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Abstract. Eight canine tumors originating from specific glandular structures in the anal region, as well as metastatic tumor tissue of two of these cases (case Nos. 7, 8), were immunohistochemically analyzed using various monoclonal antibodies (MoAbs) directed against human keratin types, vimentin, neurofilament proteins, and α-smooth muscle actin. These tumors also were stained for the broad-spectrum neuroendocrine markers neuron-specific enolase (NSE) and synaptophysin. In histologically normal canine anal structures, α-smooth muscle actin and NSE antibodies stained basally localized (probably myoepithelial) cells in the anal glands and the anal sac glands. NSE staining also was present in a limited number of luminal cells in both anal glands and anal sac glands. Synaptophysin labeling was not observed in any of these glandular structures. Histologically, the tumors were differentiated into well- and moderately differentiated perianal gland tumors (n = 5) and carcinomas without perianal gland differentiation (n = 3), corresponding to the so-called apocrine carcinomas of the anal region. Immunohistochemically, the perianal gland tumors could be differentiated from the carcinomas by marked differences in staining pattern with the various keratin MoAbs, particularly MoAbs directed against human keratin types 7 and 18. The keratin-staining characteristics of the carcinomas suggest a glandular luminal cell origin. Metastases of the carcinomas showed loss of some keratin-staining characteristics as compared with the primary tumor. Staining for NSE was only observed in solitary cells and small cell clusters in the carcinomas and their metastases, whereas the α-smooth muscle actin antibody did not react with the carcinoma cells. None of the tumors stained for neurofilament proteins or synaptophysin. An unequivocal neuroendocrine nature of the carcinomas could not be substantiated by our immunohistochemical study, although the presence of a population of neuroendocrine cells within these neoplasms seems likely. Because the immunohistochemical features of the carcinomas with respect to various keratin MoAbs and NSE are similar to those of the anal glands and the anal sac glands, both these glands might be considered as site of origin of these carcinomas.

Key words: Actins; carcinoma; dogs; immunohistochemistry; keratin; neoplasms; neuron-specific enolase; synaptophysin; tumor markers; vimentin.

Tumors in the canine anal region are fairly common and mostly originate from the specific glands in this region. They predominantly comprise the perianal gland tumors and the apocrine carcinomas. The perianal gland tumors have the highest incidence and despite their variable histomorphology associated with different ratios of reserve or basal cells to differentiated cells, generally they are benign. Perianal gland carcinomas occur sporadically and have a low rate of metastasis. The apocrine carcinomas are well known and have a characteristic biphasic morphology, i.e., a solid pattern intermingled with acinic and/or rosettelike structures and are often associated with hypercalcemia. These tumors metastasize very frequently. Solid apocrine carcinomas may be difficult to differentiate from perianal gland tumors characterized by the predominance of reserve cells. Differentiation of these tumors is of substantial importance because the apocrine carcinomas have a quite different...
prognosis. Because various specific glandular structures of the canine anal region show different staining characteristics with monoclonal antibodies raised against various human keratin types,59 immunohistochemical evaluation may aid in the differential diagnosis of these tumors. These carcinomas are assumed to arise from the anal sac glands because of their apocrine features and their often close association with the anal sacs.20,40,60 However, the anal glands are also reported to be apocrine and are also located in the proximity of the anal sacs9 and thus can also be considered as tissue of origin for such neoplasms.20,35,40 Even sweat gland tumors have histologic characteristics comparable to those of these neoplasms.25,40

A possible neuroendocrine (NE) nature of these carcinomas is indicated by the characteristic histomorphology3 and the tumor-associated hypercalcemia, thought to be induced by a humoral substance produced by the neoplastic cells.20,40,60 Recently, a parathyroid hormone-related protein has been demonstrated in such tumors.62 In human NE tumors, this protein has been associated with hypercalcemia.61 Moreover, the ultrastructural features of tumor cells are compatible with those of hormone-secreting endocrine cells.39

NE tumors in human beings have fairly characteristic but heterogeneous histomorphologic features.69 Definite and precise characterization is achieved by demonstration of their specific amines and/or peptide hormones.4 However, the concentration of these specific hormones in the tumor cells can be beneath detection levels, or the tumor cells can produce aberrant molecular forms of these hormones.49 Therefore, tumors showing histologic features of NE neoplasms immunohistochemically are primarily characterized by the demonstration of nonspecific neuroendocrine components, i.e., components independent of the production of regulatory peptides26 such as neuron-specific enolase (NSE) and synaptophysin.6,21,26,82 NSE is the γγ isoenzyme of the cytoplasmic glycolytic enzyme enolase. Although enolase has several isoenzymes with various tissue distribution patterns,67 the γγ isoenzyme is present in high concentrations in neurons and NE cells.15,26,67,82 Therefore, NSE is often used as a marker for NE tumors in human beings.35,75 Synaptophysin is an integral membrane glycoprotein that occurs in presynaptic vesicles of neurons and in similar vesicles of the adrenal medulla.81 In human beings, synaptophysin has been a valuable marker for NE tumors in different locations.15,21,82 In addition, neurofilament proteins and vimentin are expressed in some NE tumors.2,33,51

In this study, the expression patterns of several keratins, vimentin, neurofilament proteins, α-smooth muscle actin, and the broad-spectrum NE markers NSE and synaptophysin in tumors of the specific glands of the canine anal region are presented. The results are discussed with respect to the differentiation and origin of the tumors and the possible NE character of the apocrine carcinomas.

Materials and Methods

Tissues

Tumor tissue samples of eight dogs (case Nos. 1–8) presented with tumors in the anal region were used in this study. The dogs were of various breeds, and their ages ranged from 6 to 15 years. The tumors were classified according to their histologic features as described previously6 to well-differentiated perianal gland tumors showing mainly differentiated perianal gland cells (case Nos. 1–3), moderately or poorly differentiated perianal gland tumors consisting predominantly or almost entirely of reserve cells (case Nos. 4, 5), and carcinomas without histologic perianal gland differentiation showing histomorphologic features corresponding to the so-called apocrine carcinomas (case Nos. 6–8). Six tumors were submitted as surgical excisions (case Nos. 1–6). Two of the three carcinomas (case Nos. 7, 8) were found at necropsy to have widespread metastases. In these cases, metastatic tumor tissue also was sampled. The tumors with perianal gland differentiation were found in three intact male dogs, one castrated male, and one female dog. The carcinomas were from an intact female, a spayed female, and an intact male dog, respectively. Immediately after surgical excision or euthanasia, samples of the tumors were frozen in isopentane that had been precooled in liquid nitrogen; additional tumor tissue samples were fixed in 10% neutral formalin.

Formalin-fixed canine adrenal gland and canine pancreatic tissue served as positive controls for neuron-specific enolase (NSE) and synaptophysin staining. To evaluate the normal distribution of NSE, synaptophysin, and α-smooth muscle actin in the glandular structures of the canine anal region, samples from four normal healthy dogs were used (two intact female dogs, one spayed female, and one intact male dog, of various breeds, age range of 6 to 10 years).

Antibodies

The code name, specificity, original antigen, dilution, and source of the antibodies used in this study are presented in Table 1. The vimentin and NSE antibodies were polyclonal antibodies, whereas all other antibodies were monoclonal antibodies (MoAbs). The keratin MoAbs were all raised against human keratin types.43

Immunohistochemistry

The keratin and the α-smooth muscle actin antibodies were applied to frozen sections, whereas the vimentin, neurofilament proteins, NSE, and synaptophysin antibodies were used on formalin-fixed paraffin-embedded tissues. The frozen sections were fixed in cold acetone (−20 C) for 10 minutes and air dried for 5 minutes. Interaction of antibodies with the tumor specimens was blocked with 10% normal canine serum in phosphate-buffered saline (PBS) for 15 minutes at room temperature. In negative controls, the primary antibodies
Table 1. Code name, specificity, original antigen, dilution and source of the antibodies used for analysis of tumors originating from the specific glandular structures of the canine anal region.

<table>
<thead>
<tr>
<th>Code Name</th>
<th>Specificity</th>
<th>Original Antigen</th>
<th>Dilution</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCK 102</td>
<td>K* 5 + 8</td>
<td>MR 21 lung cancer cells</td>
<td>1:10</td>
<td>Euro-Diagnostics B.V.</td>
</tr>
<tr>
<td>RCK 103</td>
<td>K 5 + others</td>
<td>Hela cells and human epidermis</td>
<td>1:10</td>
<td>Prof. Ramaekers</td>
</tr>
<tr>
<td>RGE 53</td>
<td>K 18</td>
<td>Hela cells</td>
<td>1:20</td>
<td>Euro-Diagnostics B.V.</td>
</tr>
<tr>
<td>DE-K 18</td>
<td>K 18</td>
<td>Human ectocervical cytoskeletal preparation</td>
<td>1:200</td>
<td>Dr. Ivanyi</td>
</tr>
<tr>
<td>RCK 105</td>
<td>K 7</td>
<td>T24 cell cytoskeletal preparation</td>
<td>1:10</td>
<td>Euro-Diagnostics B.V.</td>
</tr>
<tr>
<td>LP2K</td>
<td>K 19</td>
<td>SV40 transformed human keratinocytes</td>
<td>1:10</td>
<td>Amersham, Ltd.</td>
</tr>
<tr>
<td>RCK 107</td>
<td>K 14</td>
<td>TR 146 cell cytoskeletal preparation</td>
<td>undiluted</td>
<td>Prof. Ramaekers</td>
</tr>
<tr>
<td>8.7</td>
<td>K 14 + 17</td>
<td>A431 human vulva squamous carcinoma cell line</td>
<td>1:200</td>
<td>Dr. Ivanyi</td>
</tr>
<tr>
<td>6B10</td>
<td>K 4</td>
<td>Human esophagus</td>
<td>1:5</td>
<td>Euro-Diagnostics B.V.</td>
</tr>
<tr>
<td>DE-K 10</td>
<td>K 10</td>
<td>Human epidermis</td>
<td>1:10</td>
<td>Dr. Ivanyi</td>
</tr>
<tr>
<td>Sm-1</td>
<td>α-smooth muscle actin</td>
<td>NH2-terminal synthetic decapetide of α-smooth muscle actin</td>
<td>1:15,000</td>
<td>Prof. Gabbiani</td>
</tr>
<tr>
<td>NSE (A 589)</td>
<td>Neuron-specific enolase</td>
<td>Human brain</td>
<td>1:20</td>
<td>Dakopatts, Inc.</td>
</tr>
<tr>
<td>Synaptophysin</td>
<td>Synaptophysin</td>
<td>Bovine brain</td>
<td>1:20</td>
<td>Boehringer Mannheim B.V.</td>
</tr>
<tr>
<td>PVI</td>
<td>Vimentin</td>
<td>Calf lens vimentin</td>
<td>1:80</td>
<td>Euro-Diagnostics B.V.</td>
</tr>
<tr>
<td>MNF</td>
<td>Neurofilament proteins</td>
<td>Human brain</td>
<td>1:10</td>
<td>Euro-Diagnostics B.V.</td>
</tr>
</tbody>
</table>

*K = keratin type according to the catalogue of Moll et al.41

were omitted in the first incubation step. The MoAbs appropriately diluted in PBS buffer containing 5% normal canine serum were incubated for 60 minutes in a humid atmosphere at room temperature. Sections were then rinsed twice in PBS for 10 minutes each time and incubated with peroxidase-conjugated goat anti-mouse IgG (Tago, Inc., Burlingame, CA), diluted 1:20 in PBS containing 5% normal canine serum for 60 minutes at room temperature. Sections were then rinsed three times in PBS for 10 minutes each time, and the antigen-antibody complexes were visualized during a 2- to 3-minute incubation step with 0.3% H2O2 and 0.5% 3,3′-diaminobenzidine tetrahydrochloride (DAB, Sigma Chemical Co., St. Louis, MO) diluted in 0.05 M Tris/HCl buffer (pH 7.6). After rinsing in distilled water for 5 minutes, the sections were counterstained with Mayer’s hematoxylin for 30 seconds. Formalin-fixed specimens were deparaffinized with xylene three times for 10 minutes each time and with 100% alcohol three times for 5 minutes each time. The MoAb against neurofilament proteins and the polyclonal antibody against vimentin were employed in an immunoperoxidase staining method as described previously.77 For NSE and synaptophysin immunohistochemical evaluation, deparaffinized sections were incubated in 3% H2O2 in H2O for 30 minutes at room temperature. The sections were rehydrated in 96% and 70% alcohol. All sections were rinsed in H2O for 10 minutes, briefly air dried, and preincubated with 20% normal swine serum in PBS for 30 minutes at room temperature. Then the NSE or synaptophysin antibody appropriately diluted in 1% bovine serum albumin in PBS (PBSA) was applied and incubated over night at 4°C in a humid atmosphere and subsequently for 1 hour at room temperature. After incubation the sections were rinsed three times for 5 minutes each time. Sections for NSE immunohistochemical evaluation were preincubated with 20% normal swine serum in PBS for 10 minutes at room temperature and incubated with swine anti-rabbit IgG (Dakopatts, Inc., Glostrup, Denmark), whereas synaptophysin sections were directly incubated with rabbit anti-mouse IgG (Dakopatts, Inc.) for 30 minutes at room temperature. Both anti-IgG antisera were diluted 1:20 in 1% PBSA. After three washing steps in PBS of 5 minutes each, the NSE sections again were preincubated for 10 minutes with 20% normal swine serum in PBS at room temperature and incubated with the rabbit peroxidase-antiperoxidase complex (Dakopatts, Inc., dilution 1:100), whereas synaptophysin sections without preincubation were incubated with the mouse peroxidase-antiperoxidase complex (Dakopatts, Inc., dilution 1:100), both in 1% PBSA. After washing in PBS three times for 5 minutes each time, the antigen was visualized using 0.3% H2O2, 0.65% imidazole, and 0.5% 3-amino-9-ethlycarbazol (AEC, Sigma) for NSE and 0.5% DAB (Sigma) for synaptophysin diluted in 0.05 M Tris/HCl buffer (pH 7.6), during a 2- to 3-minute incubation step. After rinsing twice in distilled water, all sections were counterstained with Mayer’s hematoxylin for 45 seconds.

The results of tumor immunohistochemical evaluation with respect to the various keratins and vimentin were compared with data obtained from a previous study on the immunohistochemical characteristics of the epithelial structures of the canine anal region.76 The immunohistochemical findings of the specific glandular structures in this region are shown in Table 2. The results with respect to NSE, synaptophysin,
Table 2. Immunohistochemical findings* for various anti-human keratins and anti-calf vimentin in the specific glandular structures of the canine anal region (adapted from Vos et al.76).

<table>
<thead>
<tr>
<th>Antibodies†</th>
<th>Perianal Glands</th>
<th>Anal Sac Glands</th>
<th>Anal Glands</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reserve cells</td>
<td>Differentiated Cells</td>
<td>Basal Cells</td>
</tr>
<tr>
<td>RCK 102 (K 5, 8)</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>RCK 103 (K 5, others)</td>
<td>+</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>RGE 53 (K 18)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DE-K 18 (K 18)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RCK 105 (K 7)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LP2K (K 19)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RCK 107 (K 14)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8.7 (K 14, 17)</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6B10 (K 4)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DE-K 10 (K 10)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PV1 (vimentin)</td>
<td>0</td>
<td>+/+</td>
<td>0</td>
</tr>
</tbody>
</table>

* + = positive; 0 = negative; +/0 = solitary positive cells.
† Keratin type according to Moll et al.4 given in parentheses.
‡ Incidental perinuclear staining.

and α-smooth muscle actin were compared with the findings in structures of normal dogs as described in this study.

Results

The immunohistochemical findings with the different keratin monoclonal antibodies (MoAbs) in the various tumors are summarized in Table 3. All perianal gland tumors showed an identical keratin staining pattern irrespective of the ratio of reserve (basal) cells to differentiated cells. The reserve cells were specifically stained with MoAbs RCK 102 (K 5 + 8) and RCK 103 (K 5 + others), whereas both reserve cells and differentiated cells were stained by the MoAbs RCK 107 (K 14) and 8.7 (K 14 + 17). The carcinomas without perianal gland differentiation, representing the so-called apocrine carcinomas, homogeneously stained with RCK 102 (K 5 + 8) and RCK 103 (K 5 + others). In contrast to the perianal gland tumors, they consistently stained with RGE 53 and DE-K 18 (both K 18), RCK 105 (K 7), and DE-K 10 (K 10). Additionally, a variable staining reaction was noticed in these carcinomas for the MoAbs LP2K (K 19), RCK 107 (K 14), and 8.7 (K 14 + 17) (Table 3). Two tumors were positive for RCK 107 and 8.7; one of these showed additional staining with LP2K. The third tumor also reacted with LP2K and 8.7 but appeared to be negative.

Table 3. Immunohistochemical findings* for various anti-human keratins in tumors originating from the specific glandular structures of the canine anal region.

<table>
<thead>
<tr>
<th>Antibodies†</th>
<th>Perianal Gland Tumors</th>
<th>Carcinomas without perianal gland differentiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Well</td>
<td>Moderately</td>
</tr>
<tr>
<td></td>
<td>Differentiated</td>
<td>Differentiated</td>
</tr>
<tr>
<td>RCK 102 (K 5, 8)</td>
<td>3‡</td>
<td>2‡</td>
</tr>
<tr>
<td>RCK 103 (K 5, others)</td>
<td>3‡</td>
<td>2‡</td>
</tr>
<tr>
<td>RGE 53 (K 18)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DE-K 18 (K 18)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RCK 105 (K 7)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LP2K (K 19)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RCK 107 (K 14)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>8.7 (K 14, 17)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>6B10 (K 4)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DE-K 10 (K 10)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total no. tumors examined</td>
<td>3</td>
<td>2</td>
</tr>
</tbody>
</table>

* Number of positive tumors.
† Keratin types according to Moll et al.4 given in parentheses.
‡ Only cells with reserve (basal) cell histomorphologic features were stained.
Fig. 1. Cryostat section of carcinoma without perianal gland differentiation; dog No. 8, 10 years old. Fairly homogeneous staining of tumor cells with monoclonal antibody RGE 53, directed against human keratin type 8. Immunoperoxidase, anti-human keratin type 8. Mayer’s hematoxylin counterstain. Bar = 15 μm.

Fig. 2. Cryostat section of carcinoma without perianal gland differentiation; dog No. 7, 10 years old. Fig. 2a. Heterogeneous staining of tumor cells with monoclonal antibody DE-K 10, directed against human keratin type 10. Immunoperoxidase, anti-human keratin type 10. Mayer’s hematoxylin counterstain. Bar = 10 μm. Fig. 2b. Staining of scattered tumor
with respect to RCK 107. The carcinomas stained quite homogeneously with respect to RGE 53 and DE-K 18 (both K 18) (Fig. 1). For the other keratin MoAbs, the staining pattern appeared to be more or less heterogeneous, e.g., DE-K 10 (Fig. 2a), and sometimes confined to only a small number of tumor cells, e.g., the 8.7 MoAb (Fig. 2b). The reaction patterns in the metastases corresponded with the pattern of the primary tumors except for RCK 107. Although both primary tumors were stained by this keratin MoAb, metastatic tissue did not show any positive reaction.

The α-smooth muscle actin MoAb revealed staining of muscle cells (e.g., in vessel walls) and basally located (probably myoepithelial) cells in sweat glands, anal glands, and anal sac glands. None of the tumors showed staining of tumor cells with this MoAb.

Neuron-specific enolase (NSE) and synaptophysin were clearly demonstrated in the pancreatic islet cells and the cells of the adrenal medulla. In addition, the adrenal cortex showed marked staining of the zona glomerulosa and moderate staining of the zona fasciculata and reticularis with the NSE antibody. In the normal structures of the canine anal region, no synaptophysin staining was noticed. However, NSE immunohistochemical treatment resulted in marked staining of muscle cells and axons and faint, diffuse staining of basally located (probably myoepithelial) cells in sweat glands, anal glands, and anal sac glands. Furthermore, distinctly stained NSE-positive luminal epithelial cells were observed both in the anal glands (Fig. 3) and the anal sac glands. In the anal sac glands, only few solitary NSE-positive cells were seen, whereas in the anal glands positive cells were more numerous. Differentiated perianal gland cells showed a faint diffuse, probably nonspecific staining.

The tumors, like the normal anal structures, did not show any synaptophysin immunoreactivity. In the perianal gland tumors, a faint nonspecific staining for NSE was present in well-differentiated cells. In the carcinomas without perianal gland differentiation and in their metastases, the majority of the tumor cells did not stain. However, a limited number of positive cells could be seen scattered throughout the tumor tissue, mostly as small clusters of positive cells (Fig. 4).

A positive reaction to vimentin was seen in perianal gland tumors as a perinuclear staining in a small number of differentiated cells. All tumors were negative for neurofilament proteins.

Discussion

Epithelial cells maintain in general their original keratin type (K) pattern upon malignant dedifferentiation. Appropriate keratin monoclonal antibodies (MoAbs), therefore, can be applied to evaluate tumor histogenesis. In the perianal gland tumors, the keratin staining patterns corresponded with those of normal perianal glands; in tumors and in normal glands reserve cells were selectively stained with RCK 102 (K 5 + 8) and RCK 103 (K 5 + others), whereas both basal and differentiated cells were stained with RCK 107 (K 14) and 8.7 (K 14 + 17). Immunohistochemically, the carcinomas without histologic perianal gland differentiation and their metastases were stained among others by the keratin MoAbs RGE 53 and DE-K 18 (both K 18), RCK 105 (K 7), and DE-K 10 (K 10). These keratin staining patterns of the carcinomas indicate a nonperianal gland origin. Therefore, these MoAbs could unequivocally discriminate between the carcinomas and the perianal gland tumors. In human beings, keratins 7 and 18 are considered typical of glandular differentiation. MoAbs directed against these keratins (RCK 105 and RGE 53/DE-K 18, respectively) stained in the canine anal region: in the luminal cells of the anal glands, anal sac glands, and sweat glands. The staining of the carcinoma cells with these antibodies, therefore, is consistent with neoplastic transformation of luminal cells of tubular glands. This interpretation is supported by the absence of tumor cell labeling by the α-smooth muscle actin antibody, which exclusively stained basally localized (probably myoepithelial) cells in the three glandular structures. However, the exact origin of the carcinomas could not be definitely established because RCK 105, RGE 53, and DE-K 18 react with differentiated luminal cells in all these glands. Also, the unexpected staining with DE-K 10 (K 10) makes it difficult to pinpoint the histogenesis of these tumors. In human beings, this keratin type is considered to be a marker.

cells with monoclonal antibody 8.7, directed against human keratin types 14 and 17. Immunoperoxidase, anti-human keratin types 14 and 17. Mayer’s hematoxylin counterstain. Bar = 10 μm.

Fig. 3. Anal gland; normal dog, 10 years old. Staining of luminal cells with the neuron-specific enolase polyclonal antibody. Immunoperoxidase, anti-human neuron-specific enolase. Mayer’s hematoxylin counterstain. Bar = 29 μm.

Fig. 4. Carcinoma without perianal gland differentiation; dog No. 8, 10 years old. Staining of scattered and small clusters of tumor cells with the neuron-specific enolase antibody. Immunoperoxidase, anti-human neuron-specific enolase. Mayer’s hematoxylin counterstain. Bar = 6 μm.
of keratinocyte differentiation. In the dog, DE-K 10 staining appeared to be confined to the suprabasal layer of the cornifying squamous epithelia in the skin and anal sacs. In human beings, unexpected immunolocalization of human keratin type 10 in thyroid tumors has been correlated with malignant transformation. In all carcinomas and their metastases, 8.7 (K 14 + 17) immunoreactive cells were observed. Although this keratin MoAb stained luminal cells only in the anal glands and not in the anal sac glands, the restricted number of tumor cells reactive with the 8.7 MoAb does not warrant a definite conclusion about the origin of these carcinomas.

The carcinomas differed with respect to their labeling with the keratin MoAbs LP2K (K 19), RCK 107 (K 14), and 8.7 (K 14 + 17). Heterogeneity in keratin expression pattern within a group of epithelial tumors and between primary tumors and metastases has also been observed in human beings. This heterogeneity is due to the unexpected loss of expression of specific keratin types in comparison with expression in the tissue of origin and has been associated with progression in malignancy. This phenomenon might be due to structural antigenic epitope changes in malignant transformation or the selection of specific keratin types during cell transformation and tumor development. The loss of expression of specific keratin types has also been reported in animals, for instance, during malignant transformation of experimentally induced urinary bladder carcinomas in the rat and in induced dysplastic skin papillomas and carcinomas in mice. The loss of labeling of metastatic tumor cells with the keratin MoAb RCK 107 as compared with labeling of the cells of the primary tumor is in agreement with such observation and could indicate the selection of a more malignant tumor cell population.

Neuron-specific enolase (NSE) was originally considered to be neuron and neuroendocrine (NE) cell specific. The strong reactivity for NSE in canine neurons, nerves, pancreatic islet cells, and the adrenal medulla cells is in accordance with this specificity of NSE. Moreover, as in human beings, nonspecific staining for NSE was observed in smooth muscle cells and myoepithelial cells. This nonspecific staining was possibly due to cross-reactions with other enolase isoenzymes. Although not exclusively expressed by neuronal or NE cells, NSE is the most important broad spectrum marker for NE tumors in human beings. NSE-negative NE tumors have only been reported incidentally. In rats, an NSE-positive NE tumor has been reported. In the dog, an NSE-negative NE tumor has been described; however, recently foci of NSE-immunoreactive cells in an atypical NE lung tumor also have been reported.

The carcinomas show histologic characteristics of NE tumors. Only small clusters of cells immunoreactive for NSE were seen in these tumors. Although NSE antibodies also react with myoepithelial cells, a myoepithelial origin of these NSE-immunoreactive tumor cells is unlikely because these cells were not labeled with the α-smooth muscle actin MoAb. This antibody labels myoepithelial cells as the only reacting epithelial cell type. A small number of clearly luminal epithelial cells was NSE positive in both the anal glands and, albeit a very small number of cells, in the anal sac glands. These findings suggest the presence of NE cells in these glands. The limited number of NSE-positive tumor cells in the carcinomas is compatible with the reported staining reaction for parathyroid hormone-related protein, which was also restricted to scattered or small groups of cells. In human beings, scattered NE cells can be present in a number of benign and malignant tumors. In tumors predominantly composed of non-NE cells, a subpopulation of tumor cells with NE differentiation may be present. In human beings, carcinomas originating from several organs, such as the prostate, liver, the breast, and the bladder, have been shown to contain NSE-positive cells. The endocrine features of these cells were confirmed by other NE markers by electron microscopy and by the presence of a range of hormones such as parathyroid hormone and parathyroid hormone-related protein associated with hypercalcemia. Also, prostatic carcinomas with NE differentiation are associated with hypercalcemia. Recently, parathyroid hormone-related protein has been immunohistochemically demonstrated in human breast carcinomas. Because the majority of the carcinoma cells was not stained for NSE, a genuine NE character for these tumors was not unequivocal. However, the presence of a cell population with NE differentiation within the described carcinomas is probable, especially because in epithelial tumors of non-NE origin local proliferation of NE cells may occur. In human beings, the presence of NE characteristics in carcinomas is associated with aggressive biological behavior. Because both anal glands and anal sac glands contain NSE-positive luminal cells, both glandular structures might be considered as the origin of the carcinomas without perianal gland differentiation.

The staining of canine adrenal medullary cells and pancreatic islet cells by the synaptophysin antibody is concordant with findings in human beings and other species. In human beings, synaptophysin is a good marker for NE tumors in different organs. However, nonreacting NE neoplasms have also been reported, and staining for NSE was also not always associated with synaptophysin positivity. Because synaptophysin labeling appears to be fixation
time and antigen level dependent, a low amount of synaptophysin in association with the duration of fixation used in this study may explain the absence of synaptophysin staining in the carcinoma cells.

An appreciable number of human NE tumors expresses vimentin and neurofilament (NF) proteins, particularly rectal NE tumors. Vimentin and NF protein labeling was not observed in the carcinomas. The presence of scattered solitary vimentin-positive tumor cells in the perianal gland tumors is in accordance with the staining of some differentiated cells in normal perianal glands.

In conclusion, epithelial tumors specific for the anal region in the dog can be differentiated immunohistochemically into perianal gland tumors and non-perianal gland carcinomas, based on their keratin staining pattern. Although a majority of tumor cells in the carcinomas appeared to be NSE-positive, unequivocal and complete NE differentiation of these tumors was not evident. The described immunohistochemical findings suggest that both anal sac glands and anal glands should be considered as possible locations of origin for these carcinomas.

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