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CD34 Immunoexpression in Canine Skin Follicular Tumors and Basal Cell Carcinomas: Case Series Report

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Abstract

Back ground: Basal cell carcinomas and follicular tumors are relatively common skin neoplasms both in veterinary and human medicine. Currently, it is believed that stem cells are important for skin tumor development.

Hypothesis/objective: the objective of the study was to characterize CD34 expression in canine follicular tumors and basal cell carcinoma in order to investigate the possible role of these cells in skin tumorigenesis.

Animals & Methods: eleven skin tumors including basal cell carcinomas and follicular tumors were submitted to immunohistochemistry using the stem cell marker CD34.

Results: CD34 labelling was characterized by a fine, granular and diffuses brown cytoplasmic staining both in control (endothelial cells) and neoplastic cells. Two BCC and one trichoepithelioma displayed positive neoplastic cells and/or fibroblast-like stromal cells.

Conclusion: preliminary results described here should be confirmed by large scale studies in order to clarify the potential role of stem cells on human and canine skin tumors.

Keywords: Mammary Carcinoma; Diagnosis; Histopathology; Dog

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Introduction

Skin cancer is the most common neoplastic disease among humans accordingly to World Health Organization; interestingly, dogs have a similar tumor profile [1]. Despite this high incidence, the exact nature of such tumors has been a source of controversy for many decades [2,3].

Primary hair follicle tumors accounts for up to 5% of skin neoplasms in dogs. The great majority is benign but a small percentage of these tumors may behave in a malignant fashion [4]. Some authors

categorize these tumors in trichofolliculomas, trichoblastomas, tricholemmomas, infundibular keratinizing acanthoma (IKA), and benign and malignant trichoepithelioma and pilomatricoma [1].

Basal Cell Carcinoma (BCC) is a highly aggressive human and canine skin tumor that appears to originate from the lower layer of the epidermis. There are several architectural patterns including nodular, micronodular, superficial, infiltrative, solid, clear cell and keratotic (keratinizing basal cell carcinoma, KBCC) [1].

Case number	Breed	Sex	Age (years)	Tumor type	CD34 expression (neoplastic cells/stromal cells)
1	Mixed	Female	11	BCC, solid	-/-
2	Poodle	Male	9	BCC, solid	+/-
3	Mixed	Male	12	Tricholemmoma, isthmic	+/-
4	Poodle	Female	6	BCC, solid	+/-
5	Dachshund	Female	10	BCC, solid	-/-
6	Poodle	Female	12	KBCC	-/-
7	Siberian Husky	Male	2	Trichoblastoma, ribbon type	+/-
8	Cocker Spaniel	Female	6	Trichoblastoma, ribbon type	NA
9	Boxer	Female	8	Trichoepithelioma	+++
10	Poodle	Female	12	Pilomatricoma	-/-
11	Mixed	Male	16	Trichoepithelioma	-/-

BCC (basal cell carcinoma); KBCC (keratinizing basal cell carcinoma); NA (not available).

Table 1: Patient data and CD34 immunopexpression in canine follicular tumors and basal cell carcinomas.

Antibody	Clone	Clonality	Source
CD34	C-18	Goat polyclonal	Santa Cruz Biotechnology® (code SC-7045)

Table 2: List of primary antibodies

The cellular regeneration of the skin is maintained by stem/progenitor cell subpopulations. Follicular stem cells are located in a specific area called “bulge region” which originates epidermis, hair follicles, sebaceous and apocrine glands [5].

Basal cell tumors may arise from basal epidermal cells, hair matrix cells, or stem cells in the bulge region or outer root sheath of the hair follicle [6]. Stem cell immunomarkers have shown that human follicular tumors and basal cell carcinomas may have a common cellular origin and represent different stages of differentiation [2,5,7,8]. In a letter to the editor we summarize application of stem cell markers in canine skin neoplasms including nestin, CD34, CK15, BLIMP-1, p63, CK19, and CD200 [9].

In the present study we aimed to characterize CD34 immunohistochemical expression in canine follicular tumors and basal cell carcinoma.

Materials and Methods

Tumor samples

Eleven tumors from paraffin-block archives from Department of Pathology (University of Sao Paulo) and from Laboratory of Investigativ and Comparative Pathology (Univ. Estadual Paulista) [Table 1] were stained with hematoxylin and eosin (H&E), reviewed and classified accordingly to published data [1].

Immunohistochemistry

Tissue sections were deparaffinized in xylene and rehydrated in graded ethanol to water. Following deparaffinization, sections were immersed in an antigen retrieval solution (citrate buffer pH 6.0), heated to 125°C for 30 s followed by 90°C for 30 s in a pressure chamber (Pascal®, Dako Cytomation), cooled

to room temperature for 20 min and placed in a water bath for 5 min. Further, tissue sections were submitted to a blocking step (vial 1, Envision G2 System/AP®) for 15 min and incubated with CD34 primary antibody [Table 2] overnight at 4°C in a humidified chamber at 1:2000 dilution, respectively. CD34 labeling was detected by incubating slides with polyclonal rabbit anti-goat biotinylated immunoglobulins (Dako®) in a 1:400 dilution for 30 min; after, slides were incubated with HRP/streptavidin complex (red vial, Novolink®, Novocastra Laboratories, Newcastle, England). Reaction were visualized by incubating samples with 3,3 diaminobenzidine (DAB) chromogen for 3 min. All sections were counterstained with Harris’s hematoxylin for 3 min followed by a water bath for 5 min and mounted with a permanent mounting media (Permount Fischer®). Negative controls substituted the primary antibody with antibody diluent (Novocastra®). All washes between reagents were performed with TRIS pH 7.4. Slides with excessive background and/or non-specific labeling in negative controls were excluded. The immunolabelling intensity (0=no labeling to +3=strong labeling), location and distribution were analyzed in neoplastic and non-neoplastic stromal cells.

Results

Expression of CD34 in canine basal cell carcinomas and follicular tumors

CD34 antibody presented heterogeneous results in follicular tumors and basal cell carcinomas. Labeling pattern was characterized by a fine, granular and diffuses brown cytoplasmic staining. All internal controls represented by endothelial cells in all samples were positive, except for case 8 in which there was no immunolabelling of endothelial cells. Two BBC, one KBCC, one trichoepithelioma and one pilomatricoma

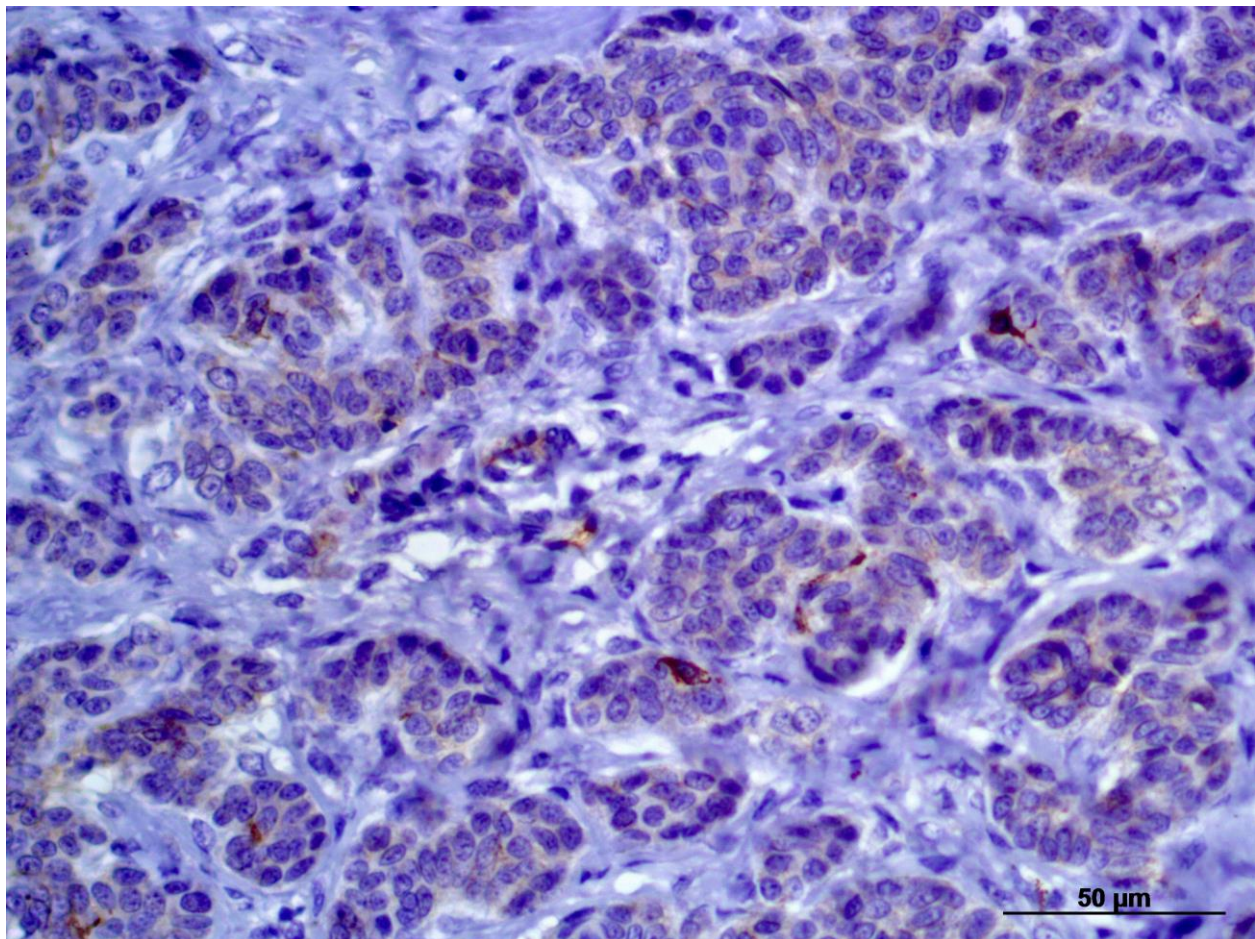


Figure 1: CD34 expression in trichoepithelioma. Note the fine, granular and diffuse brown cytoplasmic staining in neoplastic cells and fibroblast-like stromal cells 400x, 3,3` diaminobenzidine (DAB).

were negative; two BCCs cases displayed weakly stained neoplastic cells; one trichoblastoma case and one isthmic tricholemmoma case displayed weakly positive neoplastic cells. One trichoepithelioma displayed moderately stained neoplastic and clusters of fibroblast-like stromal cells [Figure 1]. Two cases 5 and 11 displayed positive nuclei. In addition, samples displayed moderately positive sebaceous and apocrine sweat glands, and also cells from outer root sheath from isthmic region.

Discussion

In this study we evaluate CD34 immunoexpression in four basal cell carcinomas (BCCs), one keratinizing basal cell carcinoma (KBCC), one isthmic tricholemmoma, two trichoblastomas, two trichoepitheliomas and one pilomatricoma.

We found a positive CD34 immunolabelling in 50% (n=10) ranging from neoplastic cells to stromal cells. One case was excluded from analysis due to lack of a positive internal control (CD34, case 8).

CD34 also known as human progenitor cell antigen is selectively expressed in hematopoietic progenitor cells and endothelial cells [10]. Using immunohistochemical and *in situ* hybridization methods, authors demonstrated that CD34 expression is restricted to basal keratinocytes from outer root sheath from isthmic region of primary follicles during all stages of follicular cycle [11]. In dermatopathology, CD34 had been used in several contexts including differentiation between BCC, trichoepitheliomas and trichofolliculomas with variable results [12-20].

The possible relationship between CD34 expression and stem cell origin of human BCC and follicular tumors was addressed by authors who states that different types of stem cells exhibiting distinct phenotypic features can give rise to different skin neoplasms [5]. In mice, CD34-positive keratinocytes are located in the bulge region; but in humans, they are located in fully differentiated keratinocytes of the follicular outer root sheath below the isthmus [21]. Thus, CD34 immunoexpression in human skin neoplasms is not related to stem cell capacity, but with degree of cellular differentiation [5].

Studies involving follicular and interfollicular keratinocytes in colony-forming assays in a canine model suggest that CD34 could be used to sort highly proliferative follicular keratinocytes [22]. In our study, we demonstrated the presence of CD34⁺/nestin⁻ neoplastic cells in one isthmic tricholemmoma, one BCC, one trichoblastoma and one trichoepithelioma.

Conclusion

Actually, the role of CD34 in BCC and follicular tumors remains largely unknown in humans and dogs. Large scale studies are needed in order to address significant conclusions regarding CD34 participation in canine and human skin tumors.

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Conflict of interests

No conflicts of interest have been declared

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