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Oral Amelanotic Malignant Melanoma in a Dog: Melan A Immunohistochemical Findings

Min-Hee Kang, Chul Park* and Hee-Myung Park[1]

Department of Veterinary Internal Medicine, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea
**Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California Davis, Davis, CA 95616, USA*

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Abstract : A 10-year-old intact male mixed breed dog was presented with a three-month history of massive oral mass. Physical examination revealed extending mass from the right upper gingiva. No metastasis was found at the time of presentation. Histopathologic examination of biopsied tissue from the oral mass was consistent with a neuroendocrine tumor with generalized epithelioid cells and few spindle cells. There were highly mitoses and no visible melanin granules with H&E staining. Immunohistochemical staining for Melan A was performed on section of tumor and was strongly positive. Diagnosis was made as amelanotic malignant melanoma based on histopathology with Melan A immunohistochemistry. This case study indicates that the Melan A immunohistochemical staining may be valuable to diagnose amelanotic malignant melanoma in dogs.

Key words **:** Amelanotic melanoma, dog, Melan A.

Introduction

Melanomas are one of the most common malignant oral tumors in dogs, representing 4 to 7% of all tumors (1). The tumor can be located in any part of the oral cavity, but the gingiva, especially pigmented oral mucosa, is the most common location for lesions (7,10). Oral melanomas are invasive, and frequently recur after surgical resection. Most oral melanomas have metastasized by the time of diagnosis (10,11). The diagnosis of melanomas may be challenging because the degree of pigmentation can be variable and tumors can be completely unpigmented (8-11). Thus, immunohistochemical confirmation of the diagnosis of melanoma is frequently necessary to estimate a prognosis and determine a therapeutic plan.

According to previously described reports (6,7), staining for Melan A and S-100 has been used in the detection of pigmented and amelanotic melanomas. However, S-100 stains various kinds of normal tissues and nonmelanocytic neoplasms, which can be confused with fusiform amelanotic melanoma. Compared to this, the staining pattern of Melan A was much more specific for melanoma cells than S-100.

This is the first case report of amelanotic malignant melanoma in the canine oral cavity diagnosed using Melan A immunohistochemistry in our country.

Case

A 10-year-old intact, mixed breed, male dog was admitted for evaluation of a massive oral mass. This mass was extending from the right upper gingiva, and had continued to enlarge over a 3-month period. Physical examination revealed a measured 5 × 2.5 × 1.5 cm oral mass on the right upper gingiva (Fig 1). There was bleeding from the firm and irregular oral mass, in addition to severe halitosis and moderate tartar. Except the oral mass, no other abnormalities were noted on the physical examination. A complete blood count revealed marked leukocytosis ($41.00 \times 10^3/\mu l$; reference range, $6\text{-}17 \times 10^3/\mu l$) with stress leukogram and mild regenerative anemia (HCT; 36.5%, reference range, 37-55%). Serum chemistry profiles also revealed no abnormal findings.

To evaluate the oral mass, we initially performed a fine needle aspiration biopsy (FNAB) and impression cytology using a excisional biopsy sample from the cut surface. Results of the FNAB showed anisocytosis, coarse nuclear chromatin

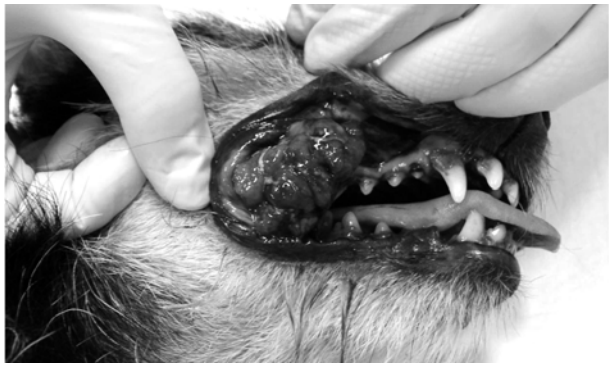

Fig 1. An ulcerative oral mass (5 × 2.5 × 1.5 cm) was noted extending from the right upper gingiva.

[1]Corresponding author.
E-mail : parkhee@konkuk.ac.kr

clumping and prominent multiple nucleoli (Fig 2). The cytological diagnosis from the FNAB was malignant round cell tumor. Examination and FNAB of the regional lymph nodes and the palatine tonsils could not demonstrate metastatic involvement at this time. Cranial radiography revealed no evidence of bony lysis or proliferation. No metastatic lesions were found on the thoracic radiography.

For histopathologic examination, the excisional biopsy tissue was fixed in 10% buffered formalin, embedded in paraffin and cut at 4 μm. Then the sections were stained with hematoxylin and eosin (H & E). On histopathologic examination, the mass was composed of generalized epithelioid cells with a few spindle cells arranged in small packets that were surrounded by thin fibrous stroma (Fig 3A & B). This was a typical neuroendocrine tumor pattern. No visible melanin granules were noted, but as the mass was in the gingiva, it was highly suspected to be an amelanotic melanoma. Immunohistochemical staining for Melan A was performed on the cut section of the tumor to distinguish morphologically similar round cell tumors and definite diagnosis. Paraffin embedded sections were stained for the monoclonal mouse anti-human Melan A protein antibody (Dako Corporation, M7196, The University of Missouri Veterinary Medical Diagnostic Laboratory, USA). The result was strongly positive (Fig 3D), which was consistent with an amelanotic malignant melanoma. Based on the histopathologic and immunohistochemistry findings, the present case was diag-

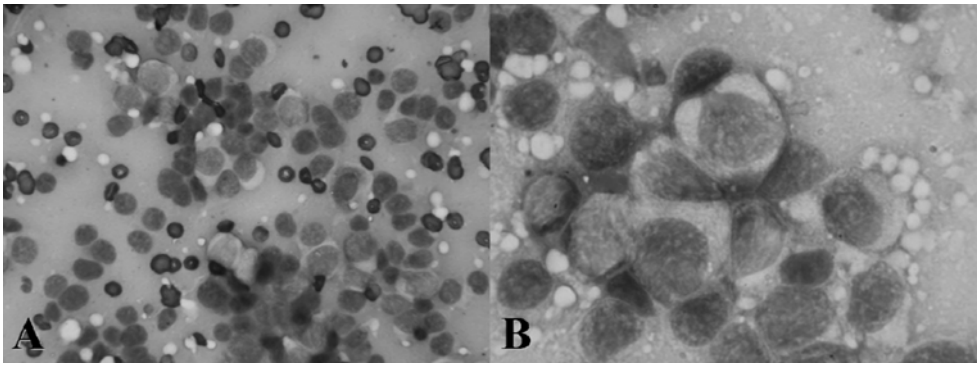

Fig 2. Photomicrographs of the fine needle aspiration biopsy (FNAB). Note the anisocytosis, coarse nuclear chromatin clumping and prominent, multiple nucleoli. Pigmentation is not apparent. Diff-Quik stain (A. × 400, B. × 1000).

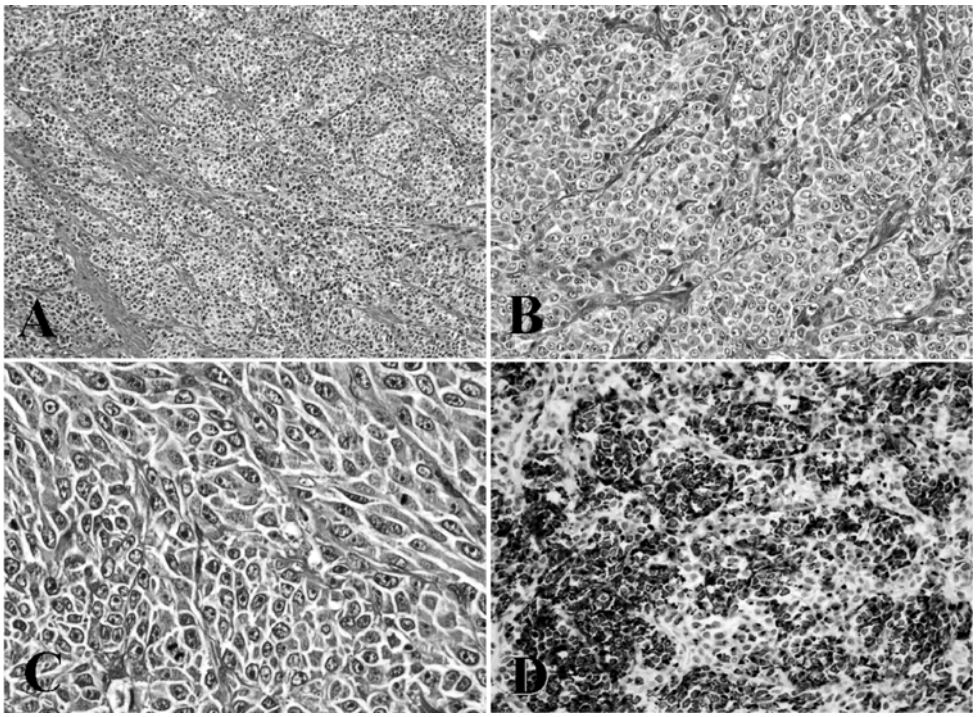

Fig 3. Histopathologic examination of excised tissue from oral mass. A-B. Note the composition of generalized epithelioid cells with a few spindle cells arranged in small packets that are surrounded by thin fibrous stroma (A. H&E, × 100, B. H&E, × 200). C. Two mitotic figures were found, which was consistent with malignancy (H&E, × 400). D. A strong positive immunohistochemical staining to Melan A in an amelanotic melanoma located in the oral gingiva (IHC, × 200).

nosed as an amelanotic malignant melanoma of the gingiva.

Given the poor prognosis for long-term survival, the owner refused further treatments, such as chemotherapy or radiation therapy. Treatment was initiated with doxycycline (5 mg/kg, PO, q 12; Dong Koo Pharm, Seoul, Korea), clindamycin (10 mg/kg, PO, q 12; Sam Jin Pharm, Seoul, Korea), celecoxib (2 mg/kg, PO, q 12; Pfizer, Seoul, Korea) and misoprostol (5 μg/kg, PO, q 12; Nelson Pharm Korea, Seoul, Korea) for palliative therapy. At four-month follow-up, there was no apparent metastasis or relapse according to thoracic radiographic examination.

Discussion

Oral melanomas are most common in dogs greater than 10 years old (10). It occurs more often in males, predominantly in small breeds (10,12). A definite diagnosis of canine melanoma is challenging, especially in poorly differentiated amelanotic melanoma, as melanocytes arise from the embryonic neuroectoderm and continue to have the ability to differentiate into spindled or epithelioid cells (2). It is important to identify amelanotic melanomas and to distinguish them from other tumor types, as the lack of pigmentation in melanomas may be associated with malignancies, which can affect the prognosis and therapy (8,9).

Immunohistochemistry to various antibodies against melanocytic markers such as S-100, Human Melanoma Black (HMB) 45, NKI/C3, Human melanosome-specific antigens (HMSA) 1 and 5, and Melan A, has been used to confirm the diagnosis of amelanotic melanoma in dogs (2,7). Among them, S-100 is widely used, but its specificity is low. Melan A has been proven to be the most successful, but the sensitivity is low. Due to this, it is generally recommended that more than a single immunohistochemical marker be used for diagnosis (6,7).

In this case, we found typical neuroendocrine tumor patterns with generalized epithelioid cells and a few spindle cells through histological examination. There were highly mitoses and no visible melanin granules on H&E staining (Fig 3C). To confirm the diagnosis, the staining for Melan A was performed and the result was strongly positive. Although Melan A or a cross-reacting antigen has been detected in some normal tissues and nonmelanocytic tumors in humans and dogs (3,4,5,6), the probability of confusing those with melanomas is relatively low. Although it is recommended to use more than one immunohistochemical stain to diagnosis amelanotic melanoma, we used only Melan A due to the strongly positive result, negating the need to do further staining for the diagnosis of this case.

In a recent study, a new approach for detecting melanoma using tyrosinase-related protein-2 (TRP-2) immunoreactivity was performed (9). TRP-2 staining may be more specific for melanomas than S-100 and also appears to be more sensitive than Melan A staining in confirming amelanotic melanomas (9). Further studies will be needed to determine that TRP-2 is an effective and acceptable diagnostic method of amelanotic melanoma in dogs.

In the present case report, we described a case of an amelanotic malignant melanoma in the canine oral cavity, where tumor melanocytes were strongly positive for Melan A. In conclusion, this case demonstrates that the immunohistochemical reactivity to Melan A could be a valuable diagnostic method for the confirmation of canine amelanotic malignant melanomas.

Acknowledgement

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개에서 발생한 구강 멜라닌결핍 악성흑색종 예 : Melan A 면역화학조직 염색 고찰

강민희 · 박철* · 박희명[1]

건국대학교 수의과대학 내과학교실, *미국 캘리포니아 데이비스 수의과대학

요 약 : 10년령의 수컷 잡종견이 3개월 간의 구강내 궤양성 종양을 주증으로 내원하였다. 신체검사상 오른쪽 상악 잇몸에서 돌출된 구강내 종양이 관찰되었다. 내원당시 전이소견은 관찰되지 않았으며 구강 종양의 절제생검을 통한 조직검사결과 전반적인 상피양 세포와 일부 방추세포를 가진 전형적인 신경내분비종으로 의심되는 소견이 관찰되었다. 멜라닌 과립으로 추정되는 소견은 관찰되지 않았지만 악성종양에서 관찰되는 세포의 유사분열은 다수 관찰되었다. 확진을 위해 조직 절제 단면에 Melan A를 이용한 면역화학조직 염색을 실시하였으며, 그 결과는 강한 양성반응을 나타내었다. 조직검사와 조직면역염색을 통하여 종양은 멜라닌 결핍 악성흑색종으로 진단되었다. 결론적으로 본 증례의 경우 개에서 멜라닌결핍 악성흑색종의 진단에 Melan A를 이용한 면역화학조직염색이 유용함을 보여준다고 생각된다.

주요어 : 멜라닌결핍 악성 흑색종, 개, Melan A.