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Histopathological Findings and Proliferative Activity of Canine Sebaceous Gland Tumours with a Predominant Reserve Cell Population

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Summary

Sebaceous gland tumours represent the third most common skin tumours in dogs, but diagnostic criteria for tumours with basal differentiation (i.e. sebaceous epithelioma) are poorly defined and there is lack of correlation with biological behaviour. The aim of this study was to identify the main histological criteria associated with malignancy in 30 canine sebaceous gland tumours with a predominant reserve cell population. For each case, tumour proliferative activity was assessed by determining mitotic index and the Ki67/MIB-1 index. Additional histological features included endophytic or exophytic growth, proportion of reserve/intermediate/mature cells, connection to the epidermis, nuclear characteristics, peripheral invasion, neoplastic emboli and necrosis. Mitotic and Ki67 indexes were variable, but correlated ($R = 0.66$; $P < 0.001$), and both were significantly higher in infiltrative tumours ($P = 0.018$ and $P < 0.001$, respectively). No significant difference in histological features was observed between tumours comprised of more or less than 90% reserve cells, nor among tumours showing proliferative activity in sebocytes. This study suggests that high proliferative activity and peripheral invasion should be considered the most significant parameters for the differentiation between benign and malignant sebaceous gland tumours.

Furthermore, the incidence of circumanal gland and testicular tumours in these dogs was significantly higher compared with an age-matched control population, suggesting a potential androgen-related pathway for the tumourigenesis of canine sebaceous gland neoplasms.

Keywords: dog; mitotic activity; sebaceous epithelioma; sebaceous tumours

Introduction

Sebaceous glands are distributed over the entire haired skin surface and account for the majority of hormone metabolism in the skin (Chen and Zouboulis, 2009). In dogs, sebaceous gland tumours represent the third most common type of skin tumour, accounting for 21–35% of all cutaneous epithelial tumours (Scott and Anderson, 1990; Vail and Withrow, 2007). These tumours are subclassified according to their histological appearance into four main types: adenoma, ductal adenoma, epithelioma and adenocarcinoma (Goldschmidt *et al.*, 1998).

Sebaceous adenomas are well-circumscribed nodules composed of multiple, large lobules of sebaceous cells showing normal maturation from peripheral basal reserve cells (usually limited to a single layer) to large, pale, lipid-laden central cells (representing the prevalent population) (Goldschmidt *et al.*, 1998).

Sebaceous ductal adenomas are composed of glandular lobules and ducts in varying proportions. Since both components, ductal and sebaceous, are always present, the term 'compound sebaceous adenoma' has been proposed (Gross *et al.*, 2005). Basal reserve cells and partially-lipidized cells are more numerous than mature sebocytes in some or all of the lobules (Goldschmidt *et al.*, 1998).

Sebaceous epitheliomas are predominantly composed of basal reserve cells, with smaller numbers

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of interspersed intermediate and mature sebocytes (Goldschmidt *et al.*, 1998). Sebaceous adenocarcinomas are poorly circumscribed nodules composed of irregular trabeculae of pleomorphic and atypical polygonal cells with variable degrees of cytoplasmic lipidization. These tumours typically lack significant numbers of reserve cells (Goldschmidt *et al.*, 1998).

Epitheliomas are among the most frequently diagnosed sebaceous neoplasms. These tumours may occasionally exhibit a locally aggressive behaviour and regional lymph node metastases or distant metastases are reported rarely (Scott and Anderson, 1990; Gross *et al.*, 2005; Bettini *et al.*, 2009). For this reason, Gross *et al.* (2005) proposed that sebaceous epitheliomas should be regarded separately from tumours similarly composed of a predominance of reserve cells, but showing increased nuclear size, higher mitotic activity and presence of mitoses in intermediate or fully-lipidized cells. The latter type of tumour was termed 'epitheliomatous sebaceous carcinoma' (Gross *et al.*, 2005).

Sebaceous tumours form a continuum and distinction between them is somewhat problematic, in particular within tumours with basaloid differentiation. Gross *et al.* (2005) proposed that epitheliomas should be defined by having approximately 90% or more reserve cells. Hence, sebaceous tumours where reserve cells prevail, but do not reach 90%, are variably diagnosed as epitheliomas, adenomas or compound adenomas, and their actual biological behaviour is unclear. Furthermore, the proposed category of epitheliomatous sebaceous carcinoma is poorly defined and there are no published data on its clinical implications.

The aims of this study were to characterize the clinicopathological features of canine sebaceous gland tumours with a predominant reserve cell population and to identify histological features associated with aggressive behaviour.

Materials and Methods

Case Selection

Surgical samples of canine sebaceous tumours submitted to the diagnostic laboratory of the Department of Veterinary Medical Sciences, University of Bologna, Italy, were reviewed for eligibility. Only tumours composed with a predominance (>50%) of basal reserve cells over fully lipidized or intermediate cells were included.

Histology

All specimens were fixed in 10% neutral buffered formalin and processed routinely. Sections were stained with haematoxylin and eosin (HE). For each case, the mitotic index was assessed by counting the total number of mitoses in 10 high-power (×400) fields, selected in the areas of highest mitotic activity. The number of mitoses occurring in each cell type (i.e. reserve cells, intermediate cells and fully lipidized cells) was recorded. According to Gross *et al.* (2005), cells characterized by moderate pale eosinophilic, granular or finely vacuolated cytoplasm were identified as intermediate cells. Intermediate cell nuclei were slightly larger than those of reserve cells and were not scalloped (Gross *et al.*, 2005).

The proportion of basaloid cells (i.e. greater or less than 90%) was assessed by consensus of two authors (PB, SS) evaluating the whole tissue section. A score was also assigned to the proportion of intermediate cells (1, absent or occasional; 2, <10%; 3, >10%). Other descriptive histological features evaluated included tumour growth (exophytic or endophytic), architectural arrangement, connection to the epidermis, nuclear characteristics, peripheral invasiveness, presence of neoplastic emboli and necrosis. Tumours growing above the skin surface were defined as exophytic, while tumours growing into dermis were classified as endophytic.

Ki67 Immunohistochemistry

Additional tumour sections were immunolabelled for Ki67 in order to assess proliferative activity by using a commercial anti-human antibody (MIB-1, mouse monoclonal, Dako, Glostrup, Denmark), with validated reactivity in canine tissues. Endogenous peroxidase activity was blocked by incubation for 30 min in H_2O_2 0.3% in methanol. Antigen retrieval was by microwave heating (four cycles of 5 min at 750 W) in citrate buffer (pH 6.0). Sections were incubated overnight at 4°C in a humid chamber with the primary antibody diluted 1 in 600 in a blocking solution (10% goat serum in phosphate buffered saline).

Sites of primary antibody binding were identified by incubation with biotinylated goat anti-mouse secondary antibody (1 in 200 dilution in blocking solution, Dako) for 30 min at room temperature. Slides were then incubated with avidin–biotin–peroxidase complex (Vectastain Elite ABC Kit, Vector Laboratories, Burlingame, California, USA) for 30 min at room temperature. 3,3′ diaminobenzidine was used as the chromogen. Sections were counterstained with Papanicolaou's haematoxylin and an additional eosin contrast was applied to aid the differentiation of lipidized cells from reserve cells. Sections of canine intestinal mucosa were used as positive controls. The basal layer of the epidermis served as an internal positive control. Negative controls were performed by substituting the primary antibody with an unrelated serum.