

Case Report

A Mixed Apocrine Gland Tumor with Metastases to the Bone and Bone Marrow in a Miniature Poodle

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Abstract: A 10-year-old female miniature poodle had a mass in its carpal joint of the left forelimb. The tumor was divided into small multiple lobules by delicate connective tissues, and necroses were found in some of the central lobules. In some connective stromal areas, chondroid and osteoid tissues were formed. The tumor cells were similar to the structure of apocrine gland epithelial cells with apical blebs resembling apocrine secretion and eosinophilic secretory materials within the luminal space, and spindle cells were sometimes found in the basal area of the glandular structure. In some areas, tumor cells invaded in the blood vessels, bone and bone marrow. Immunohistochemically, the tumor cells forming tubulo-acinar to solid structures were intensely positive for cytokeratin and keratin K8/K18, and the spindle cells were positive for vimentin and alpha-smooth muscle actin. This case was diagnosed as a malignant mixed apocrine gland tumor with metastases to the bone and bone marrow. (*J Toxicol Pathol* 2010; 23: 95–98)

Key words: apocrine gland tumor, dog, mixed tumor

Since apocrine glands are the major type of sweat glands in dogs and are located in almost all the skin, apocrine gland tumors in dogs occur at any site of the skin and represent 2.0% of canine skin tumors^{1,2}. The morphological characteristics of the apocrine gland carcinoma are similar to those of the eccrine gland carcinoma¹. Thus, determination of the site of origin is required for the differential diagnosis, since eccrine glands are normally located only in the footpad³. Histologically, apocrine sweat gland tumors can be classified as simple, complex or mixed types. In dogs, mixed carcinomas of the sweat gland are rare⁴. In addition, to our knowledge, there are no published reports on mixed apocrine gland tumors invading the bone and bone marrow. Here, we report a malignant mixed apocrine gland tumor in the forelimb of a dog invading the bone and bone marrow.

A 10-year-old female miniature poodle with a mass in the left forelimb showed lameness and enlargement of the carpal joint of the left forelimb. No gross abnormal masses were found in any site of the dog including the foot pad except for the carpal joint's mass, and no white nodule or

lesion was seen in the chest on the x-ray examination. An examination of the neighboring lymph nodes to detect the presence of a metastasis was not carried out. Since an osteosarcoma was suspected based on the x-ray examination of the mass, the forelimb was amputated surgically. On the cut section, a wine-colored bone-like area was observed in the center of the mass. Around this bone-like area, white solid elastic nodules were observed.

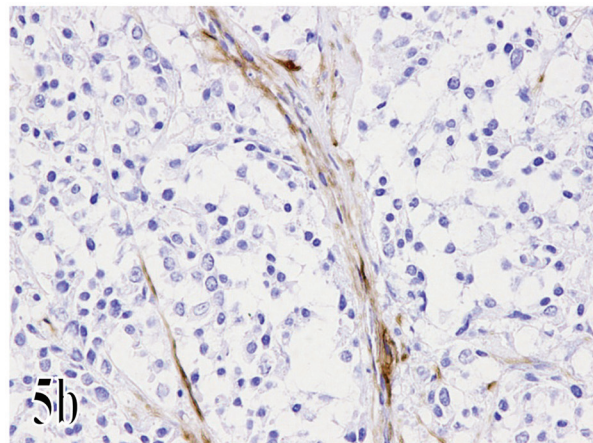
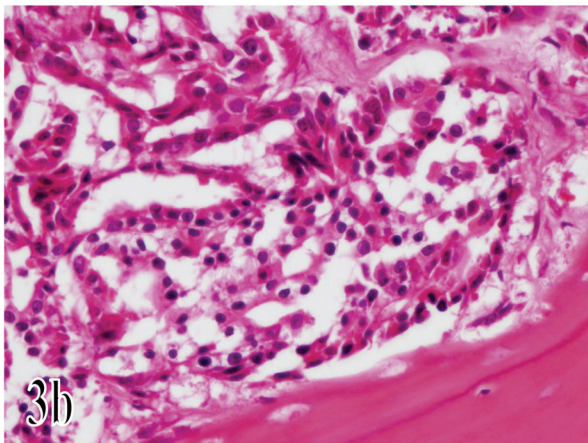
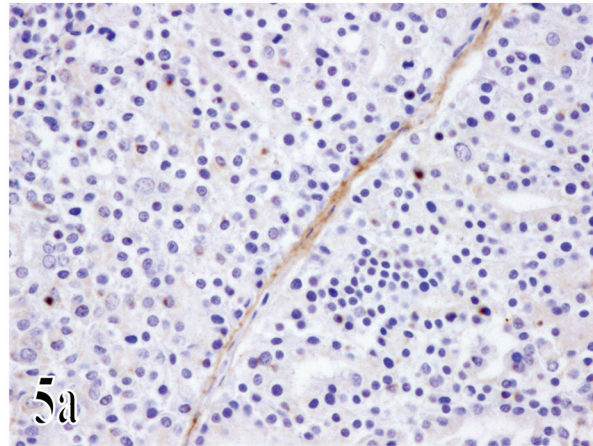
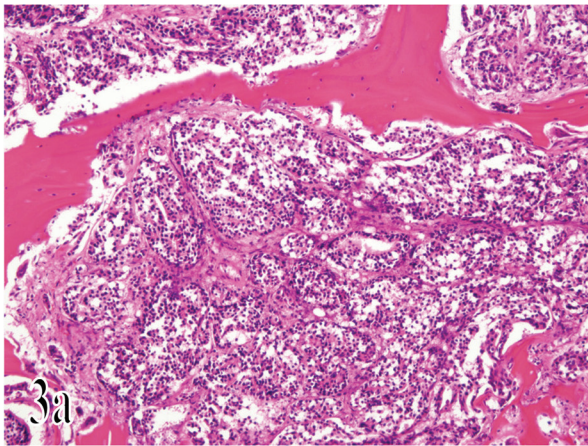
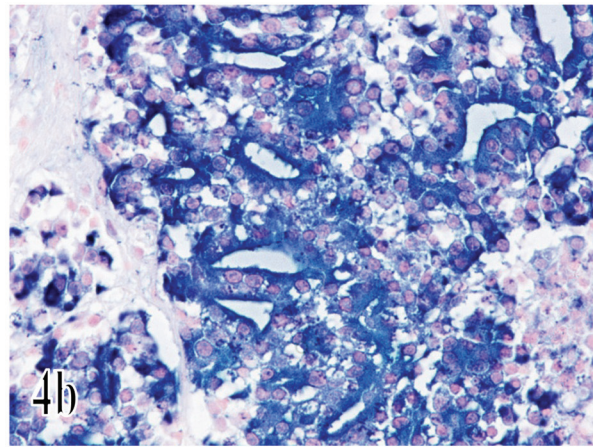
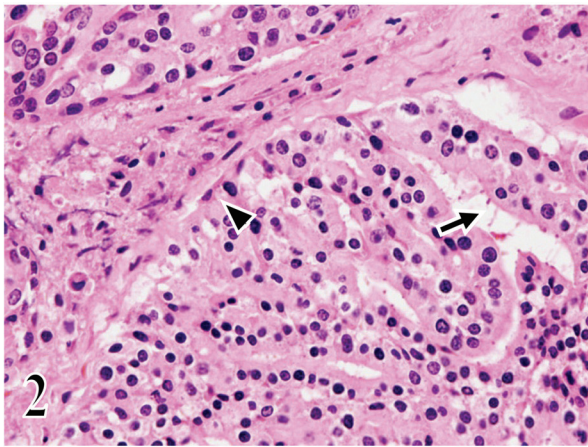
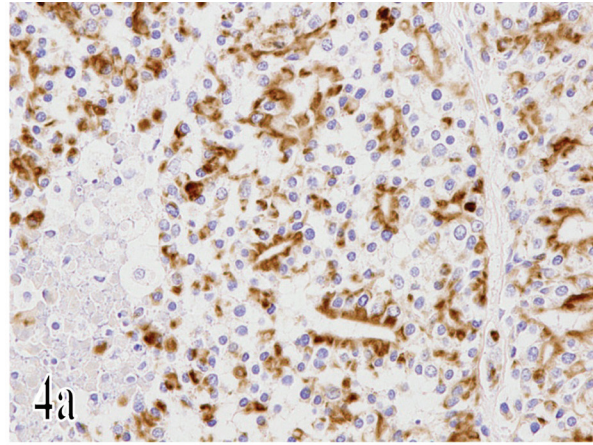
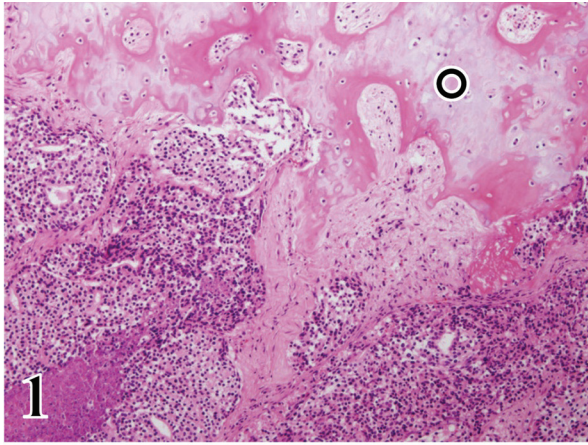
The tissue sample was fixed in 10% neutral buffered-formalin and embedded in paraffin. Sections 4 μ m-thick were made and stained with hematoxylin and eosin (HE). For immunohistochemistry, sections except for that used for the section of keratin K8/K18 immunostaining were incubated with 0.3% hydrogen peroxidase in methanol for 30 min at room temperature to block endogenous peroxidase activity. All antigen retrievals were performed in a microwave at 90°C in 10 mM citrate buffer (pH 6.0) for 10 min. The primary antibodies used in the present study were mouse monoclonal antibodies against cytokeratin (DakoJapan, Kyoto, Japan; clone MNF116, 1:100), desmin (Biocompare, South San Francisco, CA, USA; clone D33, 1:50) and alpha-smooth muscle actin (DakoJapan; clone 1A4, 1:100) and polyclonal antibodies against vimentin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA; clone C-20, 1:200) and keratin K8/K18 (PROGEN Biotechnik GmbH, Heidelberg, Germany; 1:100). Sections were incubated with these primary antibodies at 4°C for

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Figs 1-5.

- Fig. 1.** Chondroid and osteoid tissues are formed in some connective stromal areas of the tumor (O). HE stain. Original magnification: $\times 100$.
- Fig. 2.** Apical blebs resembling apocrine secretion and eosinophilic secretory materials in the luminal space (arrow) are observed on the surface of the tumor cells forming glandular structures, and spindle cells are found in the basal area of the glandular structure (arrow head). HE stain. Original magnification: $\times 400$.
- Fig. 3.** Tumor cell invasion in the bone and bone marrow (a, b). Tumor cells invade and diffusely proliferate in the bone marrow so as to replace the marrow (b). HE stain. Original magnification: $\times 100$ (a) and $\times 400$ (b).
- Fig. 4.** Tumor cells forming tubulo-acinar to solid structures are intensely positive for cytokeratin (a) and keratin K8/K18 (b). Immunohistochemical stain. Original magnification: $\times 400$.
- Fig. 5.** Spindle cells surrounding the glandular structure of the tumor are positive for vimentin (a) and alpha-smooth muscle actin (b). Immunohistochemical stain. Original magnification: $\times 400$.

overnight. Some immunodetections, except for keratin K8/K18, were carried out by the avidin-biotin complex method (VECTASTAIN[®] Elite ABC kit, Vector Laboratories Inc., Burlingame, CA, USA) with 3,3'-diaminobenzidine as a chromogen, followed by light counterstaining with hematoxylin. For keratin K8/K18, immunodetection was carried out by the avidin-biotin complex method (VECTASTAN[®] Elite ABC-Alkaline Phosphatase kit, Vector Laboratories Inc.) with alkaline phosphatase chromogen (VECTASTAIN[®] BCIP/NBT kit, Vector Laboratories Inc.), followed by light counterstaining with Fast red.

Histopathologically, the tumor was observed in the subcutis as a large mass surrounded by connective tissues and consisting of proliferating tumor cells invading into the adjacent tissue in the low-power field. The tumor was subdivided into small multiple lobules by delicate connective tissues, and necroses were detected in some of the central lobules. In some connective stromal areas, chondroid and osteoid tissues were formed (Fig. 1). In the high-power field, the tumor cells, which are similar to the structure of apocrine epithelial cells with middle to large, round to oval nuclei and abundant eosinophilic cytoplasm, showed tubulo-acinar, nodular and solid growth. In the areas showing tubulo-acinar growth, apical blebs resembling apocrine secretion and eosinophilic secretory materials within the luminal space were observed on the surface of the tumor cells forming glandular structures, and spindle cells were sometimes found in the basal area of the glandular structure (Fig. 2). The nuclei of these tumor cells were irregular in shape and size, but showed little mitotic figures. In some areas, tumor cells invaded in the blood vessels, bone and bone marrow (Fig. 3a). Tumor cells invaded in the distal end of the radius, inducing bone destruction, and diffusely proliferated in the bone marrow so as to replace the marrow. In the bone marrow, tumor cells showing glandular and solid growth formed small multiple lobules by irregular connective tissues (Fig. 3b), and spindle cells were sometimes found in the connective tissue. Immunohistochemically, the tumor cells forming tubulo-acinar to solid structures were intensely positive for cytokeratin and keratin K8/K18 (Fig. 4) but not for desmin and vimentin. The spindle cells surrounding the glandular structure showed positive immunoreactivity for vimentin (Fig. 5a) and alpha-smooth muscle actin (Fig. 5b).

In dogs, the eccrine gland is located only in the foot

pad, while the apocrine gland is located in all sites of the skin. Since the present tumor occurred in the carpal joint of the left forelimb, the tumor probably originated from the apocrine gland. It has been previously reported that the origin of the tumor is the apocrine gland, based on at least 2 of the following criteria: (i) decapitation "blebs" at the apical surface of the epithelial lining, (ii) eosinophilic secretion within lumen and (iii) double layer of epithelial lining (one layer of cuboidal/columnar cells and another layer of fusiform cells originating from myoepithelial cells)⁵. In addition, Kato *et al.* (2006)⁶ reported that the external root sheaths of the hair follicles and glandular epithelial cells of the apocrine gland are only positive for keratin K8/K18 in the normal skin of dogs, and the tumors derived from the apocrine gland in dogs are 100% positive for keratin K8/K18 immunohistochemistry. In the present study, there were apical blebs from the surface epithelial tumors and eosinophilic secretion of eosinophilic materials within the luminal space in the gland formed by the tumor cells in tubulo-acinar areas. These epithelial tumor cells in the present tumor were positive for keratin K8/K18. Furthermore, spindle-shaped cells surrounding the glandular structure were positive for alpha-smooth muscle actin, suggesting that they are originated from myoepithelial cells. These findings strongly suggest that the tumor in the present case is derived from the apocrine gland. In addition, necrosis was often observed in the central area of tumor nodules, and tumor cells invaded into blood vessels. Thus, the tumor was recognized as a malignant apocrine epithelial tumor.

Some chondroid and osteoid tissues were observed in the present tumor. It is well known that the osseous metaplasia is often observed in mammary gland carcinomas of dogs. In human's sweat gland tumors, cartilaginous and osseous metaplasias are observed in a rare mixed skin tumor called pleomorphic adenoma⁷. In animals, there is one report of osteoid formations accompanied by an apocrine gland tumor in a cow, and this tumor was diagnosed as "a malignant mixed apocrine gland tumor"⁸. On the other hand, in apocrine gland tumors of the dog, there are few reports on such a tumor, but a report of mixed carcinoma of the sweat gland in the foot pad of a dog has previous been published⁹. In that report, it was difficult to make a differential diagnosis in regard to whether that tumor was derived from the apocrine or eccrine gland in the foot pad because there was no clear direct evidence that it should be diagnosed as a

mixed apocrine gland tumor⁹. In the present tumor, the findings suggest that the tumor was composed of both epithelial components of the apocrine gland and stromal components of myoepithelial cells and chondroid/osteoid tissues, that it was not derived from the foot pad and that it should be diagnosed as “a mixed apocrine gland tumor”. Furthermore, the tumor cells invaded in the bone and bone marrow, which is a rare finding in dogs, and there is no description of this in the literature, as far as we know. Based on the results of the morphological and immunohistochemical studies on this tumor, this case was diagnosed as a malignant mixed apocrine gland tumor in a dog with metastases to the bone and bone marrow.

References

1. Maxie MG. Pathology of Domestic Animals, Vol. 1, 5th ed. Saunders W. B. Co., Philadelphia. 757–758. 2007.
2. Kalaher KM, Anderson WI, and Scott DW. Neoplasms of the apocrine sweat glands in 44 dogs and 10 cats. *Vet Rec.* **127**: 400–403. 1990.
3. Nibe K, Uchida K, Itoh T, and Tateyama S. A case of canine apocrine sweat gland adenoma, clear cell variant. *Vet Pathol.* **42**: 215–218. 2005.
4. Meuten DJ. Tumors in Domestic Animals, 4th ed, Iowa State Press, Iowa. 70–78. 2002.
5. Simko E, Wilcock PB, and Yagar AJ. A retrospective study of 44 canine apocrine sweat gland adenocarcinomas. *Can Vet J.* **44**: 38–42. 2003.
6. Kato K, Uchida K, Nibe K, and Tateyama S. Immunohistochemical studies on cytokeratin 8 and 18 expressions in canine cutaneous adnexa and their tumors. *J Vet Med Sci.* **69**: 233–239. 2007.
7. Yim YM, Yoon JW, Seo JW, Kwon H, and Jung SN. Pleomorphic adenoma in the auricle. *J Craniofac Surg.* **20**: 951–952. 2009.
8. Gulbahar MY, Alkan I, and Golen I. Mixed apocrine sweat gland tumor of the tail in a cow. *Vet Pathol.* **39**: 281–285. 2002.
9. Kawabata A, Ando J, Yasuno K, Baba T, Uchida N, Nagata M, and Shirota K. A canine case of mixed carcinoma of the sweat gland in the footpad. *Jpn J Vet Dermatol.* **14**: 3–7. 2008.