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CASE REPORT

Nailbed malignant melanoma in three dogs

Hyoung-Nam Jo[1,†], Myeong-Won Suh[1,†], Joo-Ok Lee[2], Jae-Hoon Kim[1,*]

[1]College of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, Jeju 63243, Korea
[2]Ekklim Anima Hospital, Daegu 41165, Korea

Abstract: Three 8-12-year-old male cocker spaniels presented with an appendicular mass accompanied by pain, inflammation, lameness, and loss of nail in the digits of the forelimb or hindlimb. A histological examination revealed dermal masses of the digit composed of numerous neoplastic cells with marked pleomorphism and high mitosis. The neoplastic cells showed a strong invasive tendency into the epidermis and adjacent bony tissues, such as distal phalanx. Immunohistochemistry of the neoplastic cells in three masses revealed positive reactions for Melan-A. These three dogs were diagnosed as nailbed malignant melanoma in the digit based on the history, clinical signs, and histopathologic features.

Keywords: digit, distal phalanx, dog, Melan-A, nailbed malignant melanoma

*Corresponding author

Jae-Hoon Kim
College of Veterinary Medicine and Veterinary Medical Research Institute, Jeju National University, Jeju 63243, Korea

Tel: +82-64-754-3387
Fax: +82-64-702-9920
E-mail: kimjhoon@jejunu.ac.kr

†The first two authors contributed equally to this work.

ORCID
Jae-Hoon Kim
https://orcid.org/0000-0002-4410-9126

Conflict of Interest
The authors declare no conflicts of interest.

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Melanoma is a malignant tumor arising from melanocytes, which produce melanin and are derived from neuroectodermal melanoblasts in the epidermis or adnexa structures, but primarily from the external root sheath of the hair follicle [1]. Malignant melanoma is common in dogs but uncommon in other domestic animals [1]. Canine melanocytic tumors occur most frequently in the oral cavity and the haired skin, or around the lips [1,2]. In dogs, this tumor accounts for 3% of all neoplasm and up to 7% of all malignant tumors [2].

Nailbed (human term 'subungual') malignant melanoma is derived from the melanocytes of the nailbed epithelium [1]. Nailbed malignant melanoma is common only in dogs and accounts for approximately 8% of malignant melanoma cases [1]. The digits affected by subungual melanoma often have deformed nails, growth of a mass from the nailbed, or paronychia [1,2].

Several cases of malignant melanoma have been reported in the oral cavity, limbs, and digits of dogs in Korea [3-5]. Only two cases of malignant melanoma on the digit of the forelimb and lower limb in dogs have been described in Korea [3,5], but an immunohistochemical diagnosis was not applied in those cases. This report describes the histopathological and immunohistochemical features in three cases of nailbed malignant melanoma in the digits of dogs in Korea.

The first dog (case one) presented with an abnormal digital swelling mass and pain, and the second dog (case two) had a rapid grown digital mass near nail and lameness (Table 1). After being bitten by another dog one month earlier, an abnormal mass appeared in that digit of the second dog. The third dog (case 3, Fig. 1) presented to the animal hospital with a four-month history of swelling, inflammation, and loss of nail in the 4th digit of the right forelimb. No previous trauma to the digit could be recalled in case one and three. Abnormal growth of the mass without any significant changes to the distal phalange at the affected digits was observed in these dogs using a radiographic examination according to field clinicians (Fig. 2A). No recurrence of neoplasm and metastasis was observed eight months after surgery in case 3 (Fig. 2B). Unfortunately, follow-up monitoring could not be performed in the other two dogs.

The masses, with/without distal and middle phalanges in the digits of dogs, were excised completely under local anesthesia. After surgery, the masses were fixed in 10% buffered formalin and submitted to the pathology labora-

Table 1. Clinical data of canine nailbed malignant melanomas

No. of dog	Breed	Age (yr)	Sex	Body weight (kg)	Location
1	Cocker spaniel	8	Castrated M	13	2nd digit of right forelimb
2	Cocker spaniel	12	Castrated M	Unknown	5th digit of right hindlimb
7	Cocker spaniel	12	M	13	4th digit of right forelimb

M, male.

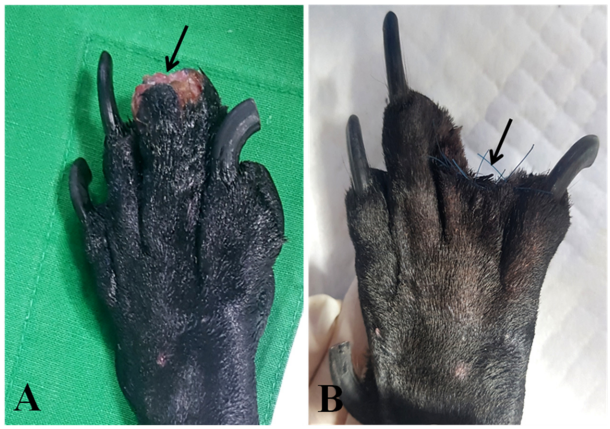

Fig. 1. Severe irregular swelling mass with a pale red color, ulceration, and loss of nail (arrow) in the 4th digit of the right forelimb (A) Case 3. Surgical biopsy including the 3rd and 2nd phalanx was performed (B).

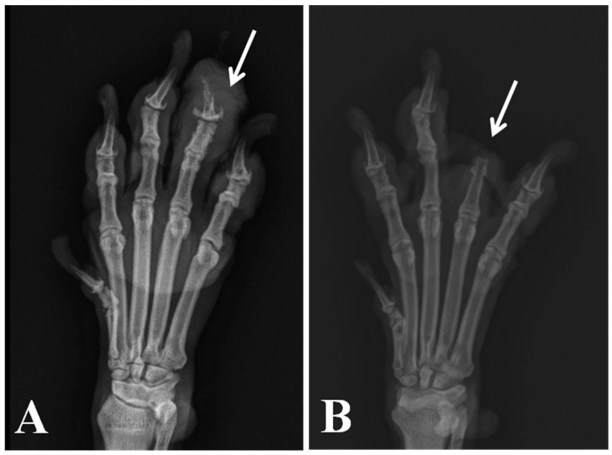

Fig. 2. Radiographs showed abnormal cutaneous mass (arrow) in the 4th digit of the right forelimb two months before the surgical excision (A) Case 3. No recurrence of the neoplasm was observed in the amputated digit eight months after surgery (B).

tory at the College of Veterinary Medicine in Jeju National University. After decalcification in formic acid-formalin solution for two or three days, the submitted digit masses were trimmed, embedded in paraffin, sectioned at 3 μm, and stained with hematoxylin and eosin for the optical microscopy examination. To determine the origin of the tumor cells, the primary antibody for Melan-A (Dako, Denmark) was used in additional paraffin-embedded sections for immunohistochemistry (IHC).

Grossly, the requested digits showed severe irregular swelling with a pale red color. A solitary pale red nodular mass was found beneath the area of the nail in the cut-surface of the affected digits in three dogs. The size of the masses ranged from 10 × 5-10 mm in two dogs (cases one and two) and 15 × 15 mm in the case three dog.

A histological examination of the digit masses revealed diffuse hyperplastic and hyperkeratotic (ortho and parakeratosis) stratified squamous epithelium with/without ulceration and underneath inflammation on the surface (Fig. 3A). The dermal masses were composed of numerous neoplastic cells (major epithelioid and minor spindle cells types) with marked pleomorphism. These neoplastic cells had enlarged or irregular sized nuclei and prominent nucleoli, as well as moderate amounts of pale cytoplasm with/without dark brown or black melanin pigmentation. Occasionally, spindle-type neoplastic cells formed interwoven or whorled pattern distribution in some areas of the mass. A large number of mitotic figures (1-2/high power field) was observed throughout the mass (Fig. 3B). The neoplastic cells had strong invasive tendency into the adjacent distal phalanx; therefore, many neoplastic cells were found in the bone marrow (Fig.

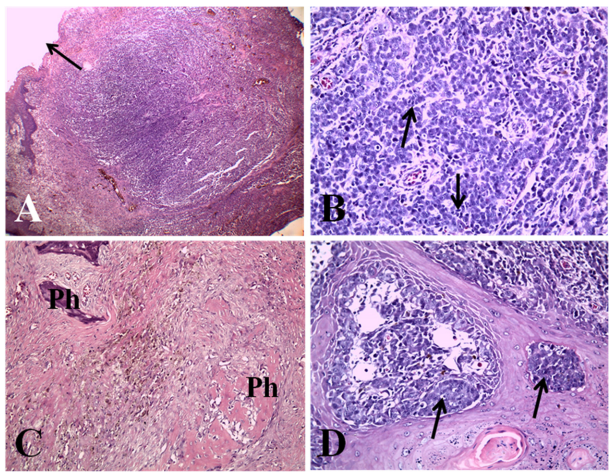

Fig. 3. Histopathologic findings of nail bed malignant melanomas in dogs. (A) Note the severe epidermal ulceration (arrow) and dermal neoplastic mass in case one (H&E stain, ×40). (B) Neoplastic cells had high mitotic figures (arrows) in case three (H&E stain, ×400). (C, D) Neoplastic melanocytes showed strong invasion to adjacent distal Ph in case one and covered epidermis (arrows) in case three (H&E stain, ×200, ×400). H&E, hematoxylin and eosin; Ph, phalanx.

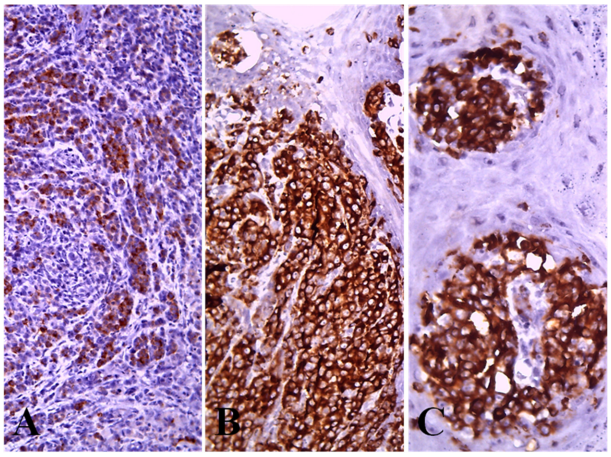

Fig. 4. Immunohistochemical results for Melan-A in digital masses. Numerous neoplastic melanocytes in the dermis (A) Case 1, (B) Case 2 and epidermis (C) Case 3 showed strong positive reactions for Melan-A (immunohistochemistry stain, ×200 [A and B] and ×400 [C]).

3C). In addition, cases two and three exhibited junctional activity, as well as the presence of small nests of neoplastic melanocytes within the epidermis (Fig. 3D). The distal phalangeal bone showed severe atrophy and surrounding fibrosis because of the invasion of neoplastic cells. According to IHC, the neoplastic epithelioid and spindle cells of the dermal mass and epidermis in three dogs showed strong positive reactions for Melan-A (Fig. 4). Based on the clinical and gross findings, histopathology, and IHC, these cases were diagnosed as nailbed malignant melanoma in the digits of dogs.

Generally, melanocytic tumors of non-haired skin in dogs, including the oral cavity and lip, have been considered to have a poorer prognosis than those of the haired skin. Compared to cutaneous melanocytic tumors in dogs, digital malignant melanomas have a poorer prognosis and higher mortality than those found elsewhere on the skin [6,7]. The majority of melanomas are in the oral and mucocutaneous junction of the lips, and approximately 50% of digital melanomas originating from the nail bed were reported to be malignant [1,8]. The reported metastatic rate of malignant melanomas from the canine digits was higher than that in the other cutaneous sites with a reported metastatic rate of 38–58% and a one-year survival rate of 42–70% [9]. Nailbed melanoma can invade the third phalanx and spread to the regional lymph nodes, particularly the popliteal lymph node and lung [1,10]. In these cases, the strong invasion of neoplastic cells in the bone marrow in the third phalanx was observed in all dogs. On the other hand, no distant metastasis was detected using a radiographic examination.

According to the literature, squamous cell carcinoma (SCC) and melanoma are the first and second most common malignant neoplasms in the canine digits, respectively [9]. In that paper, the metastatic rate of canine digital melanoma was higher, and the prognosis was poorer than that for dogs with digital SCC. The age of dogs with digital melanoma ranged from 4–14 years at the time of surgical amputation, with an average age of 9.9 years. Females (n = 31) were affected more commonly than males (n = 18) [9]. On the other hand, the three dogs with nailbed malignant melanoma in this study were all male (two castrated and one intact) with an average age of 10.7 years.

Several etiological factors, including trauma, chemical exposure, hormones, and genetic susceptibility, can be associated with the occurrence of canine melanoma [11]. The influence of these factors may result in reactive hyperplasia of the covered epithelium, with a disruption of the regular keratinocyte-melanocyte interactions and the proliferation of initiated cells. Unlike human cases, ionizing solar radiation exposure might not be related closely to the initiation of canine melanoma [12]. Based on a previous report, canine digital tumors occurred more often on the forelimbs (n = 38; 59%) than on the hindlimbs (n = 26; 41%), and more than 60% of the SCC, malignant melanoma, and other malignant tumors were found on the forelimbs [13]. Compared to the hindlimbs of dogs, plausible mechanisms for explaining this site predilection include the increased weight bearing on the forelimbs as well as the increased potential for exposure to carcinogens during digging behavior [13]. The cocker spaniel, Scottish terrier, and giant schnauzer as well as dogs with a heavily pigmented oral cavity are canine breeds with an increased risk of developing melanoma in the digits [1,9,11]. Two cases (case one and two) of nail bed malignant melanoma in this study were found in the right forelimb of cocker spaniels weighing 9 kg and 13 kg, respectively. On the other hand, the other case existed in the right hindlimb of a cocker spaniel with an unknown weight. This dog had experienced a biting wound, a type of trauma, with another dog at the 5th digit of the right hindlimb before the neoplastic mass was observed. Therefore, a previous traumatic wound might be closely related to the occurrence of nailbed melanoma in this dog.

Melanocytic tumors are generally composed of one of the following cell types: epithelioid, spindle, mixed, dendritic, and round cells [7,12]. All these cell types may occur in neoplastic masses, either alone or in combination. Melanomas may be difficult to diagnose because of the marked pleomorphism of neoplastic melanocytic cells. Therefore, IHC staining will be helpful in defining the precise origin of the tumor cells. The most common commercial antibodies used are the S-100 protein, melanoma-associated antigen (Melan-A/MART-1 or PNL2), vimentin, neuron specific enolase, tyrosinase-related proteins 1 and 2 (TRP-1 and TRP-2) [1,11,12]. According to the literature, Melan-A, PNL2, TRP-1, and TRP-2 are highly sensitive and 100% specific IHC markers for the diagnosis of canine oral melanomas [12,14]. In this study, dermal masses of canine digits were composed of two types of neoplastic cells: major epithelioid and minor spindle cells. In addition, both neoplastic cells expressed strong pos-

itive reactions for Melan-A antibody.

Nailbed malignant melanoma should be differentiated from melanocytoma, SCC, and other carcinomas of an undetermined origin. Cutaneous melanocytoma is benign tumor arising from melanocytes, and most of them are easy to diagnosis due to the presence of melanin pigments and low mitosis [1]. There is no single diagnostic method and no universally accepted criteria capable of distinguishing benign from malignant melanocytic tumors [6,12]. The histopathologic grade may help define the aggressiveness of neoplastic cells and determine the prognosis. The mitotic index (MI; number of mitotic figures per 10 consecutive non-overlapping high-power fields) is the most commonly evaluated histologic factor for the patient prognosis [6]. In addition, other parameters for a morphologic evaluation are an evaluation of the cell size and shape, nuclear size and shape, chromatin pattern, prominence of nucleoli, and lack of pigment [10]. A previous study of digital melanocytic tumors of dogs reported that the histological criteria for a malignancy were based on a high MI and nuclear and nucleolar pleomorphism [7]. Generally, in dogs, the presence of more than three mitoses per 10 high power fields is indicative of a malignancy [6]. The characteristic histopathological features, including marked cellular pleomorphism, anisokaryosis, lack of brown pigments, invasion, and high MI, indicate that the three cases of canine digit masses in this study were malignant melanoma rather than melanocytoma. In addition, the differential diagnosis for digital SCC and other carcinomas was also excluded in these cases according to the results for IHC.

For canine nailbed tumors, amputation of the affected toe is usually required due to the extensive bone involvement. Dogs treated with surgery (digit amputation or lumpectomy) alone show longer survival times. In addition, a variety of chemotherapeutic agents have been used to delay the spread of digital tumors, including dimethyl triazeno imidazole carboxamide, melphalan, doxorubicin, and cyclophosphamide [13]. To overcome the poor response rates of chemotherapy, a murine xenogeneic DNA vaccine in conjunction with local-regional control, such as amputation, was evaluated for the canine digit melanoma [15].

All canine nailbed melanomas should be considered malignant tumors. Although nailbed malignant melanoma usually grows slowly, many cases will have already invaded and destroyed the adjacent phalanx at the time of initial examination. Therefore, a histopathology examination and initial imaging analysis, such as magnetic resonance imaging and high-resolution ultrasonography with color Doppler study for nailbed mass should be performed to achieve a rapid and accurate diagnosis and select the appropriate treatment.

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