Abstract

Thirty nine canine adnexal tumours were histologically and immunohistochemically analysed. They were classified as trichoblastomas, trichoepitheliomas, sebaceous adenomas and epitheliomas, hepatoid gland adenomas and epitheliomas, and apocrine adenocarcinomas. Moreover, hepatoid gland angioadenoma and angioepithelioma were recognised. Studies of follicular tumours revealed coexpression of cytokeratin MNF116 and LP34, but sebaceous and hepatoid gland tumours as well as apocrine adenocarcinomas showed differences in the expression of both cytokeratins. All the tumours were negative for vimentin except two hepatoid gland adenomas in which coexpression of vimentin and cytokeratin was observed. Positive reaction for neuron-specific enolase (NSE) was observed in follicular tumours, whereas expression of α-smooth muscle actin (α-SMA) was found only in apocrine adenocarcinomas. Moreover, the presence of NSE observed in hepatoid gland adenomas was considered as non-specific just as both glial fibrillary acidic protein in sebaceous gland tumours and hepatoid gland adenomas, and also S100 protein in sebaceous adenomas. All the examined tumours showed lack of expression of both desmin and factor FVIII. Thus, among the used antibodies, cytokeratins, vimentin, NSE, and α-SMA play a main role in the evaluation of canine adnexal histogenesis.

Key words: dogs, skin tumours, adnexal tumours, histopathology, immunohistochemistry.
trichoblastomas, three trichoepitheliomas, six sebaceous adenomas, three sebaceous epitheliomas, 16 hepatoid gland adenomas, four hepatoid gland epitheliomas, and two apocrine carcinomas.

**Immunohistochemistry.** For immunohistochemistry, the sections were mounted on poly L-lysine glass slides, deparaffinised in xylene and rehydrated in alcohol and water. Endogenous peroxidase activity was blocked by the incubation of the sections with 3% hydrogen peroxide in water for 10 min at room temperature. Then all the sections were incubated with a panel of nine commercially-available primary antibodies (Table 1). All the sections were submitted to an antigen retrieval procedure before immunostaining. Tissue sections intended for the detection of cytokeratins (MNF116 and LP34), glial fibrillary acidic protein (GFAP), and S-100 protein (S-100) were treated with proteinase K (Proteinase K ready to use, DAKO) for 1.5-5 min at 37ºC. Those for the detection of vimentin (VIM) were immersed in unmasking solution (Target Retrieval Solution DAKO) diluted 1:10 but in cases of (VIM) were immersed in unmasking solution (Target Retrieval Solution DAKO) diluted 1:10 but in cases of lumen surface (Fig. 5). Among sebaceous and modified sebaceous gland tumours, both sebaceous adenomas and hepatoid gland adenomas consisted of small basophilic reserve cells at the periphery of the tumour lobules, which divided into mature sebocytes or hepatoid cells. In turn, epitheliomas in this group of tumours showed a preponderance of basaloid reserve cells with marked mitotic activity and scattered well-differentiated cells. Moreover, in this group of neoplasms, one case of hepatoid gland angioadenoma (Fig. 6) and two cases of hepatoid gland angioepithelioma (Fig. 7) were distinguished. The characteristics of them both were large glandular areas filled with blood, small vessels, or small amount of eosinophilic secretion. Histologically, apocrine adenocarcinomas showed the presence of tubular and cystic areas lined with single or multi-layered cuboidal cells with abundant granular eosinophilic cytoplasm and basally located large euchromatic nuclei. The lumen of glandular areas were filled with eosinophilic secretion or densely packed cluster of tumour cells (Fig. 8).

The results of immunohistochemical staining of tumour tissues with different antibodies are summarised in Table 2. All the tumours with adnexal differentiation showed positive reactivity for cytokeratins MNF116 and LP34. Neoplastic cells in trichoblastomas, trichoepitheliomas, and malignant trichoepithelioma coexpressed of both cytokeratins (Figs 9 and 10). In case of sebaceous and modified sebaceous gland tumours, basaloid reserve cells at the periphery of the lobules reacted only with cytokeratin MNF116 (Fig. 11), whereas positive staining for cytokeratin LP34 was observed only in sebocytes and hepatoid cells (Fig. 12). Apocrine adenocarcinomas outer cell layer revealed positive labelling for LP34 (Fig. 13), while luminal epithelial cells and a small cluster of epithelial cells scattered throughout the tumour matrix displayed positive reaction with cytokeratin MNF116. All the tumours were negative for VIM except two cases of hepatoid gland adenomas in which a local perinuclear staining was observed (Fig. 14). Immunoreactivity for α-SMA was found only in apocrine adenocarcinomas (Fig. 15) Neuron-specific enolase (NSE) immunoreactivity was clearly demonstrated in follicular tumours (Fig. 16), whereas hepatoid cells in six perianal gland adenomas showed a local faint diffuse staining. Immunoreactivity with GFAP was observed locally in three sebaceous adenomas, three sebaceous epitheliomas, and six hepatoid gland adenomas, while S-100 protein reactivity was found only in four sebaceous adenomas. In contrast, neoplastic cells of all the examined tumours were uniformly negative for FVIII and desmin. Immunoreactivity for FVIII was observed only in endothelial cells of large or small vessels inside the tumours. Immunostaining was not found in any of the tissue sections run as negative controls for either monoclonal or polyclonal antibodies.

**Results**

Histologically, the trichoblastomas consisted of basaloid cells with hyperchromatic ovoid to round nuclei and small to abundant granular eosinophilic cytoplasm depending on the tumour subtype. The epithelial neoplastic cells formed the long cords of cells that branched and joined together with nuclei arranged in a palisade perpendicular to the long axis of the column in ribbon subtype (Fig. 1). Medusoid subtype consisted of cords of cells radiating from a central island of densely packed cells (Fig. 2). Two cases of trichoblastomas consisted of mixed areas of different subtypes: ribbon with medusoid and granular with medusoid (Fig. 3). Trichoepithelioma was divided into benign and malignant (Fig. 4) tumours based on lymphatic invasion at the periphery of the tumour. One of benign trichoepithelioma was a cystic variant, which had several cystic areas lined by multilayered epithelium that differentiated in a trichogenic fashion towards the luminal surface (Fig. 5). Among sebaceous and modified sebaceous gland tumours, both sebaceous adenomas and hepatoid gland adenomas consisted of small basophilic reserve cells at the periphery of the tumour lobules, which divided into mature sebocytes or hepatoid cells. In turn, epitheliomas in this group of tumours showed a preponderance of basaloid reserve cells with marked mitotic activity and scattered well-differentiated cells. Moreover, in this group of neoplasms, one case of hepatoid gland angioadenoma (Fig. 6) and two cases of hepatoid gland angioepithelioma (Fig. 7) were distinguished. The characteristics of them both were large glandular areas filled with blood, small vessels, or small amount of eosinophilic secretion. Histologically, apocrine adenocarcinomas showed the presence of tubular and cystic areas lined with single or multi-layered cuboidal cells with abundant granular eosinophilic cytoplasm and basally located large euchromatic nuclei. The lumen of glandular areas were filled with eosinophilic secretion or densely packed cluster of tumour cells (Fig. 8).
Fig. 1. Trichoblastoma ribbon type HE, 200x

Fig. 2. Trichoblastoma medusoid type HE, 100x

Fig. 3. Trichoblastoma mixed granular-medusoid type HE, 100x

Fig. 4. Malignant trichoepithelioma HE, 100x

Fig. 5. Cystic variant of trichoepithelioma HE, 100x

Fig. 6. Hepatoid gland angioadenoma HE, 100x
Fig. 7. Hepatoid gland angioepithelioma HE, 100x

Fig. 8. Apocrine adenocarcinoma HE, 100x

Fig. 9. Trichoblastoma medusoid type. Positive immunostaining for cytokeratin MNF116 (HRP/LSAB+ method), 200x

Fig. 10. Trichoblastoma ribbon type. Positive immunostaining for cytokeratin LP34 (HRP/LSAB+ method), 200x

Fig. 11. Hepatoid gland adenoma. Positive immunostaining of basaloid reserve cells for cytokeratin MNF116 (HRP/LSAB+ method), 200x

Fig. 12. Hepatoid gland adenoma. Positive immunostaining of hepatoid cells for cytokeratin LP34 (HRP/LSAB+ method), 200x
Table 1
Antibody source and dilution

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<tr>
<th>Antibody</th>
<th>Source</th>
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<td>Monoclonal Mouse antismooth muscle actin, clone 1A4</td>
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Table 2
Results of immunohistochemical staining of canine tumours with different antibodies

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<th>Tumour type</th>
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<th>LP34</th>
<th>VIM</th>
<th>DES</th>
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<th>S-100</th>
<th>NSE</th>
<th>GFAP</th>
<th>FVIII</th>
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<tr>
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* number of positive cases/ total number of cases analysed

Discussion

Histological examination showed that the diversity in canine adnexal tumours mainly resulted from the capacity of basal cells to multidirectional differentiation, even in the same type of tumour. Hepatoid gland angioadenoma and hepatoid gland angioepithelioma described by Sharif (22) were not classified in the current WHO classification. Despite this, these tumours were recorded earlier by some authors as adenomas with significantly extended vessels (14, 15). The main feature of these is the tendency to more frequent recurrence after their surgical removal. Maybe for this reason, these subtypes of hepatoid gland tumours should be included into the WHO classification similarly as newly classified sebaceous, meibomian, and hepatoid gland epithelioma separated from adenomas in this group (14).

This study revealed a strong positive expression of cytokeratins in all the examined tumours, which confirm their epithelial origin. The occurrence of LP34 positive reaction in neoplastic cells in sebaceous, perianal, and apocrine gland tumours, which mark beside cytokeratin 5 and 6 also cytokeratin 18 (basic cytokeratin of the gland and duct epithelium) may suggest their glandular origin (13). Moreover, the distribution and intensity of this immunoreactivity is the same as in normal glands (10, 24) and may suggest a common origin for these tumours (29). Despite immunopositivity of the LP34 antibody in the present study, complementary reactions performed by Walter (24) with LP34 (CK5/6/18), CAM5·2 (CK8/18), and Ks18·04 (CK18) showed that LP34 recognised only cytokeratin 6 both in healthy canine skin as well as in canine epithelial skin tumours. Moreover, using antibodies that mark only one subtype of cytokeratin is more useful for the examination of the reactivity and differentiation of epithelial cells (8). Therefore, a broad panel of antibodies against different cytokeratins is needed to evaluate canine adnexal tumours differentiation.

Coexpression of the intermediate filaments of VIM and cytokeratin by epithelial and mesenchymal tumours is frequently recorded. The observed incidental expression of VIM by differentiated perianal gland cells is not the first finding of coexpression of cytokeratin and VIM in epithelial cells. It was also reported by Vos et al. (29). Moreover, the coexpression of VIM and cytokeratin was detected in poorly-differentiated squamous cell carcinomas (6, 11, 17), haemangiosarcomas (4, 26), peripheral nerve tumours (1), and tumours of the synovium and mesothelium (16). Such coexpression is normal during embryonic and foetal development (16), but during carcinogenesis it could be the result of an abnormal protein processing (4). Some authors also believe that coexpression of VIM and cytokeratin is associated with cells with secretary functions (29), which is confirmed by the presence of efferent ducts and extracellular ducts in hepatoid gland (20, 21). In addition, the coexpression of cytokeratin and α-SMA in apocrine adenocarcinomas confirmed the presence of mioepithelial cells.

Of interest is the presence of strong, positive reaction for NSE in the examined follicular tumours. The expression of NSE may be interpreted as supporting the presence of Merkel cells inside those tumours, which is observed in humans (3). This can be confirmed by the fact that Merkel cells take part in the control of hair growth and their highest concentration is inside of hair bulge, where they join together with follicular stem cells by desmosomes (3, 25). According to a suggestion by Collina et al. (3) this reaction could also reflect the presence of reactive melanocytes in follicular tumours. Moreover, trichoblastoma and trichoepithelioma resemble morphologically both carcinoma basocellularae and Merkel cell tumours, which in both humans and dogs confirmed the expression of broad spectrum cytokeratin (MNF116), NSE, CK20, and chromogranin A (9). However, negative reaction for NSE, CK20 and chromogranin A in carcinoma basocellularae confirmed the lack of Merkel cells (19). Moreover, CK20 and NSE are claimed as highly specific for Merkel cells in contrast to chromogranin A (3).
Therefore, using both NSE and CK20 antibodies would be helpful in the correct classification of these tumours. Moreover, NSE expression can confirm that follicular tumours originate from follicular stem cells. Non-specific staining for NSE was observed in perianal gland tumours, which is possibly due to cross-reactions with other enolase isoenzymes (28).

GFAP is considered as a very specific marker of glial cells, Schwann cells, axons, and tumours derived from these cells (1, 2, 27). However, in our study GFAP was also detected in sebaceous adenomas, sebaceous epitheliomas, and perianal gland adenomas. Similarly unexpected expression of GFAP was found in basal cell carcinoma and sebaceous adenocarcinoma in cat (12). Probably this was a non-specific reaction related with other soft tissue sarcomas. Vet Pathol 2004, 41, 307-318.

It is not surprising to find immunopositivity for S100 in sebaceous adenomas on account of the detection of this protein in different canine and human tumours (2, 16-18, 23, 26). The positive staining for FVIII was restricted to endothelial cells and showed a prominent vasculature of all neoplasms. The lack of desmin immunoreactivity in tumour cells supports the failure to assess any lesion as exhibiting myogenic differentiation. In conclusion, the majority of canine adnexal tumours can be easily diagnosed after a conventional histological staining. For the evaluation of their histogenesis, cytokeratins, VIM, NSE, and α-SMA have a crucial role among used antibodies. However, for the examination of adnexal tumours differentiation, it is necessary to use antibodies, which recognise other types of cytokeratins, especially those specific only for a single subtype of cytokeratin.

References