Oral mucosal melanoma: Retrospective study of a series of cases and a review of the literature

RAG Khammissa¹, M Altini², S Meer³, J Lemmer¹ and L Feller¹

Abstract

Background: Oral mucosal melanoma (OMM) is relatively rare, runs an aggressive course, affects most frequently the hard palate and the maxillary gingiva, and may be multifocal. The aetiological factors and the chain of molecular events that give rise to OMM and to its growth and metastasis are largely unknown.

Objectives: The purpose of this retrospective study was to characterize some clinical and histopathological features of OMM in a South African sample.

Materials and Methods: The histopathological reports and histological sections of 17 cases with OMM during the period 1968–2013 were retrospectively evaluated, and the data regarding age, gender, ethnicity/race and the histopathological patterns were recorded. c-Kit immunohistochemical staining was performed.

Results: OMM most frequently affected the palate and the maxillary gingiva of black persons without gender predilection. The age of the patients ranged from 20 to 78 years with the mean age at diagnosis being 56 years. Most tumours were nodular comprising a mixture of polygonal and spindle cells at different ratios with and without junctional activity. Four cases (4 of 17; 23.5%) stained positively for c-Kit. The pattern of staining was diffuse in three cases and focal in one and was predominantly membranous.

Conclusion: As almost invariably all OMM affects the palate and the gingiva of black persons, any melanotic hyperpigmentation at these sites should be biopsied and microscopically examined. Any hyperpigmentation that microscopically show proliferation of atypical melanocytes should be excised.

Keywords
Oral mucosal, melanoma, melanocytes, MC1R, c-Kit, CD117

Date received: 02 February, 2017; accepted: 21 March, 2017
OMM is rare, accounting for approximately 1% of all melanomas, and 25% of mucosal melanomas of the head and neck. They constitute only 0.5% of all oral malignancies. OMMs are usually painless and grow quickly and therefore are diagnosed late in the course of the disease when the lesions are already large. OMMs metastasize to regional lymph nodes early in the course of the disease and spread to distant sites by haematogenously. The prognosis is poor with a reported 5-year survival rate of 15–20%.

Although the pathogenesis remains largely unknown, malignant transformation of melanocytes occurs through the sequential accumulation of an unknown number of genetic and molecular alterations. The nature, number, type and sequence of these alterations differ among the various types of melanoma. Tumours arising from sun-exposed and non-sun-exposed skin, acral lesions and those arising in the mucosa of the upper aerodigestive tract or of the anogenital tract may differ substantially in their oncogenic pathways. Interaction between multiple signalling pathways activated by various ligands is required for malignant transformation to occur, and there is evidence that by-products of melanogenesis, melanin itself and microenvironmental biological mediators regulating melanogenesis and regulating the function, proliferation and differentiation of melanocytes play a role in the pathogenesis of OMM.

The aim of this study is to report a series of cases of OMM in a South African population sample from the greater Johannesburg area and to assess the frequency of expression of c-Kit (CD117) in these cases.

**Material and methods**

**Data collection**

The data of all cases of OMM recorded in the Divisions of Oral Pathology and of Anatomical Pathology, University of Witwatersrand, Johannesburg, over the 45-year period 1968–2013 were reviewed. Data on pre-existing oral melanotic lesions, alcohol consumption, tobacco, betel/areca nut use and other risk factors were not systematically documented in the clinical records accompanying the specimens submitted for histopathological examination and so had to be omitted from the study. Age, gender, ethnicity/race, oral site affected, histopathological type, pattern and degree of pigmentation and cell morphology were tabulated.

**Diagnostic criteria**

A diagnosis of OMM was made when the microscopic examination of haematoxylin and eosin-stained specimen showed radial and/or invasive proliferation of atypical melanocytes; and when in the formalin-fixed paraffin-embedded sections, the immunomarkers S100, melan A, HMB 45 and microphthalmia-associated transcription factor (MITF) were detected. The degree of melanin pigmentation was categorized into amelanotic, moderate or intense according to the judgement of the reporting oral pathologist.

**Inclusion and exclusion criteria**

Only intra-oral melanomas were included in the study. Specimens of melanoma from the lip, salivary glands or maxillary antrum and melanomas metastatic to the oral cavity were excluded.

**Immunohistochemical procedures**

Although most of the cases predated the introduction of immunohistochemistry, in the few cases where immunohistochemical staining had been performed, the tumours had stained positively for S100, melan A (Novocastra, Newcastle upon Tyne, England), HMB 45 and MITF (Dako, Glostrup, Denmark). This is the panel of staining agents which is still routinely used in our laboratory for the immunohistochemical diagnosis of melanoma.

Immunohistochemistry was performed on deparaffinized 4 μm sections with polyclonal rabbit anti-human antibody, CD117, c-Kit (code A4502, Dako, Glostrup, Denmark), at a dilution of 1:750, using standard recommended manufacturer’s procedures and protocols. Appropriate positive controls were used, along with the omission of the primary antibody as a negative control. The chromagen used was 3-amino-9-ethylcarbazole. Red staining of the cell membranes was regarded as positive.

**Ethical clearance**

The ethics clearance numbers, M131063, specific for this study and M10744 for use of archival block material obtained from human tissues were issued by the Human Research Ethics Committee (Medical) of the University of the Witwatersrand, Johannesburg.

**Results**

The study population comprised 17 persons who had a total of 22 sites affected by OMM (Table 1). Thirteen persons (76%) had one site affected, three (18%) had two sites affected and only one (6%) had three sites affected by OMM. It was not possible to determine whether those persons with multiple affected oral sites had individual lesions of multifocal origin or multiple lesions that were a manifestation of lateral spread.

The demographic data are shown in Table 1. There was a wide age distribution. All persons were Black, except for one whose ethnicity was not recorded. The female to male (F:M) ratio was 1:0.9. In all cases, either the hard palate or the gingiva was affected.

It was not possible to characterize all the histological features of OMM as most of the specimens consisted of small incisal biopsies and few included any surface epithelium. When present the surface epithelium often showed no significant pathological change and no connection to the tumour.
Two cases showed features of atypical lentigenous hyperplasia with junctional proliferation. Only one case could be classified as an in situ melanoma with a radial growth pattern. This case showed Pagetoid spread and junctional activity forming nests of atypical melanocytes at the tips of the rete ridges (Figure 1(b) and (d)).

Morphologically, the tumour cells were arranged in solid sheets, invasive nodules or trabeculae, often exhibiting alveolar and organoid histomorphological growth patterns. The tumour cells were epithelioid, plasmacytoid, spindle, round or rhabdoid in shape (Figure 1(e) to (g)). Increased and abnormal mitotic activity was present in all cases and the tumour cells invariably displayed prominent amphophilic nucleoli (Figure 1(g) and (h)). Most cases showed variable amounts of melanin, which at times was so abundant that the pigment obscured the tumour cells (Figure 1(a)). There were no amelanotic cases. Perineural infiltration was evident in a few cases.

As most of the biopsy specimens were small, it was not possible to assess features such as tumour depth (Breslow thickness), evidence of host immune response causing tumour regression and presence and number of tumour infiltrating lymphocytes. Owing to this nature of the biopsy specimens, in 13 cases (76%), it was not possible to establish with certainty whether the tumour had arisen from epithelial melanocytes or from melanocytes in the lamina propria; certainly one case (6%) arose from melanocytes in the lamina propria as there was no evidence of a physical connection with intraepithelial melanocytes; and three cases (18%) arose from intraepithelial melanocytes that showed junctional activity.

C-kit (CD117; Dako, Glostrup, Denmark) immunopositivity was noted in four cases (23.5%). In three of these, the staining distribution was diffuse while in the remaining case it was focal. The intensity of staining varied from faint to intense. Staining of the cell membranes created a ‘chicken wire-like’ staining pattern (Figure 2(a) and (b)).

Discussion
The mechanisms causing malignant transformation of normal melanocytes are unknown; but it is very likely that complex processes such as dysregulation of the cell cycle, of apoptosis

Table 1. Demographic data of patients with OMMs.

|                        | Males | Females | Total |
|------------------------|-------|---------|-------|
| Number                 | 8     | 9       | 17    |
| Age
  Mean                 | 48.43 | 62.88   | 56.13 |
  Range                 | 20–65 | 27–78   | 20–78 |
  Median                | 48    | 68      | 59    |
| Ethnicity
  Black                | 8 (100%) | 8 (89%) | 16 (94%) |
  Unknown              | 0     | 1 (11%) | 1 (6%) |
| Sites affected
  Upper alveolar mucosa | 1     | 0       | 1     |
  Upper gingiva         | 2     | 2       | 4     |
  Lower gingiva         | 1     | 0       | 1     |
  Hard palate           | 7     | 9       | 16    |
| Degree of melanin pigmentation
  Amelanotic            | 0     | 0       | 0     |
  Moderate              | 4     | 3       | 7     |
  Intense               | 4     | 6       | 10    |

OMM: oral mucosal melanoma.
Moreover, the binding of Fe²⁺ to melanins initiates a further low-level oxidative stress, which together with other environmentally derived redox-active metals within melanocytes, may promote melanomagenesis.¹⁴

Tyrosinase, tyrosinase-related protein (TRP)-1, TRP-2, p protein and melanoma antigen recognizable by T-lymphocytes are important proteins and enzymes for the formation and maintenance of the structure of melanosomes and for melanin biosynthesis.¹,¹⁶ Polymorphism of pigmentary genes encoding some of the above-mentioned proteins has been implicated in the pathogenesis of cutaneous melanoma,¹⁷ and it is possible that these gene variants also play a role in the development of OMM, but further research is needed to explore this possibility.

Pro-opiomelanocortin and its derivatives, particularly α melanocyte-stimulating hormone (αMSH) are agonist ligands of the MC1R on melanocytes. αMSH/MC1R intracellular pathway in melanocytes triggers the production of both brown-black eumelanin and of yellow-red pheomelanin and regulates some functions of melanocytes, including melanocyte proliferation and differentiation.¹,¹⁶ In Whites, MC1R gene is highly polymorphic with some genetic variants mediating the production of more pheomelanin and less eumelanin, with the generation of more mutagenic-free radicals, that may, as has been explained above, play a role in the initiation and progression of melanoma.¹³,¹₈

In addition to the unique role of the αMSH/MC1R pathway in melanin production, non-melanogenic functions of MC1R mediated via the αMSH/MC1R pathway include regulation of local inflammatory responses,¹⁷ mediation of melanocyte proliferation and survival,¹⁹ induction of DNA repair following UV-induced DNA damage¹⁷,²⁰ and diminution of oxidative stresses by reducing the generation of ROS.²¹ These important non-melanogenic functions are dysregulated in melanocytes expressing MC1R variants, hence increasing the risk of cutaneous melanomas.¹₂,¹₃,¹₉,²₀,²²

Thus, some MC1R variants also play a direct role in the pathogenesis of cutaneous melanoma apart from their role in determining the cancer-prone pigmentation phenotype of red hair, blue eyes and fair skin; and it appears that the risk for melanoma is polygenic comprising interactions between MC1R variants, other pigmentary gene variants and non-melanogenic genes including DNA repair genes, oncogenes and immuno-inflammatory genes.¹₇,²₂,²₃

Not only do the intermediate metabolites of melanogenesis play a role in melanomagenesis, but equally significantly they have immuno-suppressive properties. If released into the micro-environment, they may contribute to evasion of immune responses by the melanoma cells,² thus rendering ineffective T cell-mediated immune responses against tumour-specific antigens that may directly cause lysis of tumour cells, and therefore limit tumour growth.²⁴

About 10% of OMM are amelanotic,²⁵,²⁶ the rest exhibiting varying amounts of melanin deposits. In our case series, we did not have any cases of amelanotic OMM, but 10 cases (59%) showed intense melanin pigmentation (Table 1). It is thus clear that the quantum of melanin in most OMM is increased, but it is and of cell-to-cell interactions, other endogenous factors and environmental factors play important roles. A family history of melanoma or a personal history of melanoma, dysplastic pigmented nevi or atypical melanocytic proliferations are all important risk factors for OMM.¹¹ Melanin itself, melanin intermediates and melanocortin receptor 1 (MC1R) genetic polymorphism also appear to play a role in the pathogenesis of OMM.²,¹²,¹₃

Melanins, brown-black eumelanin and yellow-red pheomelanin are ubiquitous animal pigments, produced within melanocytes in membrane bound organelles named melanosomes.¹⁴ Melanins are polymers, enzymatically synthesized from tyrosine through a complex process. Melanins determine colour of the skin, eyes and hair and also influence the colour of the oral mucosa; they sequester metallic ions, bind drugs and organic molecules and neutralize reactive oxygen species (ROS), thus providing some protection from microenvironmental stressors.¹ However, on the other hand, melanogenesis itself, particularly the process of production of pheomelanin, if dysregulated, generates ROS that may cause DNA damage, and metabolic by-products of melanogenic processes may be cytotoxic, genotoxic or mutagenic,¹,² thus contributing to the development of melanoma.

It has been shown that in melanoma cells the melanosomes are abnormal showing loss of integrity of the melanosomal membrane with leakage of melanin fragments, of intermediates of melanogenesis and of ROS into the cell.¹⁴ These toxic agents may damage mitochondria causing increased intracellular levels of Ca²⁺ and of Fe²⁺ that is released from intracellular ferritin stores, further generating ROS, thus adding to the DNA damage and to the increased risk of genetic mutations.¹⁵ Moreover, the binding of Fe²⁺ to melanosomes creates a 'chicken wire'-like pattern (immunoperoxidase, c-Kit; original magnification ×200). (b) A different case showing moderately positive red-brown staining pattern (immunoperoxidase, c-Kit; original magnification ×200).

Figure 2. (a) Diffuse and intensely positive red-brown staining reaction predominantly of cell membranes creating a ‘chicken wire-like’ staining pattern (immunoperoxidase, c-Kit; original magnification ×200).
unclear whether the process of increased melanin production is an early biopathological event in melanomagenesis, contributing to initial transformation of melanocytes, or whether the dysregulated increased production of melanin occurs concurrently with or subsequent to the malignant transformation of normal melanocytes. If the reports that up to one-third of OMMs arise from pre-existing melanin hyperpigmentation are to be believed, it is reasonable to assume that both the dysregulated process of melanogenesis and the pre-existing increased quantity of melanin should be regarded as a risk factor for melanoma. We could not establish whether the OMM in this study arise de novo or from benign or physiological hyperpigmentations, because this information was not available.27

Particularly when they affect the palate or the maxillary gingiva, benign oral melanin hyperpigmentations including physiological/racial pigmentation, lentigo simplex, melanotic maculae, pigmented nevi, atypical melanocytic proliferation or Hutchinson’s melanotic freckles have been associated with an increased risk of OMM.25,28–30 If some of the melanocytes in these benign lesions happen to have been cytogenetically altered by one of a variety of intrinsic or extrinsic agents so that these cells are already predisposed to malignant transformation, additional molecular alterations to the melanocytes of these usually benign melanotic lesions will bring about the development of OMM.3,7 Any signs of changes in size, colour, texture or contour in histopathologically confirmed benign melanotic hyperpigmentations, should arise suspicion and prompt another biopsy and treatment based on the histopathological findings.3

Non-familial cutaneous melanomas (excluding acral melanomas) may show inactivating mutations of the tumour suppressor gene PTEN; genetic alterations of receptor tyrosine kinases and its downstream neuroblastoma rat sarcoma (NRAS)/B-rapidly accelerated fibrosarcoma (BRAF)/extracellular signal regulated kinase (ERK) pathway; upregulation of mitogen activated protein kinase ([MAPK] also known as RAS/RAF/MEK/ERK pathway); or activating mutations of BRAF or NRAS. In contrast, mucosal and acral melanomas show gain-of-function mutations of c-Kit receptor (CD117) in up to 40% of cases but lack mutations in either NRAS or BRAF.9,11,31 c-Kit, a tyrosine kinase receptor, and its ligand, stem cell factor, play an essential role in melanocyte development during embryogenesis,32 and later post-developmmentally in regulation of growth, proliferation and differentiation of melanocytes.33 In melanocytes, overexpression of c-Kit and gain-of-function mutations or amplification of c-Kit gene activate the intracellular molecules, RAS and RAF that are involved in the MAPK transduction pathway, resulting in increased cell proliferation and cell survival.33–35 These molecular events characteristic to mucosal melanomas occur independent of NRAS or BRAF molecular alterations.35 These distinct molecular alterations in mucosal melanomas indicate that the pathogenesis of mucosal and cutaneous melanomas differs, and that in those subjects with OMMs expressing KIT gain-of-function mutations, treatment with c-Kit inhibitors that target c-Kit intracellular molecular pathways may bring about beneficial clinical outcomes.36 In our series, four cases (23.5%) overexpressed c-Kit, but we have not yet confirmed whether gain-of-function mutations were present.

OMM is a relatively rare cancer, accounting for about 0.5% of all oral cancers.7,37 Only 17 confirmed cases of OMM have been diagnosed at this department of oral pathology during the 45-year period between 1968 and 2013. For unknown reasons, OMM more frequently affects Blacks, American Indians and Japanese, than Whites (Caucasians).2,26,28,29,34,38,39 Despite the fact that this laboratory provides services to all Blacks, Whites and minority ethnic groups, in this case series, 94% of the persons with OMM were Black. To the best of our knowledge, the prevalence of OMM among Blacks and Whites in South Africa is unknown.

Clinically, most cases of OMM are irregularly shaped macular, nodular or plaque-like lesions or a combination of these forms. They vary in colour from black to dark blue-grey to brown.2,40 The primary lesion is often surrounded by one or more satellite nodules. Although various authors reported either male or female predominance of OMM, in our cases, the gender distribution was equal. Most studies have reported that the anterior hard palate and maxillary gingiva are the most commonly affected sites with other oral sites occasionally affected. In all of our cases, the palate or gingiva were affected. The age of the subjects in our case series ranged from 20 to 78 years (Table 1) with a peak frequency after the age of 50 years, in agreement with the literature.4,26,31,42

The 5-year survival rate of OMM is poor (15–20%), with a mean survival time of 2 years.2,43 Larger size at diagnosis, a deeper invasive front, higher mitotic rates, vascular or neural invasion, upregulation of expression of vascular endothelial growth factor, hypoxia-inducible factor 1-alpha and CD34 at the invasion front, aberrant expression of p53 protein and loss of expression of p16 are some of the clinical, microscopical and molecular factors associated with poor prognosis.9,44,45

Histopathologically, OMM are classified as in situ in which the tumour is limited to the epithelium and the epithelial–connective tissue interface; invasive in which the melanoma cells have invaded the lamina propria or beyond; and a combined pattern in which parts of the lesion are in situ and parts have become invasive.25,38,46 It has been reported that at the time of diagnosis, about 15% of OMMs have an in situ pattern, 30% an invasive pattern and 55% having a combined pattern.25,40 Like cutaneous melanomas, the invading OMM cells are polymorphic varying from epitheloid to plasmacytoid to spindle shaped and are usually arranged in sheet-like solid, nodular, trabecular or alveolar configurations.26,28,29,40,46

OMM can arise either from melanocyte progenitors residing in the oral epithelium or from melanocyte progenitors residing in the lamina propria or submucosa.2 In this case series, owing to the inadequacy of the biopsy specimens, in 13 cases (76%), it was not possible to establish with certainty whether the OMM had arisen from epithelial or from lamina propria melanocytes. However, we are confident that one case (6%) arose from
melanocytes in the lamina propria, as there was no evidence of their physical connection with intraepithelial melanocytes breaching the basement membrane; and one case (18%) definitely arose from intraepithelial melanocytes that showed junctional activity with a radial growth pattern.

It is difficult to gain a complete picture of the entire pathological spectrum of OMM, firstly because the disease is rare, secondly the tumour is aggressive and rapidly growing and too often is diagnosed late in the course of the disease and thirdly because biopsy specimens are often incisional and small and not representative of the entire tumour. Therefore, the full depth of the tumours, the features of the lateral margins and often even their surface features cannot be characterized. All these limitations apply to our study so it was not possible to systematically classify the histopathological patterns of our OMM cases. Further research is required to identify all the oncogenic events necessary for the progression of benign fields of oral melanin hyperpigmentations to OMM and to determine whether or not extrinsic factors play any role in melanomagenesis.

Conclusion

The pathogenesis of OMM is complex. Only some of the dysregulated intracellular molecular pathways have been identified, but there are no established risk factors associated with OMM. It predominantly affects the palate and gingiva, with no gender predilection. In general, mucosal melanoma runs a more aggressive clinical course than cutaneous melanoma, with distinct molecular tumorigenic pathways. Oral melanotic hyperpigmentations should be biopsied and histologically examined to promote detection and adequate treatment of any atypical melanocytic lesions.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

References

1. Feller L, Masilana A, Khammissa RA, et al. Melanin: the biophysical-physiological of oral melanocytes and physiological oral pigmentation. Head Face Med 2014; 10: 8.

2. Thloele MM, Khammissa RA, Bouckaert M, et al. Oral mucosal melanoma: some pathobiological considerations and an illustrative report of a case. Head Neck Pathol 2015; 9: 127–134.

3. Feller L, Masipa JN, Wood NH, et al. Primary oral melanoma associated with HIV infection. SADJ 2008; 63: 016–017.

4. Sun CZ, Chen YF, Jiang YE, et al. Treatment and prognosis of oral mucosal melanoma. Oral Oncol 2012; 48: 647–652.

5. Benoist LB and van Looij MA. Images in clinical medicine. Melanoma of the oral cavity. N Engl J Med 2013; 368: e14.

6. Mihajlovic M, Vlajkovic S, Jovanovic P, et al. Primary mucosal melanomas: a comprehensive review. Int J Clin Exp Pathol 2012; 5: 739–753.

7. Lourenco SV, Bologna SB, Hsieh R, et al. Establishment and characterization of an oral mucosal melanoma cell line (MEMO) derived from a longstanding primary oral melanoma. Am J Dermatopathol 2013; 35: 248–251.

8. Rivera RS, Nagatsuka H, Gunduz M, et al. C-kit protein expression correlated with activating mutations in KIT gene in oral mucosal melanoma. Virchows Arch 2008; 452: 27–32.

9. Postow MA, Hamid O and Carvajal RD. Mucosal melanoma: pathogenesis, clinical behavior, and management. Curr Oncol Rep 2012; 14: 441–448.

10. Carlson JA, Murphy M, Slominski A, et al. Evidence of skin field cancerization. In: Dakubo GDE (ed.) Filed cancerization: basic science and clinical applications. Ontario: Nova Science Publishers, 2011, pp. 317–370.

11. Bandarchi B, Jabbari CA, Vedadi A, et al. Molecular biology of normal melanocytes and melanoma cells. J Clin Pathol 2013; 66: 644–648.

12. Han J, Kraft P, Colditz GA, et al. Melanocortin 1 receptor variants and skin cancer risk. Int J Cancer 2006; 119: 1976–1984.

13. Nan H, Kraft P, Hunter DJ, et al. Genetic variants in pigmentation genes, pigimentary phenotypes, and risk of skin cancer in Caucasians. Int J Cancer 2009; 125: 909–917.

14. Gidanian S, Mentelle M, Meyskens FL, Jr., et al. Melenosomal damage in normal human melanocytes induced by UVB and metal uptake – a basis for the pro-oxidant state of melanoma. Photochem Photobiol 2008; 84: 556–564.

15. Pavel S, Van Nieuwpoort F, Van der Meulen H, et al. Disturbed melanin synthesis and chronic oxidative stress in dysplastic naevi. Eur J Cancer 2004; 40: 1423–1430.

16. Feller L, Chandran R, Kramer B, et al. Melanocyte biology and function with reference to oral melanin hyperpigmentation in HIV-seropositive subjects. AIDS Res Hum Retroviruses 2014; 30: 837–843.

17. Scherer D, Bermejo JL, Rudnai P, et al. MC1R variants associated with susceptibility to basal cell carcinoma of skin: interaction with host factors and XRCC3 polymorphism. Int J Cancer 2008; 122: 1787–1793.

18. Palmer JS, Duffy DL, Box NF, et al. Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? Am J Hum Genet 2000; 66: 176–186.

19. Kennedy C, ter Huurne J, Berkhout M, et al. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. J Invest Dermatol 2001; 117: 294–300.

20. Hauser JE, Kadekaro AL, Kavanagh RJ, et al. Melanin content and MC1R function independently affect UVB-induced DNA damage in cultured human melanocytes. Pigment Cell Res 2006; 19: 303–314.

21. Abdel-Malek ZA, Ruwe A, Kavanagh-Starner R, et al. alpha-MSH tripeptide analogs activate the melanocortin 1 receptor and reduce UV-induced DNA damage in human melanocytes. Pigment Cell Melanoma Res 2009; 22: 635–644.
22. Feller L, Khammissa RA, Kramer B, et al. Basal cell carcinoma, squamous cell carcinoma and melanoma of the head and face. *Head Face Med* 2016; 12: 11.
23. Ghanem G and Fabrice J. Tyrosinase related protein 1 (TYRP1/gp75) in human cutaneous melanoma. *Mol Oncol* 2011; 5: 150–155.
24. Slavin S. Effector cells of experimental and clinical cellular adoptive immunobiology. In: Morstyn G and Sheridan W (eds) *Cell therapy: stem cell transplantation, gene therapy, and cellular immunotherapy*. Los Angeles: Cambridge University Press, 1996, pp. 18–42.
25. Femiano F, Lanza A, Buonaiuto C, et al. Oral malignant melanoma: a review of the literature. *J Oral Pathol Med* 2008; 37: 383–388.
26. Mohan M, Sukhadia VY, Pai D, et al. Oral malignant melanoma: systematic review of literature and report of two cases. *Oral Surg Oral Med Oral Pathol Oral Radiol* 2013; 116: e247–254.
27. Feller L, Khammissa RAG and Lemmer J. Oral mucosal melanosis. In: Blumenberg M (ed.) *Melanin*. Croatia: Intech, 2017, pp. 7–26.
28. Meleti M, Leemans CR, Mooi WJ, et al. Oral malignant melanoma: a review of the literature. *Oral Oncol* 2007; 43: 116–121.
29. Umeda M, Komatsubara H, Shibuya Y, et al. Premalignant melanocytic dysplasia and malignant melanoma of the oral mucosa. *Oral Oncol* 2002; 38: 714–722.
30. Kahn MA, Weathers DR and Hoffman JG. Transformation of a benign oral pigmentation to primary oral melanoma. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2005; 100: 454–459.
31. Furney SJ, Turajlic S, Stamp G, et al. Genome sequencing of mucosal melanomas reveals that they are driven by distinct mechanisms from cutaneous melanoma. *J Pathol* 2013; 230: 261–269.
32. Kumano K, Masuda S, Sata M, et al. Both Notch1 and Notch2 contribute to the regulation of melanocyte homeostasis. *Pigment Cell Melanoma Res* 2008; 21: 70–78.
33. Seetharamu N, Ott PA and Pavlick AC. Mucosal melanomas: a case-based review of the literature. *Oncologist* 2010; 15: 772–781.
34. Papaspyrou G, Garbe C, Schadendorf D, et al. Mucosal melanomas of the head and neck: new aspects of the clinical outcome, molecular pathology, and treatment with c-kit inhibitors. *Melanoma Res* 2011; 21: 475–482.
35. Buery RR, Siar CH, Katase N, et al. NRAS and BRAF mutation frequency in primary oral mucosal melanoma. *Oncol Rep* 2011; 26: 783–787.
36. Williams MD. Update from the 4th edition of the World Health Organization Classification of Head and Neck tumours: mucosal melanomas. *Head Neck Pathol* 2017; 11: 110–117.
37. Wang X, Wu HM, Ren GX, et al. Primary oral mucosal melanoma: advocate a wait-and-see policy in the clinically N0 patient. *J Oral Maxillofac Surg* 2012; 70: 1192–1198.
38. Mhapuskar A, Umarji H, Jain N, et al. Intra-oral malignant melanoma – a case report and review of the literature. *N Z Dent J* 2012; 108: 102–104.
39. Takagi M, Ishikawa G and Mori W. Primary malignant melanoma of the oral cavity in Japan. With special reference to mucosal melanosis. *Cancer* 1974; 34: 358–370.
40. 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.
41. Shen ZY, Liu W, Bao ZX, et al. Oral melanic macule and primary oral malignant melanoma: epidemiology, location involved, and clinical implications. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; 112: e21–e25.
42. Garzino-Demo P, Fasolis M, Maggiore GM, et al. Oral mucosal melanoma: a series of case reports. *J Craniofac Surg* 2004; 32: 251–257.
43. Hicks MJ and Flaitz CM. Oral mucosal melanoma: epidemiology and pathobiology. *Oral Oncol* 2000; 36: 152–169.
44. Kerr EH, Hameed O, Lewis JS, Jr., et al. Head and neck mucosal malignant melanoma: clinicopathologic correlation with contemporary review of prognostic indicators. *Int J Surg Pathol* 2012; 20: 37–46.
45. Prasad ML, Patel SG, Shah JP, et al. Prognostic significance of regulators of cell cycle and apoptosis, p16(Ink4a), p53, and bel-2 in primary mucosal melanomas of the head and neck. *Head Neck Pathol* 2012; 6: 184–190.
46. Vikey AK and Vikey D. Primary malignant melanoma of head and neck: a comprehensive review of literature. *Oral Oncol* 2011; 48: 399–403.

**Translational Value**

The deficiencies in this study draw attention to the fact that in order to develop a better strategy of treatment of oral mucosal melanoma, more comprehensive clinicopathological data are needed. Further research is required to identify whether or not extrinsic factors play any role in melanomagenesis.