Case Report

A Case of Canine Cutaneous Clear Cell Adnexal Carcinoma with Prominent Expression of Smooth Muscle Actin

Akiko Sakuma1, Shoko Nishiyama1, Kyohei Yasuno1, Tamio Ohmuro2, Junichi Kamiie3, and Kinji Shirota1,3

1 Research Institute of Biosciences and 3Laboratory of Veterinary Pathology, School of Veterinary Medicine, Azabu University, 1–17–71 Fuchinobe, Chuo-ku, Sagamihara, Kanagawa 252-5201, Japan
2Ohmuro Veterinary Clinic, 4262-2 Koyamacho, Machida, Tokyo 194-0212, Japan

Abstract: Cutaneous clear cell adnexal carcinoma was found in the right lip of a 14-year-old male castrated Shih Tzu. Histologically, the tumor mostly consisted of neoplastic cells with clear or vacuolated cytoplasms and contained frequent tubular structures. Neoplastic cells showed coexpression of pan-cytokeratin (CK) and vimentin by double-labeled immunofluorescence staining. In addition, immunohistochemistry revealed that the tumor cells were positive for pan-CK (AE1/AE3, KL1, CAM 5.2), CK-7, CK-8, CK-14, CK-15, CK-18, vimentin and alpha-smooth muscle actin (SMA) with varied intensity and positivity. Among these marker proteins, SMA was positive in 75% of the tumor cells. On the other hand, CK-15, which is a specific marker of follicular stem cells, was expressed in less than 1% of the tumor cells. Based on these findings, the tumor showed diverse differentiation in apocrine sweat glands and the inner and outer root sheaths of hair follicles, indicating the follicular stem cell to be the origin of this tumor. (J Toxicol Pathol 2010; 23: 265–269)

Key words: dermatopathology, dog, cytokeratin, skin, skin tumor, stem cell

Introduction

Canine cutaneous tumors that consist of clear or vacuolated cells are uncommon and include balloon cell melanoma, clear cell basal carcinoma, sebaceous carcinoma, apocrine sweat gland adenoma clear cell variant, clear cell trichoblastoma and canine cutaneous clear cell adnexal carcinoma1–5. Canine cutaneous clear cell adnexal carcinoma has been described as clear cell hidradenocarcinoma2 and follicular stem cell carcinoma2, as first proposed by Schulman et al. in 20055. This neoplasm does not differentiate into a single, special cutaneous adnexa, but has multilineage potential that enables it to differentiate into multiple cutaneous adnexa4,5. Immunohistochemical examination shows that neoplastic cells coexpress cytokeratin (CK) and vimentin, indicating that the neoplasm may be derived from follicular stem cells4–6. Follicular stem cells in dogs differentiate into cutaneous adnexa, such as the inner and outer root sheaths, sebaceous glands and apocrine sweat glands with different CK isoform expression in the respective cutaneous adnexa6–8. These differences in CK expression are useful for examining divergent adnexal differentiation of tumor5.

In this paper, we report the histological and immunohistochemical findings of a tumor in the lower lip of a 14-year-old male castrated Shih Tzu that was diagnosed with cutaneous clear cell adnexal carcinoma displaying several characteristic immunohistochemical features. We show divergent adnexal differentiation of the neoplastic cells and compare the morphology with previously reported canine cutaneous clear cell adnexal carcinomas.

The tissue specimen was fixed in 10% neutral formalin and embedded in paraffin or Optimal Cutting Temperature (O.C.T.) compound (Sakura Finetek, Tokyo, Japan). A block embedded in O.C.T. compound was snap frozen and kept at −80°C. Paraffin sections were stained with hematoxylin and eosin and reacted with periodic acid Schiff (PAS). Frozen sections were stained with Sudan III. Paraffin sections were also used for immunohistochemistry. Table 1 shows the primary antibodies used in this study. As secondary antibodies, we used peroxidase-conjugated anti-mouse (Histofine Simple Stain MAX-PO(M), Nichirei, Tokyo, Japan) and peroxidase-conjugated anti-rabbit (Histofine Simple Stain MAX-PO(R), Nichirei) immunoglobulin (Ig)G. Immunoreactions were visualized by diaminobenzidine, and the sections were counterstained with Mayer’s hematoxylin. To examine coexpression of CK and vimentin within the neoplastic cells, anti-CK (clone AE1/AE3) and anti-vimentin antibodies were used for double-labeled immunofluorescence. Anti-CK and
Canine Cutaneous Clear Cell Adnexal Carcinoma

Anti-vimentin antibodies were labeled with affinity-purified goat anti-mouse IgG fluorescein isothiocyanate (EY Laboratories, San Mateo, CA, USA) and affinity-purified goat anti-mouse IgG (Rhodamine conjugate, Chemicon, Temecula, CA, USA), respectively.

The excised mass was 8×8×10 mm in size, and its cut surface contained multiple lobules and was white (Fig. 1). Histologically, the neoplasm was located in the dermis and was composed of lobular structures separated by thin fibrous stromal tissues. The tumor mainly consisted of round to polygonal neoplastic cells. The cells varied in size and were characterized by clear or vacuolated cytoplasms with round to oval nuclei (Fig. 2a). The cytoplasm of the cells contained PAS-positive granules (Fig. 2c), which disappeared with diastase treatment. All neoplastic cells were negative for Sudan III. There were no follicular papillary mesenchymal bodies, but there were many tubular structures with or without PAS-positive basement membrane-like structures within the neoplasm (Fig. 2b). The mitotic rate was 1–2 mitoses per 10 high-power fields.

Table 2 shows the results for the special stains and immunohistochemical staining of the tumor and normal skin tissues. Immunohistochemically, the neoplastic cells were positive for pan-CK (AE1/AE3, KL1, CAM 5.2), CK-7, CK-8, CK-14, CK-15, CK-18, vimentin and α-SMA (Figs. 3, 4). The proportion of α-SMA-positive cells was more than 75% of the neoplastic cells. However, α-SMA-positive cells were rarely observed in a few neoplastic lobules (Fig. 4).

Tubular structures in the neoplasm were positive for pan-CK (AE1/AE3, CAM 5.2, KL1), CK-7, CK-8, CK-14, and partly positive for vimentin, but negative for α-SMA (Figs. 3, 4). A few neoplastic cells were positive for S-100 and PGP9.5, but negative for NSE and Melan A. MHC class II-positive cells were present in the neoplasm, which might represent dermal dendritic cells. Double-labeled immunofluorescence staining revealed that most neoplastic cells were double-positive for CK and vimentin (Fig. 5).

Histologically, neoplastic tubular structures were frequently observed, suggesting that neoplastic cells differentiated into apocrine sweat glands. However, the immunohistochemical results showed that the neoplastic cells expressed specific CK isoforms of several cutaneous adnexa. The tumor cells expressed CK-8 and CK-18, and had glycogetic granules in the cytoplasm. In normal canine skin, the outer root sheath and apocrine sweat glands stain positive for CK-8. Specifically, CK-18 is expressed in apocrine sweat glands6–8. Glycogetic granules are known to exist in the outer root sheath of normal canine skin. This suggests that the present tumor had differentiated into the outer root sheath and into the apocrine sweat gland. In addition, the neoplastic cells expressed pan-CK (KL1) and CK-14. In normal canine skin, pan-CK (KL1) is expressed in the superficial layer of the epidermis and inner root sheath, and CK-14 is expressed in the basal layer of the epidermis, outer root sheath, inner root sheath, sebaceous gland and myoepithelial cells of the apocrine sweat gland6–7. Meanwhile, the present neoplasm was negative for Sudan III. Therefore, this tumor might not have differentiated into sebaceous glands. Immunostaining indicated that the present tumor differentiated into the epidermis, inner root sheath, outer root sheath and apocrine sweat gland and that it also coexpressed CK and vimentin. This supports the hypothesis that clear cell adnexal carcinoma derives from the follicular stem cell6.

In previously reported cases of clear cell adnexal carcinomas, tumor cells have been negative for α-SMA4–6. How-
Fig. 1. A cut surface of the formalin-fixed neoplastic mass (8×8×10 mm).

Fig. 2. Histological appearances of the tumor. H&E staining. a) The tumor mostly consists of round or polygonal neoplastic cells with clear or vacuolated cytoplasms. Bar=50 μm. b) Small tubular structures are scattered in the neoplasm (arrows). Bar=20 μm. c) Fine glycogenic granules are present in the cytoplasm of the clear neoplastic cells. PAS stain. Bar=20 μm.

Fig. 3. a) Immunostaining for pan-CK (AE1/AE3): positive neoplastic cells are diffusely distributed. All tubular structures are strongly positive. b) Immunostaining for pan-CK (CAM 5.2): most neoplastic cells are positive, and tubular structures are strongly positive. c) Immunostaining for pan-CK (KL1): positive neoplastic cells including tubular cells are scattered. d) Immunostaining for CK-7: some of the neoplastic cells forming tubular structures are positive. e) Immunostaining for CK-8: most neoplastic cells are positive. Tubular structures are strongly positive. f) Immunostaining for CK-14: some areas are strongly positive. g) Immunostaining for CK-15: a few clear or vacuolated neoplastic cells are weakly positive. h) Immunostaining for CK-18: tubular structures are positive. i) Immunostaining for vimentin: most neoplastic cells, except tubular structures, are positive. Bar=20 μm.

Fig. 4. Immunostaining for α-SMA. Tubular structures are negative (inset, Bar=20 μm). The neoplastic cells are mostly positive for α-SMA; however, neoplastic cells in a nodule (*) are barely positive. Bar=100 μm.

Fig. 5. Double-labeled immunofluorescence microscopy. Nuclei are colored blue with 4,6-diamino-2-phenylindole. a) Red fluorescence indicates vimentin immunostaining. b) Green fluorescence indicates pan-CK (AE1/AE3) immunostaining. c) Merge image. Yellow color indicates colocalized pan-CK and vimentin immunoreactivity. None of the tubular structures are positive just for vimentin (arrows).
ever, in the present tumor, many neoplastic cells expressed α-SMA. Because actin is the predominant component of contractile microfilaments, which are responsible for cell motility and transport, α-SMA might play a role in cell migration and/or morphologic changes in the lower portion of the follicle during the hair cycle9. In mouse experiments in vitro, cells were found to express nestin in the bulge area and differentiated into neurons, glial cells, keratinocytes, smooth muscle cells and melanocytes9,10. These studies suggest that cells in the canine bulge area can differentiate into α-SMA-positive cells. Additionally, in normal canine skin, myoepithelial cells of the apocrine sweat gland are positive for α-SMA as well as CK, indicating that some α-SMA-positive neoplastic cells in the present case might have differentiated into myoepithelial cells. Apocrine sweat gland tumors with prominent proliferation of the myoepithelium component are considered to be a differential diagnosis due to their high positivity for α-SMA. However, most neoplastic cells also expressed vimentin, which ruled out apocrine sweat gland neoplasms. Moreover, the divergent differentiation of the neoplastic cells in this case is not seen in other cutaneous tumors, including complex apocrine sweat gland neoplasms.

In a previous report of clear cell adnexal carcinoma, tumor cells were positive for CK-8 and CK-186, thus suggesting they differentiated into the outer root sheath and apocrine sweat gland6. Other reports have shown that clear cell adnexal carcinoma is negative for α-SMA4–6. However, the present tumor was positive for CK-8, CK-18, pan-CK (KL1), CK-14 and α-SMA. Additionally, the present tumor most likely differentiated into the inner root sheath, outer root sheath and apocrine sweat gland (glandular epithelial cell and myoepithelial cell). This indicates that clear cell adnexal carcinoma has multilineage potential and differentiates into cutaneous adnexa and that this differentiation trend and the extent are different in each neoplasm.

Recently, CK-15, CK-19, CD34 and CD200 have been shown to be human follicular stem cell markers6,11,12. Canine hair follicles are reported to be more similar to human hair follicles than those of mice when considering their architecture and size12. Presumably, canine hair follicles could have stem cell machinery analogous to human hair follicles12. CK-15 and CD34 have been used as canine follicular stem cell markers in a previous study12. In the present case, CK-15 was positive in only 1% of neoplastic cells, regardless of the fact that clear cell adnexal carcinoma is suspected to be a neoplasm derived from follicular stem cells. Additionally, in normal skin, the intermediate region of the outer root sheath was positive for CK-15 (Table 2). In

Table 2. Results of Special Stains and Immunostains of Normal Skin Tissues and the Tumor*

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Epidermis</th>
<th>Inner root sheath</th>
<th>Outer root sheath</th>
<th>Sebaceous gland</th>
<th>Apocrine gland</th>
<th>Follicular papilla</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAS</td>
<td></td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Sudan III</td>
<td></td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Immunostains</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytokeratin 8</td>
<td>Ks8.7</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ (&gt;75%)</td>
</tr>
<tr>
<td>Cytokeratin 14</td>
<td>LL002</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+ (&gt;30%)</td>
</tr>
<tr>
<td>Cytokeratin 15</td>
<td>CBL272</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+ (&lt;1%)</td>
</tr>
<tr>
<td>Cytokeratin 18</td>
<td>Ks 18.4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+ (1–5%)</td>
</tr>
<tr>
<td>Cytokeratin 20</td>
<td>Ks 20.8</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Vimentin</td>
<td>V9</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+ (&gt;80%)</td>
</tr>
<tr>
<td>α–SMA</td>
<td>1A4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+ (&gt;75%)</td>
</tr>
<tr>
<td>S–100a</td>
<td>polyclonal</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ (10–20%)</td>
</tr>
<tr>
<td>NSE</td>
<td>NSE–1G4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PGP 9.5</td>
<td>13C4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+ (10–20%)</td>
</tr>
<tr>
<td>Melan A</td>
<td>A103</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>MHC class II</td>
<td>TAL.1B5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*– = negative; + = positive. b Basal cell layer is negative. c Granular and horny cell layers are negative. d Merkel cells are positive. e Langerhans cells are positive. f Intermediate region is positive. g Only myoepithelial cells show a positive reaction. h The percentage indicates the proportion of the estimated positive cells in the tumor cells.
this study, it is clear that the neoplastic cells probably differentiated into several adnexa.

**Acknowledgment:** This study was partially supported by a project grant (Creative Research Project, 2009) awarded by the Azabu University Research Services Division.

**References**