**NOTE** *Pathology*

# Complex Apocrine Carcinoma with Dominant Myoepithelial Proliferation in a Dog

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ABSTRACT. A rare case of complex apocrine carcinoma displaying dominant myoepithelial proliferation developed in the right leg subcutis of a 10-year-old male dog. The major cell population consisted of diffusely proliferating p63-expressing neoplastic cells that were largely myoepithelial in origin co-expressing α-smooth muscle actin. A small portion of the cell population consisted of concomitant basal epithelial cells lacking α-smooth muscle actin expression. The minor population consisted of p63-negative apocrine gland cells that expressed cytokeratin 8. The myoepithelial cell population showed a rather stronger proliferation activity than did the apocrine epithelial population. Thus, this tumor might have been derived from basal epithelial cells characterized by more predominant myoepithelial differentiation than luminal apocrine epithelial differentiation.

KEY WORDS: canine, diagnosis, pathology, skin, tumor.

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A hard, raised, and partly ulcerated subcutaneous tumor was found at the medial right leg of a 10-year-old male shih tzu. No other masses were found on palpation. Fine-needle aspiration cytology revealed large, round, atypical cells with variously sized nuclei and prominent nucleoli. The surgically excised tumor was approximately 2 × 3 × 3 cm. It had a whitish, solid appearance on the cut surface and was covered with connective tissue, which subdivided the tumor into irregular sized small lobules (Fig. 1). The tumor was fixed with 10% neutral buffered formalin at room temperature, dehydrated, and embedded in paraffin. Sections (4 $\mu$m in thickness) were stained with hematoxylin and eosin (HE). For immunohistochemical analysis, double-staining was performed using the primary antibodies and immunodetection kits listed in Table 1 in combination with cytokeratin 8 (CK8) and α-smooth muscle actin (αSMA); p63 and CK8; p63 and αSMA; proliferating cell nuclear antigen (PCNA) and CK8; and PCNA and αSMA. After incubation with the first primary antibody, the avidin-biotin-peroxidase complex (ABC) method was applied. To block antibody cross-reactivity, immunostained tissue slides were heated using a microwave processor (H2850; EBSciences, CT) in citrate buffer at 100°C for 10 min before incubation with the second primary antibody. Immunoreactivity was then detected using the avidin-biotin-alkaline phosphatase complex (ABC-AP) method. All slides were counterstained with hematoxylin. The ratio of PCNA-positive cells was assessed by counting cells in 10 randomly selected photomicrographs of the cellular populations positive for either CK8 or αSMA at ×400 magnification. Normal dog skin was used as a positive control tissue, and non-immunized mouse serum was substituted for the primary antibody as negative controls for immunoreactivity.

Histologically, the tumor was roughly subdivided by connective tissue into irregularly sized lobules. Each tumor lobule largely consisted of neoplastic myoepithelial cells showing moderate cellular atypia with poorly demarcated, weakly eosinophilic cytoplasm. The cells showed diffuse proliferation with or without forming small, irregularly sized cellular nests subdivided by fine, fibrous septa. These cells contained 1 round-to-oval-shaped, small-to-medium-sized nucleus with 1 or 2 small nucleoli and fine, granular chromatin and showed frequent mitotic figures (Fig. 2A and 2B). Among the neoplastic proliferations of myoepithelial cells, variously sized glandular structures

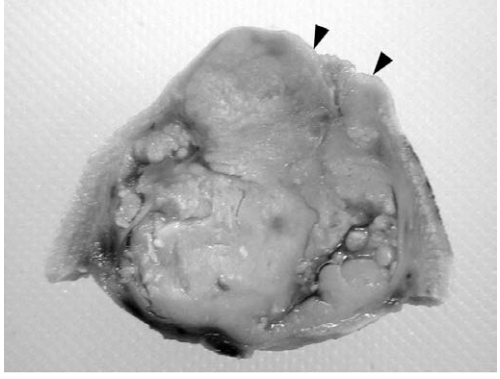

Fig. 1. Macroscopic view of subcutaneous tumor of the right leg. A hard, raised, and partly ulcerated (arrowheads) subcutaneous tumor showing whitish and solid appearance covered with connective tissue subdividing the tumor into irregular sized small lobules on the cut surface after fixation.

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Table 1. Reagents and methods for immunohistochemistry

| Primary antibodies | Antigen | Antibody type | Dilution | Antigen retrieval [a)] | Source |
|---|---|---|---|---|---|
| | Cytokeratin 8 | Mouse monoclonal, CAM5.2 | 1:1 | Microwaving | Becton Dickinson (Franklin Lakes, NJ, U.S.A.) |
| | Alpha-smooth muscle actin | Mouse monoclonal, 1A4 | 1:100 | Microwaving | Dako (Glostrup, Denmark) |
| | p63 | Mouse monoclonal, 4A4 | 1:200 | Autoclaving | Thermo Fisher Scientific Inc. (Fremont, CA, U.S.A.) |
| | Proliferating cell nuclear antigen | Mouse monoclonal, PC10 | 1:200 | Autoclaving | Dako |
| Kits for immunodetection | Detection technique | Kit | Source | | Antigen visualization |
| | Avidin-biotin-peroxidase complex (ABC) technique | VECTASTAIN® Elite ABC kit | Vector Laboratories (Burlingame, CA, U.S.A.) | | 3,3'-Diaminobenzidine (Dojindo Laboratories, Kumamoto, Japan) |
| | Avidin-biotin-alkaline phosphatase complex (ABC-AP) technique | VECTASTAIN® ABC-AP kit | Vector Laboratories | | Vector® Red (Vector Laboratories) |

a) Antigen retrieval was performed by autoclaving at 121°C for 10 min, or microwaving at 90°C for 10 min in 10 mM citrate buffer (pH 6.0).

were sometimes present, which consisted of cuboidal luminal epithelial cells that sometimes showed a multilayered structure (Fig. 2A). Neoplastic luminal epithelial cells showed moderate cellular atypia with eosinophilic cytoplasm and 1 round-to-oval-shaped, variably sized nucleus containing 1 or 2 distinct nucleoli and scanty chromatin. In part of the luminal epithelium, apocrine-like secretion of eosinophilic material was observed (Fig. 2B). Local invasion to fibrous connective tissue as well as vascular invasion of both myoepithelial and glandular epithelial cell components was frequently observed.

With regard to the immunohistochemical profile of neoplastic cells, portions of diffusely proliferating myoepithelial cells, which comprised the major part of the tumor, were largely immunoreactive for αSMA, whereas luminal glandular epithelial cells were positive for CK8 (Fig. 2C). Diffusely proliferating neoplastic cells immunoreactive for αSMA were exclusively positive for p63 in the nucleus (Fig. 2D). Clusters of neoplastic cells lacking αSMA expression but expressing p63 in the nucleus were scattered within the portion of diffusely proliferating lesions of αSMA-positive cells (Fig. 2E). Luminal epithelial cells showed positive immunoreactivity for CK8, but entirely lacked p63 expression. The distribution of PCNA-positive cells in the αSMA-positive population was much higher than that in the CK8-positive population (Fig. 2F and 2G). Based on the measurement of PCNA-positive cell counts, the αSMA-positive population attained a statistically significantly higher positive cell ratio (38.6 ± 4.5%) than that of the CK8-positive population (11.6 ± 6.6%; $P<0.01$ by Student's *t*-test).

Apocrine gland carcinomas are relatively common carcinomas in dogs between 8 and 12 years of age. Breeds at increased risk are the Old English sheepdog (4.2%), shih tzu (2.1%), and German shepherd (2.0%) [2]. According to the World Health Organization (WHO) classification of epithelial and melanocytic tumors of the skin of domestic animals [3], malignant apocrine gland tumors are classified into apocrine carcinomas, complex and mixed apocrine carcinomas, and apocrine ductal carcinomas. Among them, groups of complex apocrine carcinomas are characterized by malignant proliferation of glandular cells accompanying nonmalignant proliferation of myoepithelial cells. On the other hand, mixed apocrine carcinomas show metaplastic change of the myoepithelium to cartilage or bone, but rarely show a malignant phenotype.

Few studies have investigated the cellular phenotype of glandular epithelial and myoepithelial cells in complex and mixed apocrine gland tumors in dogs. In the normal canine apocrine gland, 3 cell populations, each with a different immunoprofile, can be recognized. The luminal layer is composed of glandular epithelial cells, which express CK8/18 [5], and basal epithelial cells, which express p63 but lack αSMA [11]. The basal layer is composed of myoepithelial cells that express p63 and αSMA [1]. In this report, we described the immunophenotypic features of a rare case of canine complex apocrine carcinoma showing predominant myoepithelial cell proliferation.

Histopathologically, diffusely proliferating myoepithelial cells formed the major population of this tumor, and apocrine glands formed a minor population, the former showing higher proliferation activity. According to the WHO classification of complex and mixed apocrine carcinomas in domestic animals, in rare instances when the epithelial and mesenchymal components both exhibit malignant changes, the tumor is referred to as an apocrine carcinosarcoma [3]. Although the mesenchymal component in this classification was not clearly defined, our present case lacked an apparent mesenchymal component and would be better diagnosed as a complex apocrine carcinoma displaying unique malignant proliferation of a myoepithelial component.

Necessary to maintain a stem cell population, p63 is a member of the p53 family of transcription factors and is consistently expressed in the basal epithelial cells of multilayered epithelia [8, 9]. Furthermore, p63 is reportedly a reliable nuclear marker for myoepithelial cells in both normal mammary glands and mammary neoplasms [1, 10]. Expression of p63 has also been detected in the myoepithelial cells in canine malignant apocrine gland neoplasms [11]. In the present case, diffusely proliferating neoplastic cells were

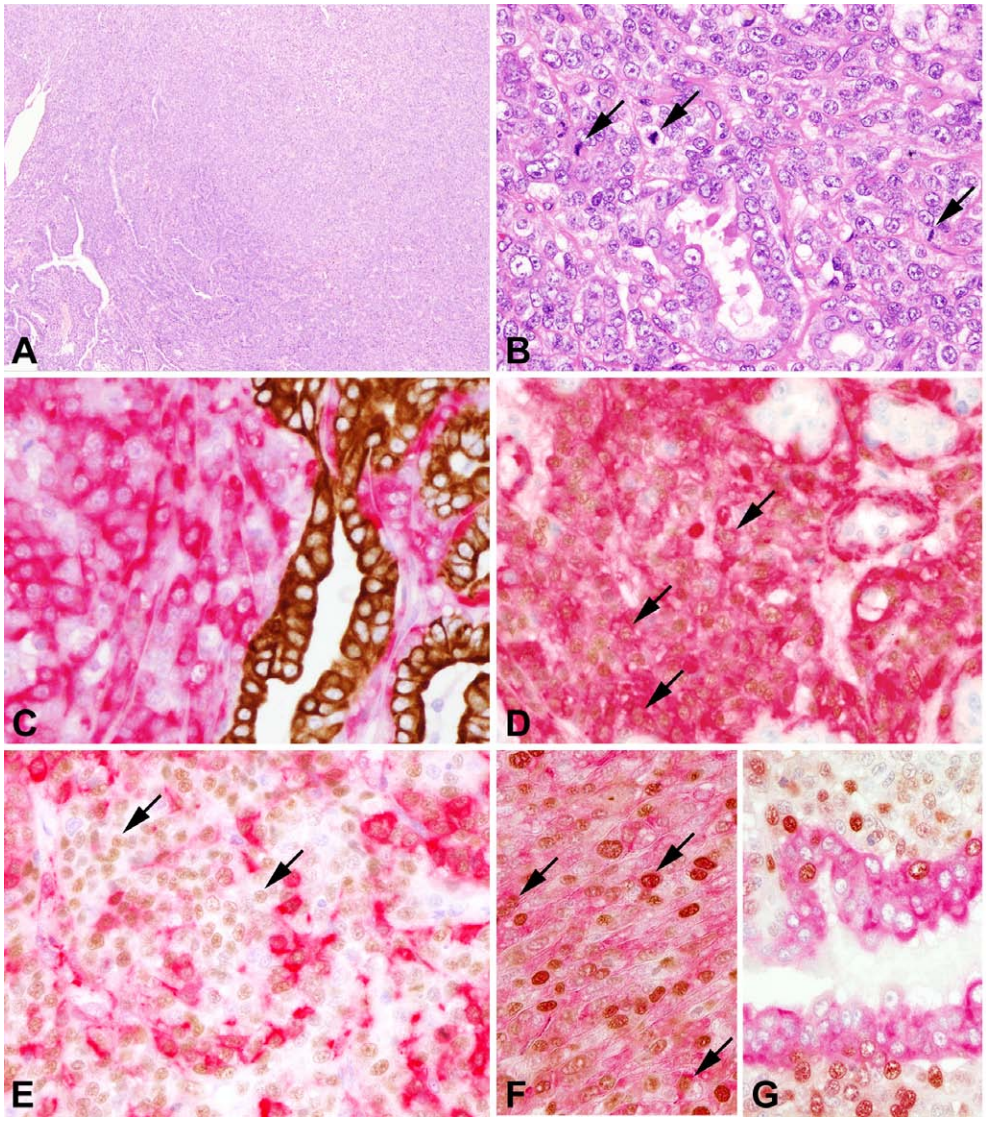


Fig. 2. Complex apocrine gland carcinoma in a dog. A. The tumor was roughly subdivided by connective tissue into lobules in low-power view. Each tumor lobule largely consisted of neoplastic myoepithelial cells showing diffuse proliferation. Among them, variously sized glandular structures were sometimes present. HE. ×4 objective. B. The tumor mainly consisted of diffuse proliferation of neoplastic myoepithelial cells that showed moderate cellular atypia. Among them, luminal epithelial cells formed glandular structures with apocrine-like secretion. Note frequent mitotic figures (arrows) in the myoepithelial population. HE. ×40 objective. C. Portions of diffusely proliferating myoepithelial cells were largely immunoreactive for αSMA (red), whereas luminal glandular epithelial cells were positive for CK8 (brown). Double immunohistochemistry of CK8 and αSMA counterstained with hematoxylin. ×40 objective. D. Diffusely proliferating neoplastic cells immunoreactive for αSMA (red) displayed exclusively nuclear p63 expression (brown; arrows). Note that luminal epithelial cells were negative for αSMA and p63. Double immunohistochemistry of p63 and αSMA counterstained with hematoxylin. ×40 objective. E. Populations of p63-positive (brown and nuclear immunoreactivity) neoplastic cells formed αSMA-negative cellular clusters (arrows) among the cellular population positive for both αSMA (red) and p63. Double immunohistochemistry of p63 and αSMA counterstained with hematoxylin. ×40 objective. F. Many αSMA-positive myoepithelial cells (red) showed nuclear PCNA immunoreactivity (brown; arrows). Double immunohistochemistry of PCNA and αSMA counterstained with hematoxylin. ×40 objective. G. CK8-positive luminal epithelial cells (red) showed less nuclear PCNA immunoreactivity (brown) than did the CK-8-negative myoepithelial population. Double immunohistochemistry of PCNA and CK8 counterstained with hematoxylin. ×40 objective.

largely positive for both p63 and αSMA, suggesting acquisition of a myoepithelial phenotype. Another population of p63-positive neoplastic cells lacking αSMA expression was scattered among the αSMA-positive myoepithelial cells. Because αSMA is expressed in myoepithelial cells but not in basal epithelial cells, a p63-positive and αSMA-negative population should be assumed to consist of basal epithelial cells. On the other hand, the CK8-positive glandular epithelial population lacked p63 expression. Their morphologically apparent apocrine features suggest that these glandular neoplastic cells showed apocrine gland differentiation.

In human mammary glands, which consist of cellular components similar to those of apocrine glands, some cases of mixed carcinomas are classified as basal-like carcinomas because the neoplastic cells of this type generally express basal epithelial cell markers as well as myoepithelial markers [7]. These neoplasms usually show higher malignancy than other types. Considering the high proliferation of the diffusely proliferating myoepithelial cell population with the concomitant distribution of basal epithelial cells, our present case might have cellular characteristics of basal-like carcinomas. However, most mammary basal-like carcinomas in humans have been shown to be invasive ductal carcinomas of high nuclear and/or histologic grades [6]. This differed from our case, in that our case involved an apocrine gland population with less aggressive proliferation.

The origin and role of myoepithelial cells have been studied in normal human mammary glands and breast cancers. Myoepithelial cells have been thought to arise from progenitor cells (basal epithelial cells) within the luminal epithelial compartment, which is bipotential and contributes to the development of both luminal epithelial and myoepithelial cells [4]. It is suggested that complex and mixed tumors of the canine mammary glands have components that originate from common stem cells with a higher capacity for divergent differentiation [10]. The structural and functional similarity between the apocrine glands and the mammary glands indicate that myoepithelial cells in the apocrine gland also differentiate from bipotential progenitor cells. Therefore, this case might have originated in the basal epithelial cells and differentiated into both luminal apocrine epithelial cells and myoepithelial cells, obtaining a higher proliferating ability in the latter component.

Recent findings suggest that myoepithelial cells are involved in mammary gland morphogenesis by modulating proliferation and differentiation of luminal acinar cells, and, in turn, function as suppressors of luminal cancers by stabilizing luminal structure [12]. Therefore, it can be hypothesized that a diffusely proliferating myoepithelial component may function to suppress neoplastic proliferation of luminal epithelial components by facilitating differentiation toward glandular structures with apocrine secretion. Indeed, in humans, a diminished number or complete loss of myoepithelial cells was associated with neoplastic proliferation of apocrine lesions of the breast [13].

In conclusion, we reported a case of complex apocrine carcinoma arising from the subcutaneous region. The present case was unusual in that both myoepithelial cells and apocrine luminal cells showed neoplastic proliferation with a higher proliferating ability in the former than the latter. In addition, both of these epithelial components might have generated from the common origin of basal epithelial cells. The reported dog suddenly died one year after the excision of the tumor. Because a necropsy was not performed, the relationship between the death and the tumor was unclear.

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