Establishment and Characterization of an Oral Mucosal Melanoma Cell Line (MEMO) Derived From a Longstanding Primary Oral Melanoma

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Abstract: Oral mucosal melanoma is rare. Its incidence peaks between 41 and 60 years of age; male/female ratio is 2:1. Preferred oral sites include hard palate and maxillary gingiva. Risk factors have not been clearly identified, but pigmented lesions may be present before the diagnosis of oral melanoma. We report an unusual case of oral mucosal melanoma of long-standing duration on hard palate and maxillary alveolar ridge in a male patient. Histopathologic features confirmed the diagnosis of invasive melanoma with a prominent in situ component. A cell lineage derived from the tumor was established and characterized, with phenotypic markers of melanocytes.

Key Words: cell culture, oral mucosa, melanoma

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INTRODUCTION

Primary malignant melanoma has been described in virtually all sites and organ systems to which neural crest cells migrate. More than 90% of melanomas occur on the skin; approximately 1% of melanomas arise from all mucosal surfaces. Oral mucosal melanoma is extremely rare, accounting for only 0.5% of oral malignancies.[1–4]

Large series of patients show that oral mucosa melanoma has its incidence peak between 41 and 60 years of age, and male/female ratio is 2:1.[5–8] Preferred sites in the oral mucosa include hard palate and maxillary alveolar ridge. Risk factors have not been clearly identified, but according to some authors, pigmented lesions may be present in one-third of the patients, before the diagnosis of melanoma.[7,9,10]

The oral mucosal melanomas are reported to be more aggressive than cutaneous counterpart. However, it is unknown if the worse prognosis of oral mucosal melanoma is because of differences in its histological features or, if late diagnosis or anatomical features are determinant of such differences.[11]

To present, only limited research has been performed to understand the behavior and physiopathology of oral mucosal melanomas. This is mainly because of its rarity and the difficulties in establishing in vitro and animal systems to perform functional and genetic investigation on this interesting type of melanomas.

We report herein a new case of oral mucosal melanoma of the hard palate and maxillary gingiva with the main characteristics of an in situ lesion and areas of superficial invasion. Additionally, a cell culture derived from this tumor was established and is presented, which may open up the possibility for new roads to melanoma investigation.

PATIENT AND METHODS

A 75-year-old Afro-Brazilian male reported a black spot on the hard palate of 7-year duration. He had noticed a slow progressive growth of the lesion during the previous 3 years. Patient denied smoking and/or alcoholism. Clinical examination revealed a large and irregularly pigmented patch affecting the right hard palate and tuber, alveolar ridge, and vestibular gingival mucosa (Figs. 1A, B). Computed tomography revealed no other internal tumor mass. Medical history was uneventful. Incisional biopsy was performed in 4 different sites for histopathological analysis. An additional biopsy specimen was used for establishment of cell culture, considering the patient's authorization and according to the Local Ethical Committee Guidelines.

Cell Culture

A fragment of the biopsy material was finely minced with a scalpel under sterile conditions and the explants were incubated in 25-mm^2 culture flasks with RPMI 1640 medium (Cultilab, Sao Paulo, Brazil), supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin, and 10% fetal calf serum. The incubation occurred at 37°C in a humidified 5% CO_2 atmosphere.

After 5–7 days, a cell monolayer could be observed growing out of the explants. When the cell monolayer reached 75%, cells were subcultured 2–5 times before cell line characterization was performed by immunocytochemistry and transmission electron microscopy. The panel of antibodies used for cell line characterization is described in Table 1.

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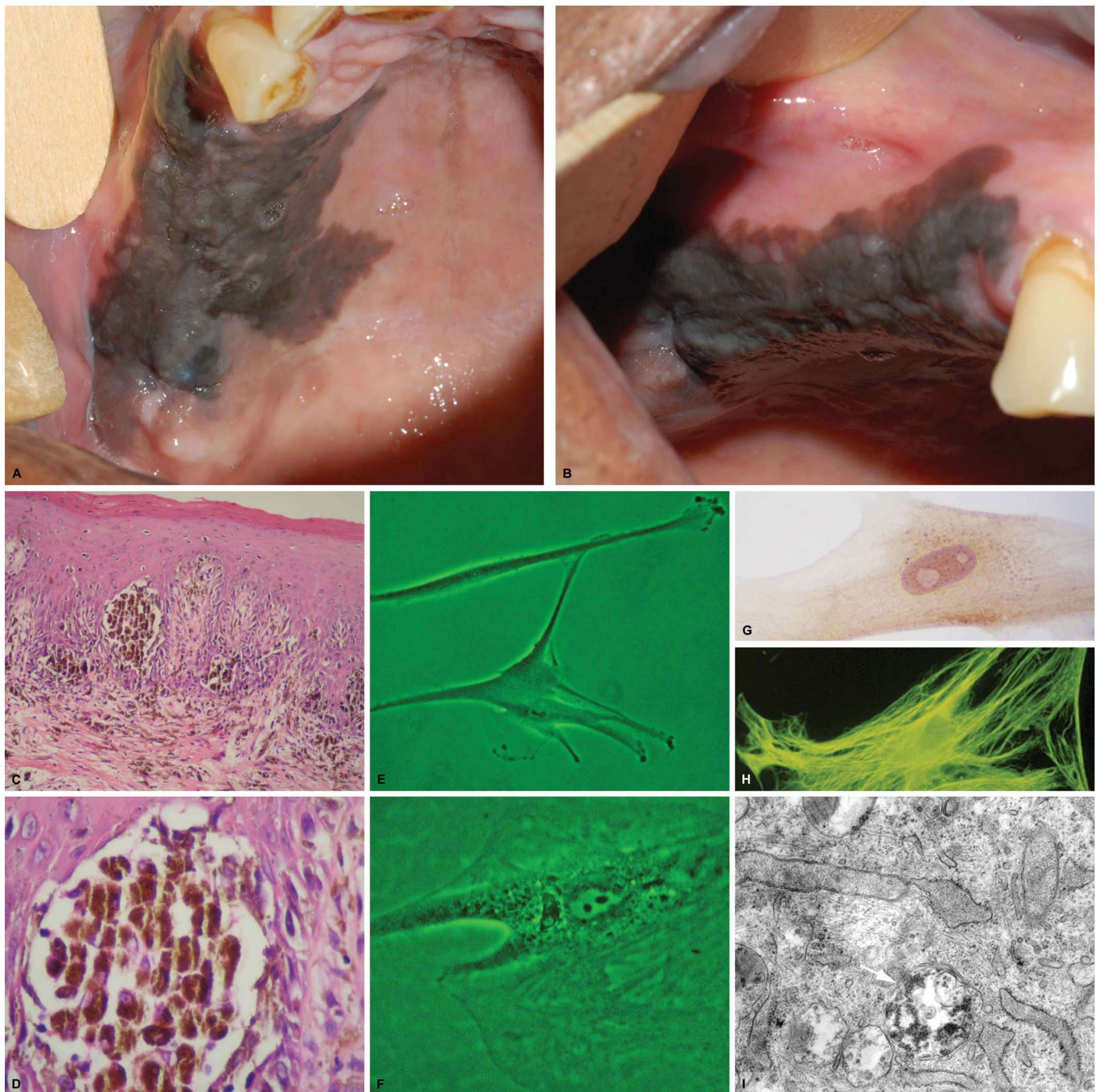

FIGURE 1. A, B, Clinical aspect of oral mucosal melanoma: irregularly pigmented patch on right palate (A) and alveolar ridge (B). C, Proliferation of melanocytes in irregular pattern occupying mostly the lower layers of mucosal epithelium. The presence of melanophages in the lamina propria (haematoxylin and eosin, original magnification, ×100). D, Detail of pleomorphic melanocytes arranged in nests in the basal layer of the epithelial crest (haematoxylin and eosin, original magnification, ×400). E, F, Human oral mucosal melanoma cell culture (MEMO): stellate and spindle cells with dark granules on the tips of the cytoplasmic prolongations (F), and polyhedral cells showing dark granules on their cytoplasm (G) (phase contrast microscopy, original magnification, ×400). G, H, MEMO cells positive for S-100 (H) and for HMB-45 (I) (streptavidin peroxidase, original magnification, ×400). I, Transmission electron microscopy of cell cultures from a primary oral mucosal melanoma: the presence of irregular melanosomes in the cytoplasm.

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TABLE 1. Antibodies, Clones, Source, and Title

Primary Serum	Clone	Source	Title
S-100	Polyclonal	Dako Cytomation	1:50
HMB-45	HMB-45	Dako Cytomation	1:50
Melan A	A103	Dako Cytomation	1:50

Cell Culture Characterization

Immunocytochemistry

Cells in passage 3 were plated on a 6-well ELISA plate containing glass coverslips, at a density of 10^3 cells per well and incubated in a 37°C in a humidified 5% CO_2 atmosphere for 48 hours. After this period, cells were fixed in 3.7% paraformaldehyde, treated with 1% Triton X-100 in phosphate buffered saline (PBS) and incubated with the monoclonal antibodies at room temperature. Incubation period and antibodies titles are described in Table 1. This was followed by incubation with the indirect dextran polymer detection system (EnVision—Dako Carpinteria, CA). Staining was completed by 3-minute incubation with 3′3 diaminobenzidine tetrachloride. Cells were then incubated with Mayer's hematoxylin for nuclear staining. All procedures were preceded by a PBS rinse. The glass coverslips containing the cell monolayer were then mounted with Vecta Shield mountant (Vector Laboratories, Burlingame, CA) and observed under an optical microscope equipped with digital camera for photographic register. Coverslips containing the cell monolayer incubated with nonimmune serum served as negative controls.

Electron Microscopy

Cells in passage 3 were plated on a 25-cm^2 flask at a density of 3×10^6 and incubated at 37°C in a humidified 5% CO_2 atmosphere for 48 hours. After this period, the cultured cells were scraped from the flask and centrifuged at 1000 RPM for 10 minutes. The pellet of cells was then fixed in 2% glutaraldehyde, washed in PBS, and postfixed in 1% osmium tetroxide. After stepwise dehydration with ethanol (70%–100%), the cells were embedded in Spurr resin, and ultrathin sectioning was performed (800–1000 Å) using an ultratome (LKB U-5 Ultratome, LKB, Sweden). The cells were stained with 4% uranyl acetate and then with Reynolds lead citrate. The grids were studied and micrographed with a Philips TEM 400 transmission electron microscope, operating at 80 kV.

RESULTS

Histopathologic analysis of the biopsied specimens revealed, for the most part, melanoma in situ, with junctional activity and intraepithelial nests of neoplastic atypical melanocytes. Neoplastic melanocytes were also seen in the superior strata of the epithelium, and some dendritic melanocytes could also be observed. Multiple small foci of invasive malignant melanoma were also present in the superficial portion of the lamina propria. Vascular and perineural invasion were not detected. Histopathological aspects of the lesion are illustrated in Figures 1C, D. Computerized tomography and cervical ultrasound showed no abnormalities in parotid, thyroid, and lymph nodes. Additional imaging investigation revealed no distant metastasis.

Culture and Characterization of Human Mucosal Melanoma Cells (MEMO)

Culture of mucosal melanoma resulted in cell growth form the explants in 48 hours. After 5–7 days, a cell monolayer was established. Cells were large with spindle, polyhedral, and star-like phenotypes (Figs. 1E, F). A few cells showed long cytoplasmic prolongations with fine and dark granules on their extremities (Fig. 1F). Other cells exhibited dark granules in their cytoplasm (Fig. 1E). All cells expressed S-100 (strong expression) (Fig. 1G), HMB-45 (Fig. 1H), and Melan-A. Transmission electron microscopy of the cells cultured revealed melanosomes at various stages, including immature melanosomes and swollen rough endoplasmic reticulum (Fig. 1I).

DISCUSSION

Oral mucosal primary melanomas are rare lesions, comprising 0.2%–8% of all melanomas and 0.5% of all malignancies.[12–16] The present report refers to a case of oral melanoma still in its early stages of invasion, which occurred in a male patient in his eighth decade of life, not commonly observed. The presence of junctional activity and transmucosal migration are some of the features that favor diagnosis of a primary oral melanoma.

Controversy surrounds the role of preexisting melanosis before development of oral mucosal melanoma, but anecdotal reports on possible transformation of benign oral pigmentation to primary oral melanoma exist.[4,10] This information may indicate that a pigmented lesion with transformed melanocytes and very slow growth rate could have been present previous to the present diagnosis. Yet, literature data state that more than one-third of oral melanomas develop from melanotic lesions, which might host the neoplastic radial growth phase many months before the diagnosis of melanoma.[7]

The pathogenic mechanisms of oral mucosal melanoma development are yet poorly understood because of the rarity of the lesion and the difficulties in obtaining fresh tissue for molecular studies. Chang et al[17] reported establishment and characterization of a melanoma cell line and phenotypic and immunophenotypic aspects of the cells reported herein are similar to their work. The development of a new cell line derived from an oral mucosal melanoma opens up new avenues for studies and understanding of this disease, as they may keep the main genetic alterations present in the tumor.

The new cell line derived from an oral melanoma (MEMO cells) shown in our study posses morphological features, ultrastructure, and differentiation markers typical of melanoma cells (S-100, melan-A, and HMB-45). They also exhibited characteristics of neoplastic cells, such as rapid replication under culture conditions. Molecular characterization of this cell line will now be important for the development of new functional studies.

Tagawa et al[18] also reported the establishment of a cell line derived from an oral melanoma, however, the melanoma cells were obtained form pleural fluids of the metastatic tumor and probably, their genetic characteristic might be different

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from cells grown out of primary melanoma as the one shown in our work. Nonetheless, Tagawa et al[18] have been performing several studies on these cells, which have been revealing some interesting molecular aspects of the disease.

The successful establishment of a new oral mucosal melanoma cell line (MEMO) in our laboratory will serve to develop studies comparing the genetic alterations of these cells with alterations already described in skin melanomas for a better understanding of the mysterious mechanisms of oral mucosal melanomas, which are reported to have distinct biological behavior. This effort may in future lead to identification of molecular targets for the development and trial of new chemotherapeutic drugs and multimodality therapies.

REFERENCES

1. Allen AC, Spitz S. Malignant melanoma: clinicopathologic analysis of the criteria for diagnosis and prognosis. *Cancer*.1953;6:1–45.
2. Chang AE, Karnell LH, Menck HR. The National Cancer Data Base report on cutaneous and noncutaneous melanoma: a summary of 84836 cases form past decade. The American College of Surgeons Commission on Cancer and the American Cancer Society. *Cancer*. 1998;83:1664–1678.
3. Hicks MJ, Flaitz CM. Oral Mucosal melanoma: epidemiology and pathobiology. *Oral Oncol.* 2000;36:152–169.
4. Garzino-Demo P, Fasolis M, Maggiore GM, et al. Oral mucosal melanoma: a series of case reports. *J Craniomaxillofac Surg.* 2004;32:251–257.
5. Regezi JA, Hayward JR, Pickens TN. Superficial melanomas of oral mucous membranes. *Oral Surg Oral Med Oral Pathol.* 1978;45:730–740.
6. Manolidis S, Donald PJ. Malignant mucosal melanoma of the head and neck: review of the literature and report of 14 patients. *Cancer*. 1997;80:1373–1386.
7. Rapini RP, Golitz LE, Greer RO Jr, et al. Primary malignant melanoma of the oral cavity. A review of 177 cases. *Cancer*. 1985;55:1543–1551.
8. Lourenço SV, A MS, Sotto MN, et al. Primary oral mucosal melanoma: a series of 35 new cases from South America. *Am J Dermatopathol.* 2009;31:323–330.
9. Batsakis JG, Suarez P, El-Naggar AK. Mucosal melanomas of the head and neck. *Ann Otol Rhinol Laryngol.* 1998;107:626–630.
10. Kahn MA, Weathers DR, Hoffman JG. Transformation of a benign oral pigmentation to primary oral melanoma. *Oral Surg Oral Med Oral Pathol.* 2005;100:454–459.
11. Gonzales-Garzia R, Gias L, Martos PL, et al. Melanoma of the oral mucosa. Clinical cases and review of the literature. *Med Oral Cir Bucal.* 2005;10:264–271.
12. Rapidis AD, Apostolidis C, Vilos G, et al. Primary malignant melanoma of the oral mucosa. *J Oral Maxillofac Surg.* 2003;61:1132–1139.
13. Lopez-Graniel CM, Ochoa-Carrillo FJ, Meneses-Garcia A. Malignant melanoma of the oral cavity: diagnosis and treatment experience in a Mexican population. *Oral Oncol.* 1999;35:425–430.
14. Chidzonga MM, Mahomva L, Marimo C, et al. Primary malignant melanoma of the oral mucosa. *J Oral Maxillofac Surg.* 2007;65:1117–1120.
15. Batsakis JG, Suarez P. Mucosal melanomas: a review. *Adv Anat Pathol.* 2000;7:167–180.
16. Barker BF, Carpenter WM, Daniels TE, et al. Oral mucosal melanomas: the WESTOP Banff workshop proceedings. Western Society of Teachers of Oral Pathology. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 1997;83:672–679.
17. Chang KW, Lim SC, Chao SY, et al. Establishment and characterization of an oral melanoma cell line (ME). *Oral Oncol.* 2001;37:301–307.
18. Tagawa T, Yatani R, Nishioka H, et al. Primary malignant melanoma of the oral cavity. Case report and establishment of melanoma cell line. *Int J Oral Surg.* 1981;10:16–20.

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