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Case Report

A case of rapid recurrence of apocrine ductal carcinoma originating from the oral scent gland of a Richardson's ground squirrel (*Urocitellus richardsonii*)

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Abstract: A 3-year-old female Richardson's ground squirrel developed a subcutaneous mass at the left oral angle. Seven days after removal of the mass, the mass recurred and metastasized to the cervical lymph node. Histologically, the primary mass was subdivided by fibrous trabeculae into various-sized neoplastic cell lobules showing a solid growth pattern with frequent mitoses and sometimes forming intracytoplasmic lumina. Large to medium-sized lobules formed a central cyst plugged by comedo necrosis. Neoplastic cells showed infiltrative subcutaneous growth. In the recurrent tumor, tubular structures lacking apparent apocrine secretion appeared within the solid growth portion. Neutrophil infiltration was evident within the tubules and intracytoplasmic lumina. Neoplastic cells were diffusely immunopositive for AE1/AE3 pan-cytokeratin (CK) in all lobules and focally positive for CAM5.2 CK in the lobules forming a central cyst and/or tubular structures, but they entirely lacked positivity for the periodic acid Schiff reaction. Ki-67-positive proliferating neoplastic cells were higher in numbers with the recurrent tumor than with the primary tumor. In addition, phosphorylated c-MYC immunoreactivity was observed in neoplastic cell nuclei, distinctly at the portion of invasive growth. Thus, the present case was diagnosed as apocrine ductal carcinoma originating from the oral scent gland, which typically shows highly aggressive biological behavior. (DOI: 10.1293/tox.2017-0071; J Toxicol Pathol 2018; 31: 189–193)

Key words: apocrine ductal carcinoma, oral scent gland, Richardson's ground squirrels, metastasis, recurrence

Ground squirrels are members of the squirrel family of rodents (Sciuridae), which generally live on or in the ground rather than in trees. There are 62 known species of ground squirrel[1]. Among them, Richardson's ground squirrels (*Urocitellus richardsonii*) are medium-sized rodents found on the prairies of North America[2]. As spontaneous tumors occur in Richardson's ground squirrels, development of hepatocellular carcinomas has been reported in wild cases in relation with ground squirrel hepatitis virus infection[3, 4]. Because these ground squirrels have become popular pets, cases with spontaneously occurring tumors, such as buccal salivary gland adenocarcinoma and mast cell tumors, have been reported[5, 6].

Many mammals communicate social information to conspecifics via the odoriferous secretions from scent glands[7]. Like other rodent species, ground squirrels have integumentary scent glands located in the oral angle area, dorsal area, and anal area[8]. In most instances, greeting behavior (which is focused on the oral gland) and the degree of sociality paralleled levels of scent marking[8]. The scent gland is composed of modified sudoriferous and sebaceous glands. The oral gland of ground squirrels in particular is an apocrine-type gland consisting of three lobes, each connected to a hair follicle by a duct. In addition, a pair of large sebaceous glands also exists adjacent to the hair follicles[9].

The present case was the first to show rapidly growing recurrent scent gland apocrine ductal carcinoma at the oral angle of a Richardson's ground squirrel. Recurrence was observed 7 days after the initial resection.

A 3-year-old female Richardson's ground squirrel with a hemispherically elevated subcutaneous mass measuring 11 × 7 × 5 mm at the left oral angle was referred to a vet-

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erinary clinic in Mie Prefecture, Japan. Because it did not respond to antibiotics, the mass was surgically removed. The cut surface was yellowish white and composed of small lobules (Fig. 1). A mass recurred at the same location 7 days after surgical removal of the primary mass, and the cervical lymph node was enlarged. Three days later, the mass, which was 11 × 9 × 7 mm in size, was surgically excised again along with the cervical lymph node and submandibular gland, but the squirrel ultimately died 8 months after the first medical examination because of intraoral invasion of the mass. The primary and recurrent masses and enlarged lymph nodes were subjected to histopathological examination. All tissue samples were fixed in 10% neutral buffered formalin and routinely processed, embedded in paraffin, sectioned at 3 µm, and subjected to hematoxylin and eosin staining and periodic acid Schiff (PAS) reaction.

Immunohistochemical analysis was performed using the avidin-biotin-peroxidase complex technique with a Vectastain® Elite ABC Kit (Vector Laboratories Inc., Burlingame, CA, USA) and primary antibodies against cytokeratins (CKs), i.e., CAM5.2 reacting with CK7 and CK8 (mouse monoclonal, ready to use; BD, Franklin Lakes, NJ, USA) and AE1/AE3 reacting with pan-CK (mouse monoclonal, 1:50; Dako, Glostrup, Denmark); vimentin (goat polyclonal, 1:200; Santa Cruz Biotechnology, Dallas, TX, USA), S-100 protein (rabbit polyclonal, ready to use; Dako); α smooth muscle actin (SMA; mouse monoclonal, clone 1A4, 1:100; Dako); chromogranin A (rabbit polyclonal, 1:3,000; Yanaihara Institute Inc., Fujinomiya, Japan); synaptophysin (mouse monoclonal, clone SY38, 1:100; Dako); Ki-67 (mouse monoclonal, clone MIB-5, 1:25; Dako); β-catenin (rabbit polyclonal, 1:100; Santa Cruz Biotechnology); and phosphorylated-c-MYC (rabbit polyclonal, 1:50, Santa Cruz Biotechnology). Prior to endogenous peroxidase blocking by incubation in 0.3% H_2O_2 solution in absolute methanol for 30 min, sections were microwaved at 90°C for 10 min in 10 mM citrate buffer (pH 6.0) for CAM5.2 CK, vimentin, and Ki-67 staining, microwaved at 90°C for 10 min in 10 mM Tris/1 mM ethylenediaminetetraacetic acid (EDTA) buffer (pH 9.0) for AE1/AE3 CK and αSMA, autoclaved at 121°C for 10 min in 10 mM citrate buffer (pH 6.0) for β-catenin, or autoclaved at 121°C for 10 min in 10 mM Tris/1 mM EDTA buffer (pH 9.0) for S-100 protein and synaptophysin. Antigen-retrieval treatment was not done with chromogranin A and c-MYC staining. The chromogen was 3,3′-diaminobenzidine, and the counterstain was hematoxylin. To confirm the specificity of the primary antibodies, skin tissues adjacent to the primary mass lesion, including the epidermis, Merkel cells, fibroblasts, blood vessels, and peripheral nerves, as well as the cervical lymph node and submandibular gland removed at the 2nd surgery, were used as positive controls for CAM5.2 and AE1/AE3 CKs, vimentin, S-100 protein, αSMA, chromogranin A, synaptophysin, proliferative activity (Ki-67), and β-catenin. Skin tissue that included apocrine gland ducts and acini of a Richardson's ground squirrel was also used as a positive control for CAM5.2 and AE1/AE3 CKs. Preneoplastic liver cell lesions induced by hepatocellular tumor promotion in a rat and a mammary simple carcinoma of a bitch were used as positive controls for phosphorylated-c-MYC. Nonimmunized sera were substituted for the primary antibody as negative controls for immunoreactivity.

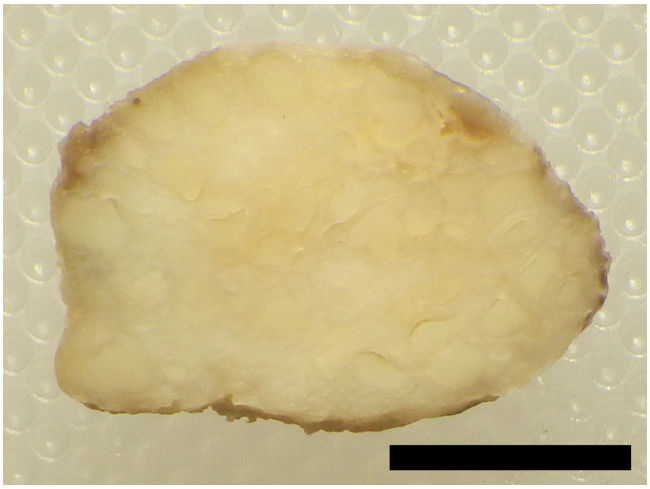

Fig. 1. The cut surface of the primary mass after formalin fixation was yellowish white and composed of small lobules. Bar = 5 mm.

Microscopically, the primary mass was demarcated by thin fibrous tissue and subdivided by irregular connective tissue trabeculae into large- to small-sized lobules of various shape (Fig. 2A). Neoplastic cells were arranged in a solid pattern, with larger lobules having a central cyst plugged by comedo necrosis (Fig. 2A), and accompanied by neutrophil infiltration. The central cysts lacked apparent apocrine secretion at the luminal surface. Individual neoplastic cells had round to cuboidal, sometimes elongated, foamy and lightly eosinophilic cytoplasm with distinct cell borders (Fig. 2B). Intracytoplasmic lumina were sparsely observed with or without eosinophilic material that was positive for the PAS reaction. The round to oval-shaped nuclei varied in size, with one or two distinct nucleoli and fine dot-like chromatin, and had a fairy high mitotic index with a mean value of 3 in ten 400× magnification fields. Neoplastic cells showed infiltrative downward growth to the subcutis. However, vessel invasion of neoplastic cells was not evident. In the recurrent tumor, large neoplastic lobules accompanying the central cyst and comedo necrosis were observed less frequently, and instead, small neoplastic lobules were mainly observed. Different from the lack of apparent tubular structure in the primary tumor, variably sized neoplastic tubules were variably distributed within the neoplastic lobules without accompanying apparent apocrine secretion (Fig. 2B). Both the central cyst and tubular structures lacked a PAS-positive reaction at the luminal surface. Neutrophil infiltration was evident within tubules and intracytoplasmic lumina (Fig. 2B). Enlarged lymph node tissue was mostly replaced by infiltrative neoplastic cells, showing marked cellular pleomorphism and a high number of mitoses.

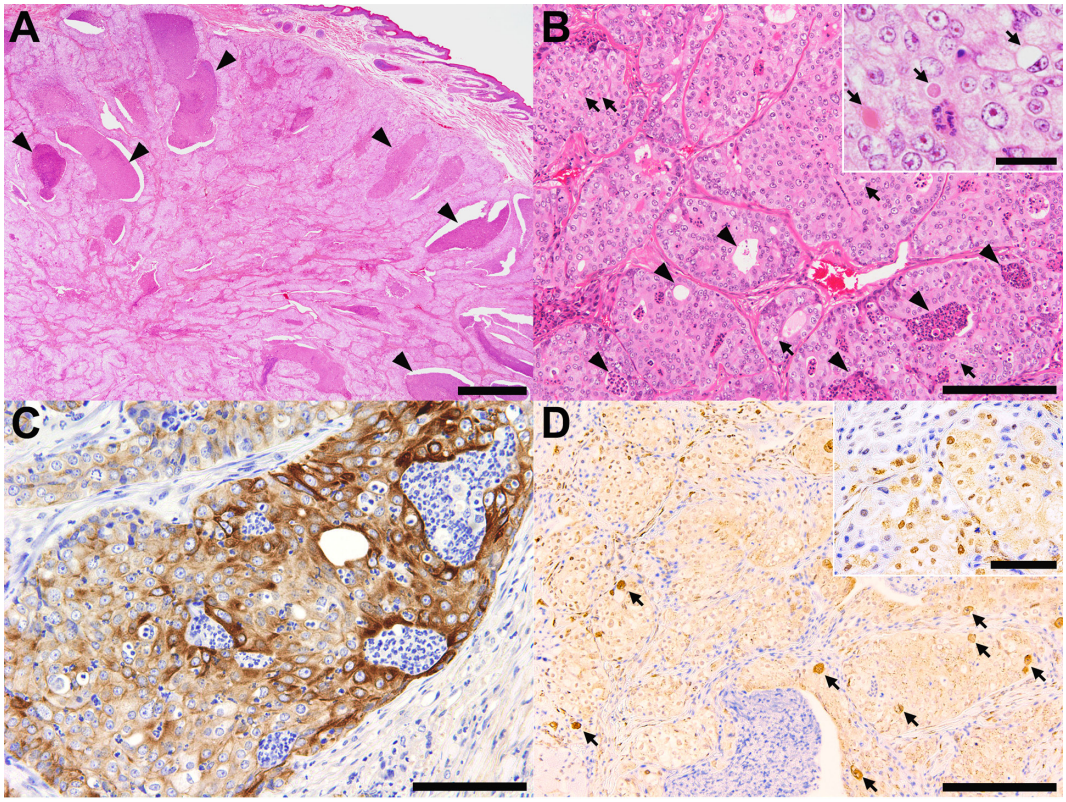

Fig. 2. A rapidly recurrent apocrine ductal carcinoma originating from the oral scent gland of a Richardson's ground squirrel (*Urocitellus richardsonii*). (A) Light microscopic view of the subcutaneously located primary mass subdivided by irregular connective tissue trabeculae into large to small-sized lobules of various shapes. Neoplastic cells are arranged in a solid pattern, with large to medium-sized lobules having central cysts plugged by comedo necrosis (arrowheads). Hematoxylin and eosin staining. Bar = 1 mm. (B) Medium to small-sized lobules of neoplastic cells in the recurrent tumor, accompanying tubular structures (arrowheads), and intracytoplasmic lumina (arrows) with or without eosinophilic material among the solid sheet arrangement of neoplastic cells. Small foci of neutrophil infiltration are evident within tubules and intracytoplasmic lumina. The inset shows a high-power view of the intracytoplasmic lumina (arrows) with or without eosinophilic material. A small focus of neutrophil infiltration in a tubular structure is also evident. Hematoxylin and eosin staining. Bar = 200 µm (inset: 20 µm). (C) Neoplastic cell lobules with tubular structures reacted positively for CAM5.2 in the recurrent tumor. Avidin–biotin complex method with hematoxylin counterstaining. Bar = 100 µm. (D) Neoplastic cells at the portion of tumor invasion (left upper portion of the photomicrograph) reacted positively for phosphorylated c-MYC intensively in the nucleus, in addition to diffuse and weakly positive reactivity in the cytoplasm. Arrows indicate mitotic cells showing hyperphosphorylation of c-MYC. The inset shows a high-power view of the c-MYC-positive neoplastic cells at the portion of tumor invasion. Avidin–biotin complex method with hematoxylin counterstaining. Bar = 200 µm (inset: 50 µm).

With regard to the immunohistochemical profile, neoplastic cells diffusely reacted positively for AE1/AE3 pan-CK but were negative for vimentin and αSMA. Neoplastic cells forming central comedo necrosis or tubular structures reacted positively for CAM5.2, especially at the luminal front (Fig. 2C). Neoplastic cells were sparsely positive for chromogranin A in the primary and recurrent tumors but diffusely positive for approximately 20% of all neoplastic cell lobules in the lymph node metastasis. Neoplastic cells were negative for synaptophysin. Spindle cells in the connective tissue surrounding neoplastic lobules gave a positive reaction for vimentin and αSMA. Ki-67-positive proliferating neoplastic cells were distributed diffusely in the neoplastic tissue, showing a mean Ki-67-positive cell ratio of 9.8% in ten 400× magnification fields of the recurrent tumor in contrast to the mean ratio of 6.4% in the primary tumor. In both of the primary and recurrent tumors, as well as the lymph node metastasis, all neoplastic cells reacted positively for phosphorylated c-MYC weakly in the cytoplasm, and among them, strongly positive mitotic neoplastic cells were scattered (Fig. 2D). Neoplastic cells distinctly at the portion of tumor invasion reacted positively for phosphorylated c-MYC intensively in the nucleus (Fig. 2D). While almost all of the neoplastic cells showed cell surface membrane immunoreactivity with β-catenin, both cell membrane immunoreactivity and cytoplasmic immunoreactivity were observed at the portion of tumor invasion in both of the primary and recurrent tumors, as well as the lymph node metastasis.

In rodent species, tumors originating from scent glands are common in gerbils[10]. However, in species of ground squirrels, only one report of two cases of adenocarcinoma of the dorsal glands in European ground squirrels has previ-

ously been published[11]. In those cases, the neoplasms were composed of columnar epithelial cells arranged in anastomosing tubules, irregular acini, and focally in solid sheets with fine collagenous stroma. In light of the anatomical localization and histopathological characteristics, immunohistochemistry results, and histochemistry findings, the authors concluded that these tumors were of apocrine secretory epithelium origin.

Histopathologically, the present oral angle tumors lacked the components of sebaceous glands but showed neoplastic cell proliferation forming lobules, which was accompanied by a central cyst plugged with central comedo necrosis in both the primary and recurrent tumors, suggestive of a ductal cell origin as observed in ductal carcinomas of the mammary gland and salivary gland in humans[12, 13]. Some neoplastic cells had intracytoplasmic lumina. In the recurrent tumor, tubular formation appeared in addition to a solid pattern of neoplastic cell proliferation; however, apocrine secretion and a PAS-positive reaction at the luminal surface were lacking, suggesting a ductal origin of the neoplastic cells. Immunohistochemically, the neoplastic cells were positive for both AE1/AE3 pan-CK and CAM5.2 CK, similar to the positive pattern for both CKs in the ductal cells as well as in the acinar cells of the apocrine gland of a Richardson's ground squirrel. On the basis of these typical histological and immunohistochemical findings and the anatomical site of the tumor development, the present case was diagnosed as apocrine ductal carcinoma originating from the scent gland at the oral angle. The lack of ulceration of the present case, unlike that typically seen in this type of neoplasm of the skin, may be due to the subcutaneous location of the scent gland.

Carcinoma of apocrine duct origin is a rare malignant tumor that shows differentiation to the apocrine ductal epithelium and has been reported in dogs and cats[14]. Solid-cystic apocrine ductular carcinoma, which appears to occur exclusively in cats and is rare, has been proposed as a newly recognized subtype in this category[15]. This entity was most likely previously included in the basal cell carcinoma category in veterinary pathology[15]. In the present case, histological features of multilobular neoplastic cell proliferation forming a central cyst and comedo necrosis within the lobule and extra- or intracellular luminal differentiation, as well as CAM5.2 CK-positive immunoreactivity especially at the luminal front of the cyst and tubular structures, may be consistent with solid-cystic apocrine ductular carcinoma[15]. The neoplastic cells in the present case typically consisted of a single type of cells. Unlike its benign counterpart, carcinoma of this type lacks a bimorphic poroid cell population or clusters of large clear cells[15].

Rapidly recurrent biological behavior and a pleomorphic cellular nature accompanying multiple necrotic areas and prominent mitoses suggested a highly malignant nature for the present case. The present case also revealed lymph nodal metastasis, a point of distinction from the very uncommon distant metastases and lymphatic invasion in canine and feline apocrine ductal carcinomas[14]. We also observed nuclear immunolocalization of phosphorylated c-MYC in neoplastic cells, distinctly at the portion of invasive growth. In contrast, Ki-67-positive proliferating cells were distributed diffusely in the neoplastic tissue, showing a higher number of Ki-67-positive cells in the recurrent tumor than in the primary tumor. Somatic amplification and overexpression of c-MYC is seen in invasive tumors and is associated with poor outcome in different human tumor types, and it is becoming increasingly clear that c-MYC either directly or indirectly controls cellular invasion and migration and thus metastasis by regulating the expression of specific downstream programs[16]. Many transforming oncogenes ultimately drive c-MYC expression[16]. For example, c-MYC is one of the target proteins of β-catenin-mediated transcription[17]. It is reported that induction of Wnt/β-catenin signaling upregulates c-MYC in the invasive front of a tumor[18]. Considering the increase in the nuclear immunoreactive phosphorylated c-MYC in the portion of tumor invasion, c-MYC activation in the present case may be related to neoplastic cell invasion rather than facilitation of cell proliferation. However, involvement of the Wnt/β-catenin signaling in the c-MYC activation was not evident in the present case. The strong immunoreactivity to phosphorylated c-MYC in mitotic neoplastic cells in the present case may be the reflection of mitosis-specific hyperphosphorylation of this protein[19].

Although the cellular origin of the aforementioned cases of the dorsal scent gland in European ground squirrels was the apocrine secretory epithelium[11], contrary to the ductal origin in the present case, the rapid and locally invasive growth pattern in those tumors was similar to that of the present case. Because ground squirrels have become popular pets, further studies may be necessary on the histopathological characteristics, incidence, and biological behavior of scent gland tumors in these animals.

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