

Scientific Report

Immunohistochemical characterization of sebaceous epithelioma in two dogs

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Summary

This report describes two cases of sebaceous epithelioma and its immunohistochemical characterization with CK 14, CK18, p63, Ki67 and Bcl-2 immunostaining. Case 1 was a 12-year-old, intact English Cocker spaniel female presenting with multiple skin nodules over one year. Case 2 was a 7-year-old, spayed poodle female with a five-month history of solitary mass. Hematoxylin and eosin (H&E) staining showed that the nodules in both cases were irregular lobules comprised of epithelial cells around well-differentiated sebocytes. Neoplastic cells were positive for CK14 and p63 but were negative for CK18 cell marker. In addition, immunostaining for Ki67 proliferation marker showed 13.1% and 12.4% positive cells in the two cases, respectively. Furthermore, Bcl-2, which is highly expressed in human benign sebaceous tumors, was seen in basaloid cell nuclei and cytoplasm. CK14, CK18, p63, Ki67, and Bcl-2 antibody application provided further information for diagnosing sebaceous epithelioma and for prognosis in these two cases.

Key words: Bcl-2, Cytokeratin, Dog, Immunohistochemistry, Sebaceous epithelioma

Introduction

Sebaceous gland tumors can be divided into five main types: hyperplasia, adenoma, ductal adenoma, epithelioma, and carcinoma, according to their histopathological features and clinical presentation (Gross *et al.*, 2005). Sebaceous hyperplasia is the most common sebaceous tumor in dogs and is composed of proliferation of mature sebaceous lobules around one or more central squamous ducts. Sebaceous adenoma consists of lobules of normal mature sebocytes and fully-lipidized cells with few basaloid cells. Sebaceous ductal adenoma is characterized by multiple enlarged ducts with few sebocytes or basaloid cells. Sebaceous carcinoma, which presents with the most malignant features in sebaceous tumors, consists of poorly defined lobules of atypical neoplastic cells showing cytoplasmic lipidization reminiscent of sebaceous cells (Gross *et al.*, 2005; Bongiovanni *et al.*, 2012).

Sebaceous epithelioma is firm nodules that are either solitary or multiple. It is mostly seen in middle-aged or older dogs and often occurs on the head, ears, and dorsum. Breeds at higher risk for sebaceous epithelioma include Cocker spaniel, Lhasa Apso, Shi-tzu, Siberian husky, Irish setter, and Alaskan malamute (Gross *et al.*, 2005). Histopathological findings are characterized by moderate lobular irregularity, basaloid cell proliferation with few well-differentiated sebocytes surrounded by interlobular stroma (Gross *et al.*, 2005; Bettini *et al.*,

2009).

In the present report, two sebaceous epithelioma cases and their immunohistochemical characterization were investigated using anti-CK14, CK18, p63, Ki67, and Bcl-2 antibodies in order to determine whether those markers are useful for diagnosing sebaceous epithelioma and evaluating potential malignancy in dogs.

Case presentation

Case 1 was a 12-year-old, intact English Cocker spaniel female presenting with multiple skin nodules. Her medical history showed that an initial nodule was found on the ear pinnae, then multiple nodules formed on the trunk, neck, limbs, paw and face over the course of the next year. Nodules were moderate to firm in consistency, round to verrucous, and some were ulcerated (Fig. 1A). At the time of surgical excision, 22 nodules were present.

Case 2 was a 7-year-old, spayed poodle female with a five-month history of solitary mass in the hind limb. The mass was firm, round, and 1-2 cm in size (Fig. 1B). All nodules in both cases were surgically removed. Two nodules on the trunk and paw of case 1 and the one nodule in case 2 were subjected to histopathological analysis.

Tissue specimens were fixed in 10% neutral buffered formalin. Tissue sections about 4 mm were stained with hematoxylin and eosin (H&E). Immunohistochemical

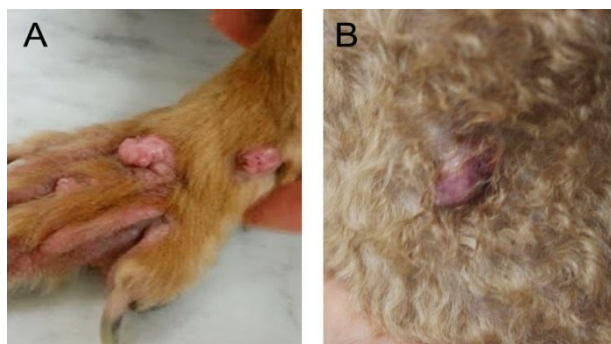


Fig. 1: Nodules in two dogs. A: Case 1, a Cocker spaniel, showed multiple and verrucous nodules in the paw. B: A solitary, round nodule was confirmed in case 2, a poodle

analysis was performed using antibodies for CK14, CK18, P63, Ki67, and Bcl-2. Antigen-antibody complexes were detected using the avidin-biotin complex procedure. Detailed immunohistochemistry antibody information is shown in Table 1. After immunoreaction, sections were colorized with 3-amino-9-ethylcarbazole and counterstained with Mayer's hematoxylin. In order to evaluate the proliferation index, Ki67 immunolabeling was quantified as the Ki67-labeling index (the number of positive cells per 1000 nuclei in neoplastic cells) in five high power fields (HPF). Histopathological analysis showed case 1 nodules to be composed of irregular lobules with few scattered mature sebocytes surrounded by interlobular stroma (Fig. 2A). In the lobules, the majority of cells resembled epithelial basaloid cells with scant eosinophilic cytoplasm, and the nuclei were round to ovoid and fairly uniform with one to three small nucleoli. Six to eight mitotic figures were seen per each HPF (Fig. 2A). Similar to the findings in case 1, case 2 H&E-stained sections also showed irregular trabecules infiltrated by epithelial basaloid cells with scattered mature sebocytes, surrounded by fibrous stroma (data not shown). Epithelial basal cells showed scant eosinophilic cytoplasm, as seen in case 1, and the nuclei were uniform and round to ovoid with one to two small nucleoli (data not shown). Mitotic figures were rare (0 to 2 per HPF). Squamous differentiation and keratinization were also noted in case 1 and case 2 sections. In order to confirm that the neoplastic cells originated from basaloid reserve cells of sebaceous glands, immunohistochemistry using CK14 and p63 sebocyte and basaloid cell markers in combination with CK18 luminal cell markers was performed. Basaloid and sebocytic cell cytoplasm showed strong positive CK14 expression (Fig. 2B), and basaloid cell nuclei were positive for p63, in both cases (Fig. 2C). CK18 was rarely stained in both cases (data not shown). In order to investigate malignant potential in these cases, immunohistochemistry for Ki67 and Bcl-2 was performed. Neoplastic cell nuclei in both cases were stained with anti-Ki67 antibody and the Ki67 index was 13.1%, and 12.4%, respectively. Furthermore, anti-apoptotic factor Bcl-2 was also strongly expressed in basaloid cell nuclei and cytoplasm in both cases (Fig. 2D). Based on these findings, both cases were diagnosed as canine sebaceous epithelioma. In order to determine if

metastatic lesions were present, thoracic and abdominal radiography was performed in both cases, but no active lesions were found.

Table 1: Detailed antibody information

Antigen/Antibody	Clone	Source	Working dilution
Cytokeratin 14	LL002	Abcam	1:100
P63	SC8431	Santa Cruz	1:100
Cytokeratin 18	C-04	Abcam	1:100
Ki67	MIB-1	Dako	1:100
Bcl-2	50E3	Cell signaling	1:50

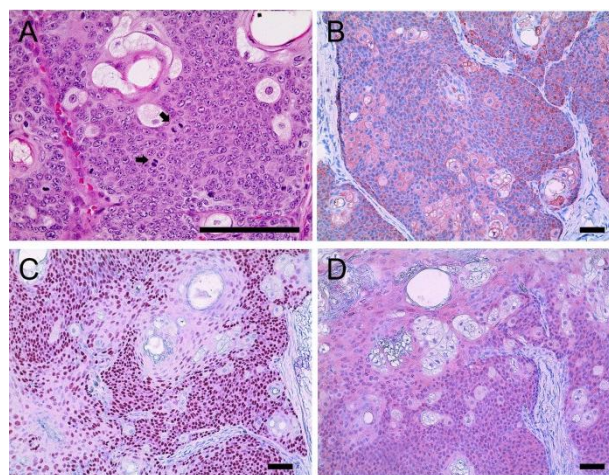


Fig. 2: Histopathologic findings in case 1. A: Nodules are comprised of irregular trabecules with few mature sebocytes, the majority of cells resemble epithelial basaloid cells with scant eosinophilic cytoplasm and the nuclei were ovoid and fairly uniform, with one to three small nucleoli. Mitotic figures (arrows) were frequently seen per high power field, hematoxylin and eosin staining. B: Cytoplasmic staining for CK 14 was largely observed in the basaloid cells. C: P63 was largely stain in the nuclei of the basaloid cells. D: Large amounts of cytoplasmic and nucleic staining for Bcl-2 anti apoptotic factor were noted in the basaloid cells of nodules, (Bar indicates 100 μ m)

Discussion

Sebaceous epithelioma is a common sebaceous tumor comprising 37.2% of all sebaceous tumors in dogs (Gross *et al.*, 2005). However, immunohistochemical characterization of sebaceous epithelioma has rarely been reported in dogs. This study investigated immunohistochemical characteristics in two sebaceous epithelioma cases using anti-CK14, CK18, p63, Ki67, and Bcl-2 antibodies.

Case 1 showed multiple nodules in a Cocker spaniel, a breed reported to have a high incidence of sebaceous epithelioma. Case 2, a poodle, presented with a single solitary nodule. Studies on the immunohistochemical findings of human sebaceous tumors suggested that anti-cytokeratin (CK) antibodies could be useful in diagnosing sebaceous tumors (Ansai *et al.*, 1994; Ansai *et al.*, 2011). Sebaceous carcinoma and sebaceoma (a benign sebaceous epithelioma) are positive for anti-CK5 and anti-CK14 antibodies and negative for anti-CK1, CK10, CK15, CK17, CK18, and CK20 antibodies (Ansai

et al., 1994). In dogs, a small number of case reports have described immunohistochemical characterization of sebaceous tumors. It has been reported that normal and neoplastic sebocytes are stained with CK14 but not CK18 or CK19 (Saraiva *et al.*, 2008; Yasuno *et al.*, 2011). Basal sebaceous gland reserve cells also stain positive for CK14 and p63 (Yasuno *et al.*, 2011). On the other hand, CK18 is considered a luminal epithelial marker and is expressed in normal and neoplastic apocrine sweat glands (Kato *et al.*, 2007; Yasuno *et al.*, 2011). Histological and immunohistochemical findings of nodules in the present cases were consistent with canine sebaceous epithelioma. Strong positive expression for CK14 and p63, and negative expression for CK18 were observed, supporting the premise that neoplastic cells originated from basaloid sebocytic cells not from apocrine luminal cells. Immunohistochemical examination for CK14 and p63 in combination with CK18 may be useful when diagnosing sebaceous epithelioma and excluding other adnexal luminal tumors.

Sebaceous epithelioma can be categorized as low-grade malignancy, and local recurrence can be seen after surgical excision (Gross *et al.*, 2005). However, sebaceous epithelioma is an occasionally aggressive tumor that metastasizes to regional lymph nodes and distant organs (Bettini *et al.*, 2009). In order to differentiate between malignant sebaceous carcinoma and benign sebaceous tumor in humans, proliferating cell nuclear antigen and Ki67 markers are used (Hasebe *et al.*, 1994; Cabral *et al.*, 2006). In these cases, no metastases to regional lymph nodes or distant organs were found. The Ki67 index in these two dogs were 13.1% and 12.4%, respectively, which are somewhat lower than those previously reported for sebaceous epithelioma with metastases in a canine and for sebaceous carcinoma in a human (Bettini *et al.*, 2009; Ansai *et al.*, 2011). This finding indicates that a low Ki67 index level might reflect non-metastasis and relatively good prognoses for the present cases. A large retrospective study to evaluate proliferation markers in sebaceous epithelioma would be useful to identify histological criteria for low- to high-grade differentiation and to evaluate patient prognosis.

Furthermore, anti-apoptotic factor Bcl-2 is rarely expressed in malignant sebaceous carcinoma and is also a useful marker for distinguishing between malignant and benign sebaceous tumors (Cabral *et al.*, 2006). Weak Bcl-2 expression was observed in sebaceous carcinoma, while benign sebaceous tumors highly express Bcl-2 (Ansai *et al.*, 2011). This suggests that anti-apoptotic Bcl-2 may reflect a lack of apoptotic sensitivity in malignant sebaceous tumor (Ansai *et al.*, 2011). In dogs, Bcl-2 expression in sebaceous tumors has not been investigated. In the present cases, similar to benign sebaceous tumor findings in humans, strong Bcl-2 staining was noted; suggesting that sebaceous epithelioma in dog also highly expresses the anti-apoptotic Bcl-2 factor. Further study to compare Bcl-2 expression in sebaceous epithelioma and malignant sebaceous carcinoma will provide information on the role of Bcl-2 in determining malignancy in dog sebaceous tumors.

In this report, we diagnosed sebaceous epithelioma in Cocker spaniel and poodle by clinical and histopathological findings. The neoplastic basaloid cells of sebaceous epithelioma were positive for CK14, p63, Ki67, and Bcl-2, as shown by immunohistochemistry. Application of anti-CK14, CK18, p63, Ki67 and Bcl-2 antibodies in the present cases provided further information for diagnosing sebaceous epithelioma and determining prognosis. A further large-scale study investigating these markers in sebaceous tumors would be helpful for diagnosing sebaceous tumor and distinguishing between benign and malignant sebaceous tumors.

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