Original article

Diagnostic Value of Fluorescence Method on Melanoma in Dogs

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Abstract

A malignant melanoma, a giant pigmented tumor of a 10-year-old, male mixed-breed dog at the oral mucosa, and a benign melanoma, a black macule of a 14-year-old, female Shetland sheepdog at the pinna, were investigated. Histopathologic examinations revealed severe proliferation of the pigmented cells in both cases. Fluorescence method of formalin-fixed paraffin-embedded unstained specimens (fluorescence method) was performed, and some pigmented cells showed specific fluorescence in the malignant melanoma, but not in the benign melanoma. The pigmented cells of the pigmented oral mucous in the malignant case were also examined by the fluorescence method, but failed to show any specific fluorescence. It was demonstrated that pigmented cells in canine malignant melanoma showed specific fluorescence as well as those in the case of humans, which indicated that the specific fluorescence of melanocytes was associated with malignant proliferative properties.

Key words: Malignant Melanoma, Melanocytoma, Skin neoplasia

INTRODUCTION

Melanoma is a proliferative disorder of melanocytes, and is one of the most hazardous cutaneous malignancies in humans¹²⁾. Melanoma is generally diagnosed with clinical and histopathologic findings, but can be evaluated by biological behavior of the cells for a definitive diagnosis in some cases¹²⁾. Melanogenic activity of these pigmented cells has been demonstrated by means of the fluorescence method using formalin-fixed paraffin-embedded unstained specimens

in humans⁷⁾. The fluorescence method has also proven to be valuable for differentiating malignant melanomas from other pigmented tumors⁷⁾. In dogs, the morphologic features of melanoma are similar to those in humans, but most cases are benign⁸⁾. In the veterinary nomenclature system, it has been proposed that all non-congenital, benign proliferations of melanocytes are to be designated melanocytoma, and the term melanoma is used as synonymous with a malignant proliferation of melanocytes^{14, 15)}. It seems that the function of the canine melanocytes is not synergistic with the morphology in some cases, and it is not always easy to predict the biological behavior. To resolve these discrepancies between the morphology and biologic behavior, in the present

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study the pigmented cells of benign and malignant melanoma diagnosed clinically and histopathologically were investigated by the fluorescence method.

CASES

Case 1

A 10-year-old, intact male mixed-breed dog presented with a 2-month history of sore. A giant, irregularly shaped, and pigmented tumor, over $40 \times 50 \times 100$ mm in size, was recognized around the middle pharynx based on the palatum molle (Fig. 1A). X-ray examination did not reveal metastatic lesions in any thoracic or abdominal organs. The elevated portion of the tumor and the pigmented oral mucous membrane (Fig. 2A) were excised, and a histopathologic examination was performed. The tumor widely invaded the dermis, and it was composed of nests of atypical epithelioid melanocytes, surrounded by spindle melanocytes (Fig. 1B). A marked regional variation in the amount of pigment was observed, and Masson-Fontana stain revealed abundant melanin granules in most of the cells. The nuclei were spherical or ovoid, and contained dispersed chromatin. Mitotic figures were occasionally observed. The pigmented oral mucosa did not show any abnormalities, except hyperpigmentation in the basal layer of the epithelium (Fig. 2B), where Masson-Fontana stain revealed melanin granules.

Case 2

A 14-year-old, intact female Shetland sheepdog presented a mildly elevated pigmented maculae, approximately 5 mm in diameter, on the inside of the pinna (Fig. 3A). Owner had noted the lesions recently. The nodule was excised, and a histopathologic examination revealed a symmetric, wedge-shaped configuration composed of nests and clusters of heavily pigmented, epithelioid melanocytes localized to the superficial dermis and the epidermis (Fig. 3B). Masson-Fontana stain revealed abundant melanin granules in the epithelioid cells. Mitotic figures were rare, and mitotic atypia was not observed.

Fluorescence method

Biopsy specimens from the lesion were fixed in 10 percent

Fig. 1. Malignant melanoma in Case 1. A large, irregularly shaped, pigmented tumor around the middle pharynx (A). Nests of atypical epithelioid melanocytes surrounded by spindle melanocytes (B) (H & E, $\times 1765$). Intense green specific fluorescence in a number of atypical tumor cells (C) (Fluorescence method, $\times 1765$).

neutral-buffered formalin, embedded in paraffin, then cut into $3 \mu\text{m}$ sections. The tissue sections were deparaffinized with xylorol and enclosed with mineral oil. Unstained specimens were examined under a fluorescence microscope (Olympus IMT2-RFL) with light (USH 102D, Ushio Electric Co.), mirror (BP490, DM500, AFC+O515), and filter (515W)⁷.

A number of atypical tumor cells of Case 1 showed an intense green specific fluorescence, while the others exhibited weak or no fluorescence (Fig. 1C). The pigmented mucus membrane of Case 1 and the pigmented cells of Case

Fig. 2. The pigmented oral mucosa of Case 1 (A). Normal epithelium, except hyperpigmentation at the basal layer of the epithelium (B) (H & E, $\times 1765$). Pigmented cells with no specific fluorescence (C) (Fluorescence method, $\times 1765$).

2 did not show specific fluorescence (Figs. 2C and 3C).

DISCUSSION

In dogs, it is difficult to predict the biological behavior of a melanoma arising in the haired skin unless at the time of clinical evaluation there is already evidence of metastasis to

Fig. 3. Benign melanoma in Case 2. A mildly elevated and pigmented macule on the inside of the pinna (A). Lesion is composed of heavily pigmented, epithelioid melanocytes arranged in nests and clusters at the upper dermis and epidermis (B) (H & E, $\times 1765$). Pigmented cells with no specific fluorescence (C) (Fluorescence method, $\times 1765$).

the lymph nodes and/or lungs⁸). Histopathologically, malignant melanoma is characterized by atypical melanocytes in sheets, packets, and cords, and mitotic

activity is the primary feature used to differentiate between benign and malignant melanocytic proliferative disorder of the skin. When the tumor has more than 3 mitotic figures/10 high-power fields, it should be diagnosed as malignant³⁾. However, it is considered that all melanomas arising from the mucous membrane of the lip, mucous membranes at other mucocutaneous junctions, and in the subungual region are malignant^{1, 8)}. In this study, biopsy specimens of classic malignant and benign melanocytic disorder of dogs were used to evaluate the fluorescence method, and specific fluorescence was found to exist in the cells of malignant melanoma. It was suspected that this finding was associated with malignant behavior, suggesting that the fluorescence method might be valuable in distinguishing malignant melanoma from benign pigmented disorders.

The fluorescence method has been widely used as a highly sensitive and specific method for the histochemical demonstration of catecholamines and 5-hydroxytryptamine and their immediate precursors, DOPA and 5-hydroxytryptophan, at the cellular level^{4, 5, 9)}. As a sensitive tool for the demonstration of DOPA containing compounds, this method is useful in human dermatology for examining melanocytes, nevus cells, and melanoma cells^{10, 11)}. As fluorogenic catechols, two main catechol amino acids, DOPA and 5-S-cysteinyldopa (5-S-CD), have been identified in melanoma, although normal human skin contains 5-S-CD which was originally described as a precursor in the formation of pheomelanin^{2, 6)}. At present, 5-S-CD is believed that these substances are formed and excreted by active melanocytes in humans, and is found only in the cytoplasm and nucleolus of the melanin-producing cells by this fluorescence method^{7, 13)}.

It was demonstrated that pigmented cells in canine malignant melanoma showed specific fluorescence as do those of humans, and specific fluorescence of the melanocytes was clearly associated with malignant proliferative properties. Since the same enzymes in the malignant pigmented cells could be associated with their behavior, fluorescence method appeared to be a simple and valuable means to demonstrate the melanogenic activities in melanoma. Further investigation is needed on understanding of biological behavior of melanocytes and

factors associated with malignancy in humans and dogs.

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犬メラノーマにおける蛍光法の診断的意義

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要約: 10歳齢の雑種犬雄から採取し悪性メラノーマと診断した巨大な口腔内黒色腫瘍と, 14歳齢のシェットランド・シープドッグ雌から採取し良性メラノーマと診断した耳介内側の皮膚黒色斑を用いて蛍光法の診断的意義を検討した。組織学的にマッソンフォンタナ染色陽性色素細胞の増殖が認められたこれら検体のホルマリン固定パラフィン標本における蛍光所見を検討したところ, 悪性例では色素細胞の一部で蛍光が認められたが, 良性例では蛍光を認めなかった。また悪性例の健常黒色口腔粘膜も検討したが色素細胞に蛍光は認められなかった。以上より, 犬の悪性メラノーマにおいて色素細胞が人と同様に蛍光陽性所見を示す例があることが確認され, この蛍光陽性細胞の存在が悪性増殖の示標となることが明らかになった。

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