

Histopathologic and electron microscopic findings of canine malignant melanoma from the lower limb and digit

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Abstract

A 16-year-old female mixed dog was submitted for examination at the pathology division of national veterinary research and quarantine service (NVRQS). Grossly, white or grayish spherical, multinodular, firm to friable masses were present in the tibiofibula to the pharyngeal area of the right limb, and dysphagia, breathing difficulties and tachypnea were shown. Various-sized white or grayish black masses were scattered in lungs and diaphragm and one mass was observed in the trachea and in the jejunum, respectively. Histopathologically, the neoplastic cells were composed of polygonal or spindle shaped cells with various sized round to oval nuclei and abundant cytoplasm. These cells formed lobules or nests separated by fine connective tissue and contained little amount of melanin pigments. Melanin pigments were stained dark gray or black with Fontana-Masson method. Melanosomes were also ultrastructurally demonstrable by electron microscopy. Based on above results, the present case was diagnosed as canine malignant melanoma originating from the lower limb and digit.

Key words: Malignant melanoma, Melanin, Canine, Digit

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Introduction

Melanomas have been reported in dogs¹⁻⁵⁾, cats⁶⁾, and some domestic animals including horse⁷⁾, cattle⁸⁾, sheep⁹⁾, alpaca¹⁰⁾ and pig¹¹⁾ as well as rats¹²⁾ and bird¹³⁾ occurred spontaneously. In dogs, melanomas are relatively common, accounting for 3% of all neoplasms and up to 7% of all malignant tumors¹⁾. The most common sites are the oral cavity including lips and the skin pigmented heavily. Oral melanomas predominantly arise in the gingival and account for 33% of malignant tumors in the oral cavity⁴⁾. On the other hand, melanocytic neoplasms in the digit are rare; which have a high metastatic rate. The digits affected by the melanoma often have deformed nails, and show growth of a mass from the nailbed, or paronychia. The digit melanoma may result in destruction of phalangeal bone³⁾. In this case, the malignant melano-

noma from the digit of a dog was examined by histochemical stain, histopathology and electron microscopy.

Case Report

A 16-year-old female mixed dog with skin ulceration and elevation of right digit was delivered to the local animal hospital in August 2006. The animal had been treated with antibiotics, steroid and antifungal drugs for 3 months. After eight months posterior to presentation, the dog appeared again to the same hospital. Several white or grayish yellow masses with severe ulceration were observed in the tibiofibula to the pharyngeal area of the right limb and the dog showed dysphagia, breathing difficulties and tachypnea. According to a poor prognosis, the animal was submitted for examination at the pathology division of NVRQS and sacrificed by exsanguinations under deep



Fig 1. Melanoma; canine. A white or grayish yellow spherical, multinodular, firm to friable mass, originating from the right digit extended into the limb

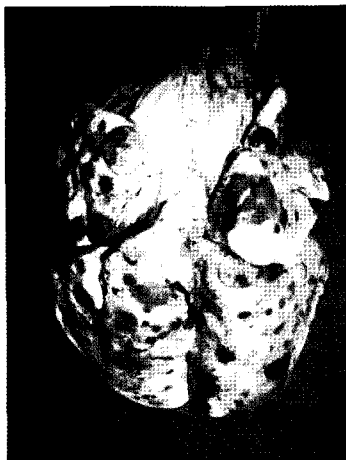


Fig 2. Metastatic melanoma; canine. Multiple, white or grayish raised shiny nodules disseminated throughout the lungs

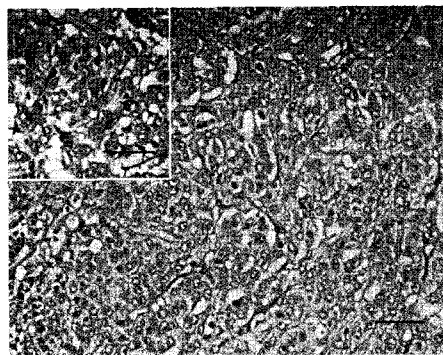


Fig 3. Digital mass; canine. Neoplastic cells consist of polygonal or spindle shaped cells with various sized round to oval nuclei and abundant cytoplasm and form lobules or nests surrounded by fine connective tissue. *Insert*: A few neoplastic cells have black pigment. HE. Bar=100 μ m



Fig 4. Digital white mass; canine. Neoplastic cells containing melanin are stained with dark gray or black. Fontana-Masson stain. Bar=100 μ m

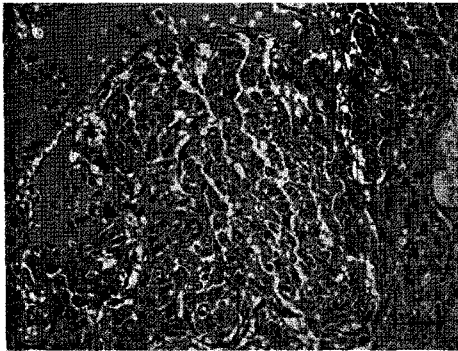


Fig 5. Lung; canine. Tumor emboli are present in alveoli. HE. Bar=100 μ m

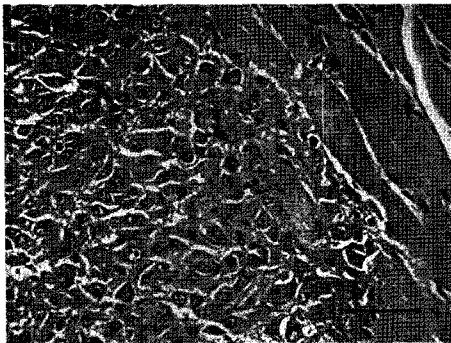


Fig 6. Diaphragm; canine. Spindle or polygonal cells are invaded into muscle bundles. HE. Bar=100 μ m

ether anesthesia. There was a 6 \times 3cm white or garyish yellow spherical, multinodular, firm to friable mass, or iginated from the

right digit, into which it extended (Fig 1).

Various-sized masses were scattered in the lungs (Fig 2). and diaphragm and only one same mass was observed in the trachea and in the jejunum, respectively. All organs and tissues including the neoplastic mass was fixed in 10% neutral buffered formalin and embedded in paraffin. Sections (4 μ m were cut and stained with hematoxylin and eosin (H&E) or traditional histochemical stain for melanin pigment such as Fontana-Masson method using a commercial kit (Diagnostic BioSystems, CA, USA). The neoplastic cells consisted of polygonal or spindle shaped cells with various sized round to oval nuclei and abundant cytoplasm. These cells formed the lobules or nests separated by fine connective tissue and had little amount of melanin pigments (Fig 3). Melanin pigments were stained to be dark gray or black with Fontana-Masson method (Fig 4). The polygonal or spindle shaped cells were ob-

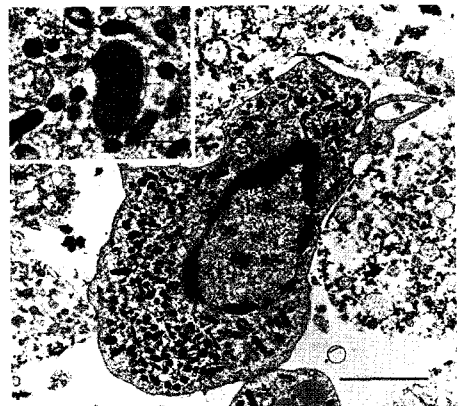


Fig 7. Digital mass; canine. Aberrant melanosomes are observed in the cytoplasm of neoplastic cells. Bar=2 μ m. Insert: There are various sized melanosomes in cytoplasm. EM. Bar=500nm

served in some organs such as the lungs (Fig 5), the diaphragm (Fig 6), the trachea or the jejunum. Mitotic figures were high. For electron microscopy, paraffin block were deparaffinized, post-fixed in 1% osmium tetroxide in 0.1M phosphate buffer (pH 7.4), and embedded in epoxy resin (TED PELLA, Inc, CA, USA). Semithin sections were stained with toluidine blue for light microscopic survey. Ultrathin sections of the selected are as were double-stained with uranyl acetate and lead citrate and then observed under an electron microscope (Hitachi H-7100FA, Tokyo, Japan). The neoplastic cells had filopodia and contained various amounts of dense melanin in aberrant melanosomes surrounded by the membrane (Fig 7).

Discussion

Canine melanomas have been reported to arise in oral cavity, skin, digit, eye and mucocutaneous junctions including lips, prepuce, vulva, anus, nose and eyelid. Subungual melanoma from the digit causing destruction of phalangeal bone accounts for 8% of melanoma³⁾. Most affected animals were more than 10 years old with a range of 8 to 13 years. Breeds most commonly affected are Scottish terrier, standard schnauzer, Irish setters, miniature schnauzer, rottweiler and golden retrievers^{2, 14, 15)}, while mixed breed animals are low. No sex predilection has been demonstrated. In oral melanoma, however, males are more commonly affected than females, and the male/female ratio is 2-3:1¹⁶⁾.

Digit melanoma has a high rate of meta-

stasis via lymphatics to regional lymph nodes and lungs. Marino et al⁵⁾ also reported that 32% of digit melanoma metastasized to lungs on presurgical radiography and 26% developed pulmonary metastasis after surgery. In this case, metastatic foci were observed in diaphragm, trachea and jejunum as well as lungs.

Amelanotic or rare-pigmented melanomas should be differentiated from other neoplasms such as trichoblastoma, trichoepithelioma, pilomatricoma, sebaceous and apocrine neoplasma. To identify melanin pigments, traditional histochemical stains such as Fontana-Masson or dihydroxyphenylalanine oxidase (DOPA) test and electron microscopy have been used. Fontana-Masson stain was applied for the argen-taffin reaction to reduce silver in ammo-niacal solutions. Melanin acts as the reducing agent in this reaction and is stained to be brown or black. However, the specificity of this technique is reduced because this stain could cross-react with lipofuscin¹⁷⁾. Amelanotic melano-cytic tumor cells, as well as normal melanocytes, have melanosomes identified ultrastructurally by electron microscopy¹⁸⁾. Melanosomes producing melanin pigments, must be differentiated from compound melanosomes such as secondary lysosomes extruded by melanocytes and phagocytized by keratinocytes and macrophages^{18, 19)}. Real melanosomes usually contain crossstriations, whereas secondary melanosomes do not¹⁸⁾.

In conclusion, the neoplastic cells composed of polygonal or spindle shaped cells had few blackish pigments. These

melanin pigments were confirmed with traditional histochemical stain using Fontana-Masson method and electron microscopy. We therefore reported the present case of a canine malignant melanoma originated from the lower limb and digit.

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