buffer (pH 6.0), and those for CK19 were pretreated by trypsin at 37°C for 20 min for antigen retrieval as previously described [2]. Sections were incubated with 3% H_2O_2 in phosphate-buffered saline (PBS) for 10 min to quench endogenous peroxidase. Thereafter, the sections were treated with 5% skimmed milk in PBS for 30 min and incubated with each primary antibody for 1 hr at room temperature, followed by an incubation with peroxidase-conjugated secondary antibody (Histofine Simple Stain MAX PO; Nichirei, Tokyo, Japan). Positive reactions were detected with 3, 3′-diaminobenzidine (DAB Substrate Kit; Nichirei). Sections were counterstained lightly with hematoxylin. Normal labial glands were used as a positive control.

Microscopically, the mass was well-circumscribed and located in the submucosa of the inner lip (oral mucosa) without any clear evidence of local invasion. It was composed of reticular pattern of anastomosing cords of short spindle cells with oval nuclei

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Table 1. Immunoreactivity of the normal labial gland and neoplastic cells

Cell types		CK AE1/AE3	CK19	α-SMA	p63	Vimentin	PCNA (%)	Phenotype
Normal labial gland	Acinar epithelium	+	+	–	–	–	–	
	Duct epithelium	+	+	–	–	–	–	
	Myoepithelium	+	±	+	+	–	–	
	Basal cell	+	±	–	+	–	–	
This tumor	Reticular	+	±	+	+	–	5.1	Myoepithelium
	Duct	+	+	–	–	–	4.7	Duct epithelium
	Small island	+	±	–	+	–	5.5	Basal cell
	Mesenchymal cell	–	–	–	–	+	5.2	

–, negative; ± , weakly positive; +, positive.

and eosinophilic cytoplasm (Figs. 1 and 2). Myxoid or edematous stromal tissue with scattered spindle to elongated mesenchymal cells was seen between the neoplastic cords (Fig. 2); some mesenchymal cells were in close proximity of the neoplastic cords. The myxoid stroma was positive for alcian blue (Supplementary Fig. 2). Additionally, cuboidal to ovoid neoplastic cells were arranged in ducts or small islands (Fig. 3). Some ducts were connected to large ducts communicating with the overlying oral mucosa (Supplementary Fig. 3). The results of immunohistochemistry in the normal labial gland and this tumor are shown in Table 1. The short spindle cells showing reticular pattern were positive for CK AE1/AE3, α-SMA (Fig. 4) and p63, weakly positive for CK19, and negative for vimentin, showing a myoepithelial phenotype. The ductal neoplastic cells were positive for CK AE1/AE3, CK19 (Fig. 5) and negative for α-SMA, p63 and vimentin, showing a duct epithelial phenotype. The neoplastic cells in small islands were positive for CK AE1/AE3 and p63 (Fig. 6), weakly positive for CK19, and negative for α-SMA and vimentin, showing a basal cell phenotype. Mesenchymal cells in the myxoid stroma were positive for vimentin (Supplementary Fig. 4) and negative for CK AE1/AE3, CK19, α-SMA and p63. There were some vimentin-positive and α-SMA-negative cells within the neoplastic cords (Supplementary Figs. 5 and 6). The PCNA-positive index of the neoplastic epithelial cells was around 5%; it was almost the same as that of the mesenchymal cells in the myxoid stroma (Table. 1).

The tumor of the present case consists of an admixture of luminal epithelium, myoepithelium and basal epithelial cells, characterized by reticular arrangement of myoepithelial cells. Odontogenic tumors, such as ameloblastoma and ameloblastic fibroma, should be considered in the differential diagnosis as they often show cord or palisading arrangement [5]. Odontogenic tumors usually involve the maxillary and mandibular bones and gingiva; the involvement of the lip is not described [5, 8]. The present tumor is considered to arise from the labial gland, based on its anatomical location and the connection to the overlying oral mucosa. Additionally, there was no clear evidence of odontogenic differentiation. Therefore, the possibility of odontogenic tumor was excluded.

According to the WHO classification of salivary gland tumors in animals, benign epithelial tumors are classified into pleomorphic adenoma, oncocytoma, canalicular adenoma, sebaceous adenoma, ductal papilloma and cystadenoma [6]. The malignant epithelial tumors are classified into acinic cell carcinoma, mucoepidermoid carcinoma, cystadenocarcinoma, adenocarcinoma, malignant myoepithelioma, malignant mixed tumor and squamous cell carcinoma. Unlike mammary and apocrine gland tumors [3, 4, 7], there is no classification of "complex" tumors in the salivary gland [6, 8]. The tumor consisting of both epithelial and myoepithelial cells is classified as benign pleomorphic adenoma or malignant mixed tumor. In the present case, based on the low proliferative activity and absence of tumor invasion, recurrence or metastasis, the tumor was diagnosed as pleomorphic adenoma of the labial gland.

In dogs, reported cases of pleomorphic adenomas in the salivary gland are composed of multicystic arrangement of luminal epithelium surrounded by vacuolated myoepithelium with nodules of mesenchymal (fibrous, cartilaginous) cell proliferation [11]. In humans, epithelial cells in pleomorphic adenomas show various histological patterns, such as ductal, basaloid and squamous cell appearance, while myoepithelial cells show spindle, plasmacytoid and clear cell appearances [1]. The proliferation patterns of myoepithelial cells are myxoid, solid, reticular/anastomosing, pseudocystic and pseudoglandular [1, 10]. In rodents, spontaneous pleomorphic adenomas of the salivary gland are reported in prairie dogs (*Cynomys ludovicianus*) [9]. The tumors in prairie dogs consist of ductal or trabecular proliferation of luminal epithelium admixed with solid or diffuse proliferation of myoepithelium. They also contain alcian blue-positive myxomatous stroma. The present study is the first report describing the reticular pattern of myoepithelium in salivary gland tumor of animals.

In pleomorphic adenoma of humans and animals, mesenchymal/stromal cells are described as a tumor component [1, 8]. In the present case, the presence of vimentin-positive and α-SMA-negative cells within the myoepithelial cords may indicate a transition from myoepithelial to mesenchymal cells. This hypothesis is further supported by the alcian-blue positivity in the stroma, as myoepithelial foci are positive for alcian-blue in canine pleomorphic adenomas [11]. Moreover, the PCNA index of the mesenchymal cells is almost the same as that of the neoplastic epithelial cells. Therefore, the mesenchymal cells in the present case are considered as a tumor component, and they might be originated from myoepithelial cells.

Malignant tumor can arise in pleomorphic adenoma; it is called carcinoma ex pleomorphic adenoma in humans [1] or carcinoma in pleomorphic adenoma (malignant mixed tumor) in animals [6, 8, 13]. Malignant mixed tumor has been reported in a dog [12]

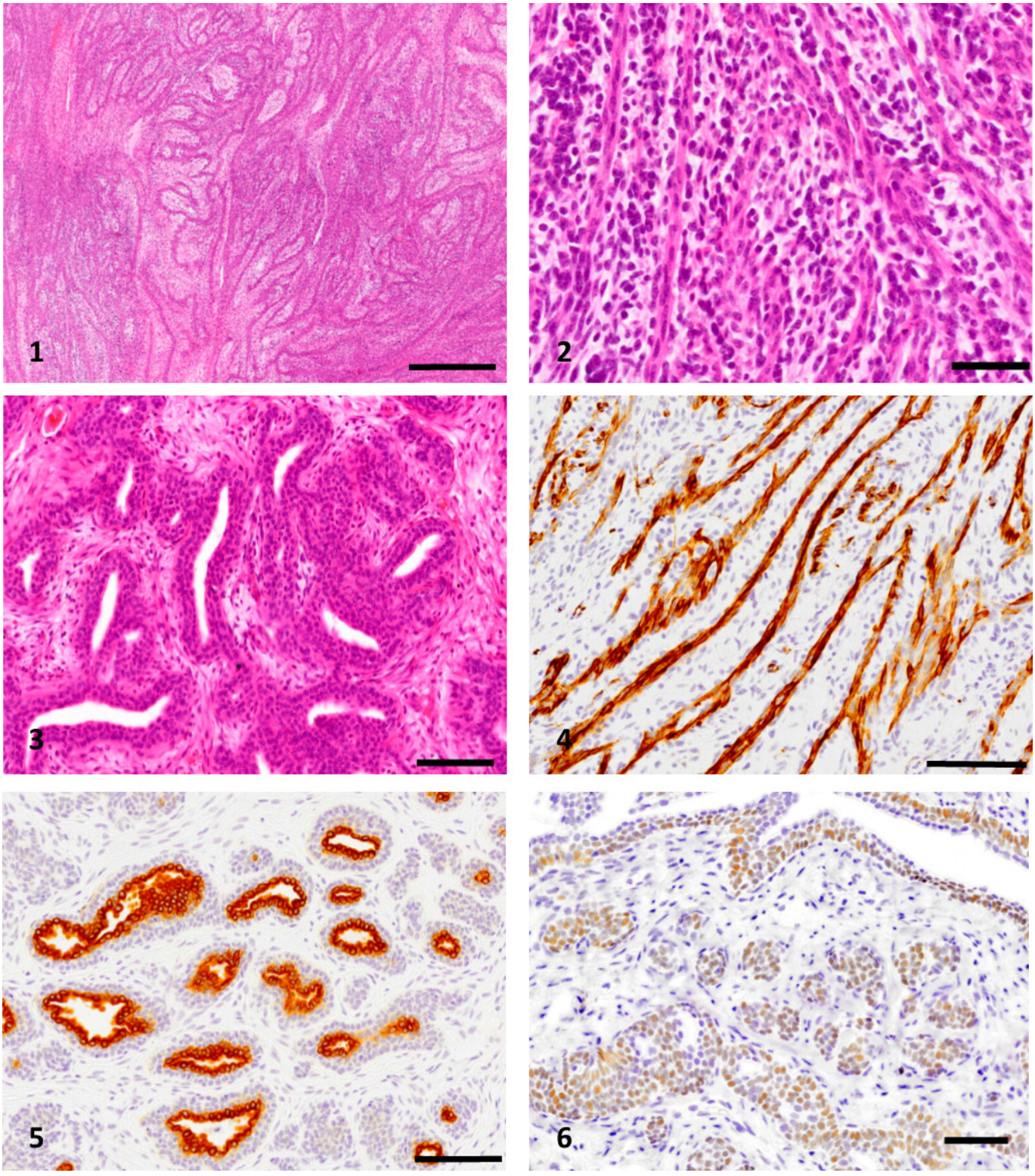

Fig. 1. The tumor is composed of reticular pattern of anastomosing cords of neoplastic cells with stromal tissue. HE stain, bar=500 μm.

Fig. 2. Higher magnification of Fig. 1. The neoplastic cords consist of short spindle cells with eosinophilic cytoplasm. Moderately to highly cellular stroma is seen between the neoplastic cords. HE stain, bar=50 μm.

Fig. 3. Neoplastic cells are arranged in ducts or small islands. HE stain, bar=100 μm.

Fig. 4. Neoplastic cells arranged in reticular pattern are positive for α-smooth muscle actin. Bar=50 μm.

Fig. 5. Neoplastic cells forming ducts are positive for CK19. Bar=50 μm.

Fig. 6. Neoplastic cells arranged in small islands and in the basal part of ducts are positive for p63. Bar=50 μm.

and a cat [14]. These tumors are composed of atypical and invasive epithelial cells and/or sarcomatous mesenchymal cells [1, 6, 8, 12–14]. The present tumor did not show such malignant aspects.

In this study, we reported a canine pleomorohic adenoma of the labial gland with characteristic reticular pattern of myoepithelial proliferation; the histologic features are different from pleomorphic adenomas in animals reported to date. As salivary gland tumors are known to have diverse histologic patterns, further accumulation of cases is required to understand the histopathology of canine salivary gland tumors.

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