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Cellular and biological evaluation and diagnostic immunohistochemistry of cytokeratin 15/19 expression in distinguishing cutaneous basal cell carcinoma

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ABSTRACT

Recent studies have investigated the expression of proliferative markers, but little is known about the expression of cytokeratin 15 and 19 in different histological subtypes of basal cell carcinoma (BCC). We conducted cellular, biological, clinicopathological and immunohistochemical analysis on the manifestations of 8 BCC by hematoxylin and eosin stain (H&E) staining and immunohistochemistry and reviewed relevant literature. Microscopically, the tumor cells were multiple remarkable foci of epidermolytic hyperkeratosis with large pleomorphic nuclei and scant cytoplasm together with peripheral palisading and forming solid nests. Furthermore, the most tumors were composed of highly cellular areas with a homogenous population of round, ovoid and spindle cells, hyperchromatic nuclei, high cellular pleomorphism, high mitotic index and various morphologic patterns. Moreover, the tumors displayed an invasive growth, with positive expression of Cytokeratin 19 (CK19) and negative expression of CK15. Our study revealed that the expression of CK19 was associated with progression and invasion in cases with BCC and immunohistochemistry is indispensable in distinguishing this tumor from other types of cutaneous carcinoma. To our best knowledge, it may be a considerable biomarker to assess invasiveness of cutaneous-surface BCC and to guide clinical management of such tumors.

1. Introduction

Cutaneous neoplasms with ballooning degeneration of neoplastic cells are uncommon in human and animals. They include balloon cell melanoma, balloon cell nevus, clear cell basal cell carcinoma, and clear cell acanthoma [1–3]. Basal cell carcinoma (BCC) is unusual in dogs, usual in the cat, and rare or unknown in other species [4]. In dogs, accounting for less than 0.3% of cutaneous neoplasms, and its etiology remains unknown [5,6]. In contrast, human BCC is the most common skin malignancy of the Caucasian population, having a significantly increased prevalence over recent decades [7]. BCC is low-grade malignant epithelial tumors that show no epithelial or adnexal differentiation. Furthermore, BCC in canine, cats and human is currently defined as a low-grade tumor arising from the basal cells of either the interfollicular epidermis or the hair follicles and may represent a neoplastic transformation of stem cells [6–8] and or it arises from the basal cells of the epidermis and pilosebaceous units. In human beings, BCC is the most common malignant tumor, with increasing outbreak [9,10]. Histologically, tumor cells resemble basal cells, are intensely stained with hematoxylin, and have scant cytoplasm. Basal cell carcinomas do not metastasize but show extensive local invasion.

The etiology of BCC is still obscure but appears to be of multifactorial origin. UV radiation (UVR), and especially UVB, is

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responsible for the feck of skin damage and is believed to be the primary established risk factor in the advancement of BCC [11,12]. Other extrinsic risk factors, beyond UVR, predisposing to BCC, include ingestion of arsenic acid (medicine, pesticides), ionizing radiation, X-ray and grenz-ray exposure, topical nitrogen mustard administration and thermal burns. Constitutional factors include gender, age, immunosuppression and genetic predisposition (a family history of BCC, genetically inherited nucleotide excision repair [NER] defects such as xeroderma pigmentosum [XP]). And also, pigmentary traits, such as fair cutaneous, blond or red hair, light eye color, tendency to sunburn and poor tanning ability (skin Type I), have all been associated with a higher risk of BCC [13].

The correct diagnosis between these tumors is very significant because BCC is a locally aggressive neoplasm and must be totally excised with safe margins [14]. Diagnosis of BCC can be easily made by histopathological investigations, however sometimes errors occur, particularly when trying to differentiate BCC from certain squamoubasal cell carcinoma, trichoepithelioma, desmoplastic epithelioma, metatypical carcinoma. In such cases immunohistochemical stains are utilized. Immunohistochemistry (IHC) combines anatomical, immunological and biochemical techniques for the identification of specific tissue components by means of a specific antigen/antibody reaction tagged with a visible label. IHC makes it possible to visualize the distribution and localization of specific cellular components within a cell or tissue [4]. CK are intermediate filaments characteristically found in epithelial cells and their tumors. CK comprised a family of at least 20 different polypeptides, numbered consecutively from 1 to 20, according to differences in molecular weight and isoelectric pH [15]. Most importantly, determination of the CK profile of a particular carcinoma is useful in the differential diagnosis of carcinomas; studies have shown that these tumors tend to recapitulate the CK profiles of tissues from which they arise [15–17]. CK typing, therefore, can be of practical importance in analyzing tumors presenting as metastatic disease from clinically unknown primary sites of origin.

Immunohistochemistry and light microscopy are often required to definitively categorize these tumors. The aim of this paper is to characterize histologically and immunohistochemically a type of cutaneous carcinoma unique to the dog.

2. Materials and methods

2.1. Animal welfare

This study was approved by the Animal Ethics Committee at the Private Veterinary Clinic (Ethics code permit no. TU2013-1-11-007Y). The animals were placed in shade, in standard conditions, water ad libitum, and without restriction of movement according to the guidelines of the Institutional Animal Ethical Committee of the Private Veterinary Clinic. Surgeries were performed under aseptic conditions and sedation by injection of xylazine hydrochloride (0.05 mg/kg) followed by 2% lignocaine hydrochloride.

2.2. History of patients

In December 2013 to September 2014, 8 cases with the age range 4–9 years-old, and weight 8–12 kg, both sex, and different breeds were referred to the veterinary clinic with a history of cutaneous lesions on the ventrolateral and dorsolateral abdominal, thorax and facial area, and these included alopecia, erythema, erosions, ulcers, crusts, thickening and wrinkling of the cutaneous, each with a diameter of 1.5–2.5 cm, and these enlarged progressively and became ulcerated. Furthermore, these lesions were neglected by the dogs and therefore progressed forming indurated tumor lesions, partly ulcerated and covered by an adherent crust on the surface. Moreover, the dogs lived outdoors, spending much time lying in lateral recumbency in the sun.

2.3. Hematological profile

The blood cell count was undertaken manually and calcium and phosphorus concentration were measured by commercial kits (Pars Azmoon, Alborz, Iran) using a semi-automatic analyzer (EMP 168 Vet Biochemical analyzer; Shenzhen Emperor Electronic technology Co. Ltd, Shenzhen, China). Hematological and biochemical profiles (including blood cell count and serum calcium and phosphorus concentrations) were within normal ranges.

2.4. Surgical and pathological analysis

For partial excisional biopsy, an intravenous combination of diazepam (0.27 mg/kg) (Tamin, Tehran, Iran) and ketamine hydrochloride (5.50 mg/kg) (Alfasan, Woerden, the Netherlands) were used for induction and maintenance of anesthesia and the dogs received normal saline solution 0.9% intravenously, to a total of 0.5 L (12 mL/kg/h) (Samen, Mashhad, Iran). Atropine sulfate (0.03 mg/kg) (Tamin, Tehran, Iran) was given subcutaneously as pre-medication. The cutaneous lesions were removed surgically and processed routinely. The samples were fixed in formalin and embedded in paraffin for sectioning. The sections were stained with hematoxylin and eosin.

2.5. Immunohistochemical analysis

For further study, paraffin sections were stained immunohistochemically with cytokeratin 15 and 19 markers (Abcam Co., Cambridge, USA). One formalin-fixed, paraffin wax-embedded section of each lesion was stained with H&E. Tumor was classified independently by two pathologists in order to confirm the diagnosis according to the WHO criteria [18].

3. Results

3.1. Histopathological findings

Microscopically, the tumors' growth, which was composed of cells resembling basal cells, was observed in the dermis and subcutis. The histopathological examination of multiple masses of the cutaneous indicated a solid and sheet-like proliferation of relatively non-uniform and small to large-sized atypical epithelial cells having hyperchromatic nuclei and scant eosinophilic cytoplasm (Fig. 1A and B) and supported by a fibrous stalk together with epidermolytic hyperkeratosis. The palisading basaloid neoplastic cells were characterized by moderate to high mitotic activity (Fig. 1C and D). In some area, microscopically, tumors issue showed the presence of groups of irregular cells with cytological irregularities and acidophilic cytoplasm. Moreover, histologically, the cutaneous tumors were well-demarcated with densely cellular. Neoplastic cells varied in size, and ranged in shape from round to polygonal to spindle-shaped (Fig. 1C and D). Moderate to abundant amounts of pale eosinophilic cytoplasm were present along with a large nucleus and prominent nucleolus and also, cells showed predominantly atypical morphologies with a high mitotic index (Fig. 1A and B).

In some area, tumors cell nests consisted mainly of basaloid cells with hyperchromatic nuclei and little cytoplasm together with solid sheets of tumor cells were interspersed with cribriform areas composed of spaces lined by a single layer of cuboidal to spindle cells morphologically distinct from surrounding basaloid cells (Fig. 1C and D). Furthermore, the stroma was infiltrated by

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