KIT, NRAS, BRAF and *FMNL2* mutations in oral mucosal melanoma and a systematic review of the literature

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Abstract. Oral mucosal melanoma (OMM) is an aggressive malignant tumor derived from melanocytes in the oral cavity. The genetic etiology of OMM has not been extensively investigated to date. In the present study, the aim was to detect novel gene mutations in patients with OMM. Mutation analysis of *KIT*, *BRAF* and *NRAS* was conducted by polymerase chain reaction. In addition, the relevant literature was searched using the PubMed database, and previous findings were compared with the results of the present study. Among the 9 patients with OMM examined, *KIT*, *BRAF* and *NRAS* mutations were detected, and these mutations were all observed at a frequency of 11.1% (1/9 patients). Notably, a novel *FMNL2* mutation in 2 patients with OMM was identified by exome sequencing. In conclusion, the current study observed *KIT*, *BRAF*, *NRAS* and *FMNL2* mutations in patients with OMM, which may be of benefit for elucidating the underlying mechanism of OMM pathogenesis.

Introduction

Malignant melanoma (MM) is an aggressive tumor derived from melanocytes, which has a high degree of malignancy with rapid progression and early metastasis. Patients with this tumor have a poor prognosis and low survival rate (1,2). Despite the majority of MMs originating from the skin (cutaneous epithelia), they can arise in almost any part of the body. Cutaneous melanoma, which comprises 91.2% of all melanoma cases, is always associated with sun exposure (3). However, mucosal melanoma is another type of MM that is observed in regions with low sun exposure. Mucosal melanoma is rare, with the head and neck being the most common sites, accounting for 1.4% of all melanomas (4). In total, ~6.5% of mucosal melanomas may arise in the oral cavity and differ from their cutaneous counterparts in terms of pathogenesis, biological behavior and prognosis (5).

Oral mucosal melanoma (OMM) is estimated to account for 0.2-8% of all MM cases, and appears to be more common in Asia, including Japan and India, as compared with Western areas (6,7). In recent years, the incidence of mucosal melanoma has increased significantly, particularly in Asia. The prognosis of OMM is worse compared with cutaneous melanoma, with a greater tendency to metastasize and a 5-year survival rate of only 8-15% (5,7). In contrast to cutaneous melanoma, which is associated with sun exposure among other factors, the precise etiology of OMM has not been defined due to various different influencing factors including ingested tobacco, ingested alcohol and inhaled environmental carcinogens, including smoke and formaldehyde (8). Although the use of tobacco, mechanical trauma and denture use have been reported as possible risk factors for OMM, the genetic etiology has not been extensively investigated, and the pathogenesis remains unclear (7,9).

Considering its highly aggressive biological behavior, the pathogenesis of melanoma has been examined in numerous studies to identify more effective treatment approaches (10-12). In 2002, Davies *et al* (13) reported that ~50% of all melanomas harbored an activating mutation in *BRAF*. Among all *BRAF* mutations, the V600E mutation accounted for >90% of cases. This study led to a melanoma genomic revolution, and the genetic etiology and potential therapeutic targets of this disease have become a focus for research. A number of *BRAF* inhibitors have been applied to clinical practice and have been demonstrated to inhibit melanoma proliferation (11,12).

Melanoma arising in different parts of the body may be associated with diverse molecular genetic profiles, suggesting that those tumors may represent a distinct pathogenesis. In recent years, an increasing number of studies have been focused on *KIT, BRAF, NRAS* and other mutations. The *KIT* mutation is more common in mucosal and acral melanomas than in cutaneous melanomas, and the percentage of *KIT*

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Abbreviations: MM, malignant melanoma; OMM, oral mucosal melanoma; MAPK, mitogen-activated protein kinase

Key words: oral mucosal melanoma, *KIT, BRAF, NRAS, FMNL2*

mutations detected in mucosal melanoma has been widely recognized to be 10-35% (14). The *BRAF* mutation is the most common mutation in cutaneous melanoma with a high incidence. However, several studies have demonstrated a low incidence of *BRAF* mutation in melanomas arising from non-hair-bearing skin, mucosa and internal organs that are totally sun protected, while the *BRAF* mutation is also rare in uveal melanomas (15-17). Furthermore, mutations in *NRAS* have been detected in 15-25% of melanoma patients (18). The frequency of all these mutations vary according to different methods of sourcing the analyzed tumor and differences in processing methods, although there appears to be no significant difference between cutaneous and mucosal melanoma.

While the genetic etiology of cutaneous melanoma has been widely reported, few studies have described the distribution pattern of mutations in OMM and investigated the cause of its variety. Thus, the aim of the present study was to detect the presence of *KIT*, *BRAF* and *NRAS* mutations in 9 OMM patients. In addition, exome sequencing was conducted to identify the genes associated with OMM.

Table I. Patient information.

Patient	Age, years	Sex	Location	Lymph node metastasis	Recurrent melanoma
1	61	M	Upper gingiva	+	-
2	69	M	Upper gingiva	+	-
3	57	M	Upper gingiva	+	-
4[a]	56	M	Upper and lower gingiva	+	-
5	44	M	Lower gingiva	+	-
6	47	M	Lower gingiva	+	-
7	61	F	Buccal mucosa	+	+
8	51	M	Upper lip vermilion	+	+
9	42	F	Lower gingiva	+	+

[a]Patient presented multiple melanomas at the upper and lower gingiva, and the paraffin-embedded sample was obtained from the upper gingiva. M, male; F, female; +, present; -, absent.

Materials and methods

Tumor samples and clinical background. Information on the characteristics of the patients included into the present study is listed in Table I. In total, 9 patients with a histologically proven diagnosis of OMM were selected from the Department of Oral and Maxillofacial Surgery, Peking University Hospital of Stomatology (Beijing, China) between July 2009 and November 2014. Diagnosis was based on the criteria provided by the World Health Organization on the classification of head and neck tumors (19). The study was reviewed and approved by the Ethics Committee of the Peking University Hospital of Stomatology (Beijing, China). Written informed consent was obtained from all subjects prior to tissue collection.

Genomic DNA extraction and mutation analysis. The tumor and para-carcinoma tissues were transported to the Center Laboratory (Peking University Hospital of Stomatology) within 30 min of surgery in 4˚C Krebs-Ringer Hepes (KRH) solution (containing 120 mM NaCl, 5.4 mM KCl, 1 mM $CaCl_2$, 0.8 mM $MgCl_2$, 11.1 mM glucose and 20 mM HEPES, pH 7.4) and aerated with 95% O_2. For DNA isolation, tissues were minced and homogenized in 80 μl PBS using Tissue Ruptor (IKA, Staufen, Germany). Then, the genomic DNA was extracted using a QIAamp DNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's protocols. The concentration of the extracted DNA was determined using the NanoDrop 8000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA). Exons 11 and 15 of the *BRAF* gene (NG_007873.3), exons 1 and 2 of the *NRAS* gene (AH001530.2), and exons 11 and 13 of the *KIT* gene (NG_007456.1) were amplified by polymerase chain reaction (PCR) with six pairs of primers that covered the entire coding region of OMM. Primer sequences from previous studies used for PCR amplification are presented in Table II (20,21). PCR assays were performed in a 30 μl reaction with KOD-plus enzyme (Toyobo Life Science, Osaka, Japan), as previously described (22). Following an initial denaturation step at 95˚C for 5 min, 30 cycles of amplification consisting of denaturation at 94˚C for 1 min, annealing at 55˚C for 30 sec and extension at 72˚C for 45 sec were conducted, followed by a final extension step at 72˚C for 10 min. The PCR products were separated by 1% agarose gel electrophoresis and purified using an AxyPrepDNA gel Extraction kit (Axygen Scientific, Inc., Union City, CA, USA). Products were then sequenced using an ABI 3730XL DNA analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.). Mutations were analyzed using Polymorphism Phenotyping (version 2; http://genetics.bwh.harvard.edu/pph2/dbsearch.shtml) and the probability of missense mutations being damaging based on scores allocated to a combination of properties (including single-nucleotide polymorphisms (SNPs), maps coding SNPs to gene transcripts, extraction of protein sequence annotations and structural attributes, and construction of conservation profiles) was estimated, with a score closer to 1 indicating a greater incidence of missense mutation (23).

Exome sequencing and variant calling. The genomic DNAs of patients no. 6 and 10 (tumor and para-carcinoma tissue), as presented in Table I, were randomly fragmented into 150-200 bp. Next, library construction, exome capture and sequencing were performed according to the protocol of the Sure SelectXT Target Enrichment System for Illumina Paired-End Sequencing Library, Illumina HiSeq and MiSeq Multiplexed Sequencing Platforms (Illumina, Inc., San Diego, CA, USA). Sequencing by synthesis was performed on a HiScanSQ sequencer (Illumina, Inc.) using the paired-end method. BAM files summarizing Burrows-Wheeler alignments that were mapped to the hg19 reference sequence were generated using the Genome Analysis Toolkit (GATK) (22,23). The raw reads with all characteristics that were not confidently aligned, exhibited a ratio of N >10%, a length <25 bp without adapter and a quality score of <20 by Phred-scaled mapping

Table II. Primer sequences of *KIT, BRAF* and *NRAS* exons used in polymerase chain reaction.

Gene	Exon	Size (bp)	Forward primer (5'-3')	Reverse primer (5'-3')
KIT	11	289	TGTTCTCTCTCCAGAGTGCTCTAA	AAACAAAGGAAGCCACTGGA
KIT	13	294	CATCAGTTTGCCAGTTGTGC	AGCAAGAGAGAACAACAGTCTGG
BRAF	11	204	CTCTCAGGCATAAGGTAATG	CACTTTCCCTTGTAGACTGTT
BRAF	15	209	CCTAAACTCTTCATAATGCTT	ATAGCCTCAATTCTTACCAT
NRAS	1	174	CGCCAATTAACCCTGATTACT	CACTGGGCCTCACCTCTA
NRAS	2	196	CCCCTTACCCTCCACAC	AGGTTAATATCCGCAAATGAC

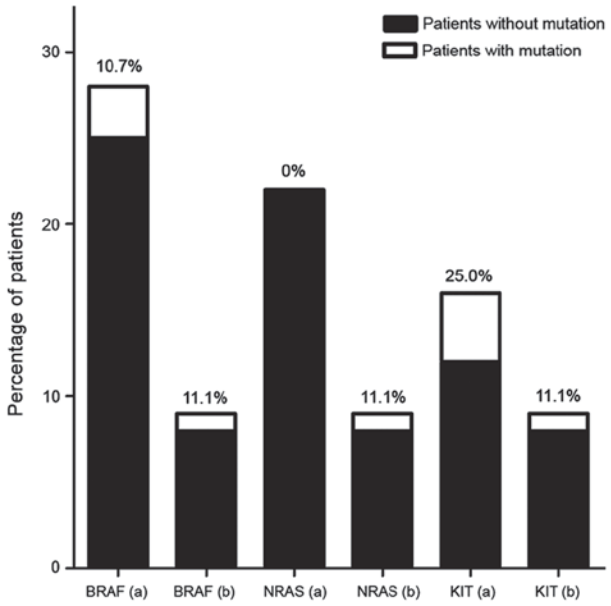

Figure 1. *KIT*, *BRAF* and *NRAS* mutations in oral mucosal melanoma. The percentage at the top of the histogram represents the rate of gene mutations observed in patients reported in the literature, denoted by (a) and the current study, denoted by (b).

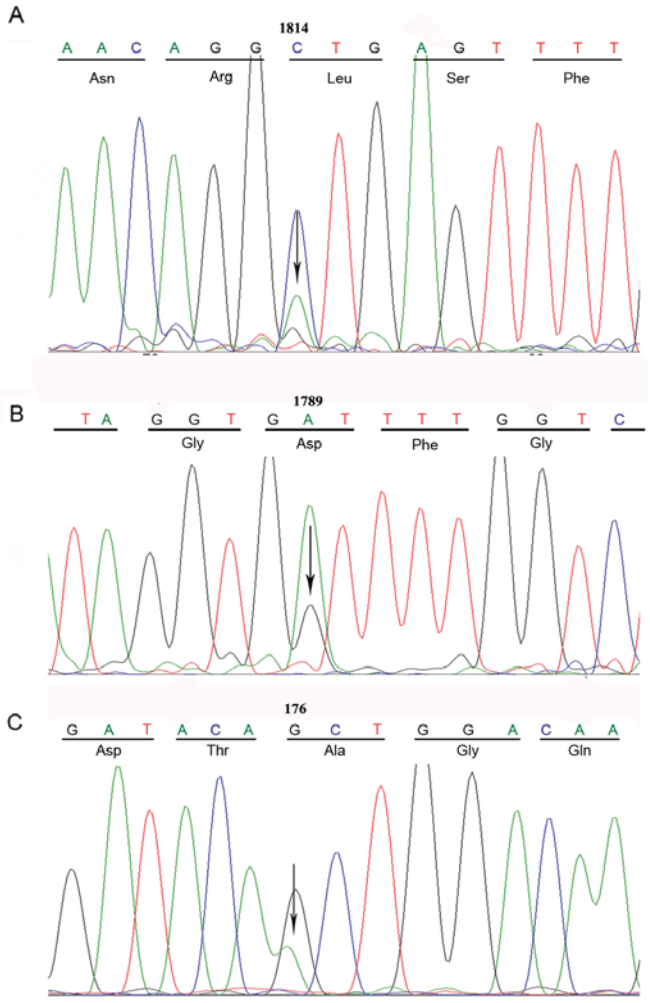

Figure 2. Comparison between mutation analysis and literature review results. (A) Alteration of *KIT* at nucleotide position 1814 of exon 11 is a heterozygous mutation from C to A and causes the p.L589M mutation. (B) Alteration of *BRAF* at nucleotide position 1789 of exon 15 is a heterozygous mutation from A to G and causes the p.D594G mutation. (C) Alteration of *NRAS* at nucleotide position 176 of exon 2 causes the p.A59T mutation.

were excluded from further analysis. The single nucleotide variants (SNVs), insertions and deletions were detected and annotated by the GATK and Annovar software (release date Feburay 1st, 2016; http://www.openbioinformatics.org/annovar/) (24).

Results

KIT, BRAF and NRAS mutations in patients with OMM. Among the 9 patients included into the present study, there were 7 males and 2 females with a median age of 54 years (age range, 42-69 years). Out of the 9 melanoma samples, 6 were obtained from the gingiva, 1 from the hard palate, 1 in the upper lip vermilion and 1 in the buccal mucosa (Table I).

Among the 9 primary oral mucosa melanoma samples that were analyzed in the present study, 3 patients (33.3%) exhibited *KIT, BRAF* or *NRAS* mutations. *KIT, BRAF* and *NRAS* mutations were each identified in 1/9 patients (11.1%; Fig. 1). Furthermore, a novel mutation was identified, namely L589M in exon 11 of the *KIT* gene, which had not been previously detected in melanomas or any other tumor types. The alteration in the *BRAF* gene was detected in exon 15 (D594G), which has been described in arcal melanoma (25). The *NRAS* mutation involved codon 59, and although a mutation at this position has been previously identified (25), a novel amino acid substitution (A59T) was detected in the current analysis (Table III and Fig. 2). No mutations in the adjacent tissues were observed.

Comparison between mutation analysis and literature review results. The present study performed a

Table III. *KIT*, *BRAF* and *NRAS* gene mutations in oral mucosal melanoma.

Case no.	Age/sex	Gene	Exon no.	Nucleotide definition	Amino acid definition	Functional effect	Polyphen-2 score
9	61/F	*KIT*	11	c.1814C>A	p.L589M	Missense	0.981
1	61/M	*BRAF*	15	c.1789A>G	p.D594G	Missense	0.983
7	44/M	*NRAS*	2	c.176G>A	p.A59T	Missense	0.745

M, male; F, female.

Table IV. *KIT*, *BRAF* and *NRAS* genes in oral mucosal melanoma mutations from the literature.

Authors, year	Gene	Exon no.	Amino acid definition	Functional effect	(Refs.)
Cohen *et al*, 2008	*KIT*	11	W557R, V569G	Missense	27
Cohen *et al*, 2008		13	K642E	Missense	27
			S645S, P585P	Synonymous	
Soma *et al*, 2014	*BRAF*	15	V600L, V600E	Synonymous	5
Buery *et al*, 2011					6
Buery *et al*, 2011	*NRAS*	2	K166K, F66F	Synonymous	6

systematic search of studies published between July 1969 and March 2015 (https://www.ncbi.nlm.nih.gov/pubmed). Additional information was identified by searching the reference lists of the included publications. Inclusion criteria for the literature review were as follows: i) Studies that included the key words 'oral and melanoma and (mutation OR mutated)'; ii) studies that examined gene mutations in patients with oral malignant mucosal melanoma; iii) studies that were only published in English. A preferred reporting items for systematic reviews and meta-analyses flowchart for literature attrition was also included (26). Only a single entry was made if the same mutation or patient was reported across multiple studies. Separate entries were made for each patient when the same mutation was detected in unrelated patients. In the systematic review conducted in the present study, a total of five studies (5,6,14,27,28) on gene mutations in OMM were identified, which satisfied the eligibility criteria. The distribution pattern of mutated genes is summarized in Fig. 1. The *KIT, NRAS* and *BRAF* mutations were detected in 3/28 (10.7%), 0/22 (0.0%), and 4/16 (25.0%) patients with OMM, respectively (5,6,14,25,26). In these articles, the 11 mutations observed were distributed on 5 exons (including exons 1, 2, 11, 13 and 15), of which there were 4 synonymous mutations and 6 missense mutations (Table IV). Of the 6 *KIT* mutations, 2 cases harbored the same mutation in exon 13, leading to a replacement in position 642 of a lysine by a glutamic acid (K642E) (6,29). In addition, 2 synonymous mutations (S645S and P585P) were detected in these 2 cases; however, they did not result in amino acid changes (28). A single mutation occurred at codon 557 in exon 11 and resulted in the replacement of a tryptophan by a glutamic acid L-arginine (W557R), which has already been previously described in gastrointestinal stromal tumors (29). Furthermore, the V569G mutation was identified in 1 case (28), which has not otherwise been previously reported. In regards to *BRAF*, the classical V600E substitution that is typically identified in cutaneous melanoma was detected in 2 patients previously (5,13). There was also another mutation located at codon 600, namely the V600L mutation, resulting in the replacement of the valine by leucine (6). No missense mutation of *NRAS* was reported in a previous study investigating OMM, but two synonymous mutations, *K166K* and *F66F*, were demonstrated in 2 patients (6).

FMNL2 mutations detected by exome sequencing. Exome sequencing was used to identify the potential pathogenic gene mutation in 6/9 patients in the present study, which were revealed not to be mutations in the *KIT, BRAF* or *NRAS* genes. However, only 2 of the 6 enrolled patients met the sequencing requirements. The exome sequencing in these 2 cases resulted in $2.1x10^8$ reads, yielding >99% exome coverage, with the exome average depth of each sample being between 58 and 70%. A total of 37 SNVs, as well as 5 insertion and deletion mutations, were detected in the 4 samples, while 22 mutations were observed in patient no. 6 and 15 mutations in patient no. 10. These mutations were only detected in the pathologic tissue, but not in the para-carcinoma tissue. More detailed information is listed in Fig. 3. These results revealed that OMM has an increased number of somatic mutations. Among the 37 SNVs, there was a *FMNL2* gene detected in the 2 patients (Fig. 3), which demonstrated a non-frame-shift insertion mutation, and these insertion mutations involved the area of exon 15. While this mutation was predicted to have no influence on the protein level, it may serve a role in transcription.

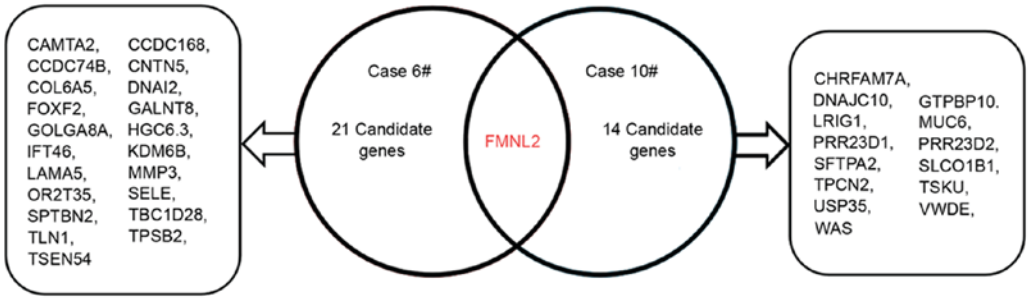

Figure 3. Genetic mutations associated with oral mucosal melanoma identified by exome sequencing in two patients of the present study.

Discussion

The RAS/RAF/mitogen-activated protein kinase kinase/extracellular signal-regulated kinase (ERK), also known as the mitogen-activated protein kinase (MAPK)/ERK pathway is the most common cell-signaling pathway in the progression of melanoma by regulating cell proliferation, differentiation and survival (30). The *BRAF* gene, encoding a serine/threonine protein kinase, is a well-known downstream molecule participating in this signal cascade. The V600E (Val600Glu) mutation that accounts for >90% of all BRAF mutations in cutaneous melanoma has been demonstrated to be activated by the RAS-guanosine triphosphate (GTP) protein, leading to ERK activation and stimulating the growth of melanoma cells (13,31). Another molecule that leads to the activation of the MAPK pathway is *NRAS*, leading to accumulation of RAS-GTP and affecting various downstream molecules, including phosphoinositide 3-kinase and RAF kinases (32,26). Codon 61 is the major position for *NRAS* alterations in melanoma, such as Q61H, Q61K and Q61L (32). Furthermore, the *KIT* gene, one of the receptor tyrosine kinases, is an important receptor for melanocyte proliferation and migration (33). The MAPK pathway may also be triggered by the activation of a *KIT* mutation, which participates in melanoma development through the induction of signaling proteins (34).

The *KIT* and *BRAF* mutations detected in the present study are consistent with the results of the literature review, while the mutation rate of *NRAS* was slightly different. This discrepancy in the results may be due to the inclusion of adjacent tissues in the samples used in previous studies. In addition, the small sample sizes utilized in the present study and systematic review may be another cause of this difference. However, in general, the results of the current study corresponded with the findings of previous studies included in the systematic review. The *BRAF* and *NRAS* mutations are the main genetic mutations in cutaneous melanoma cases, with a high incidence rate of 30-70 and 15-25%, respectively (15-18). In contrast, a low incidence of *BRAF* and *NRAS* mutations has been described in mucosal melanoma patients. It appears that the *BRAF* mutation is quite rare in mucosal melanoma, whereas the *NRAS* mutation has a higher frequency in comparison with *BRAF* in this melanoma, although its percentage remains <20%. With regard to the mutation frequency in OMM, 1/9 patients (11.1%) presented a *BRAF* mutation, while the same incidence (1/9; 11.1%) of *NRAS* mutations was detected in the present study. According to the results of the systematic review, 3/28 OMM patients (10.7%) were reported to exhibit a *BRAF* mutation, while no missense mutation of *NRAS* was reported among the 22 OMM patients (0.0%) included in previous studies (6,25). Thus, it is clear that the *BRAF* mutation in OMM occurs with a low incidence of <10%, which is consistent with the observations of previous studies on mucosal melanoma (20,35,36). The distinction of *BRAF* mutations existing between cutaneous melanoma and OMM may therefore reveal a different molecular pathogenesis between these two melanoma types, and the different frequency of the *NRAS* mutation in mucosal melanoma and OMM may indicate that OMM has a distinctive pathogenesis and is a separate subtype. Furthermore, the novel *NRAS* mutation (namely A59T) detected in the current study may provide a molecular target for further investigation. Additionally, missense mutations of *NRAS* (p.S39F) with a relatively low peak on the sequencing map were identified in 6 patients, which may be associated with the tumor heterogeneity phenomenon.

Prevalence differences in *KIT* mutations between melanomas were observed at different sites (20,35), with *KIT* mutations occurring less frequently in head and neck melanomas. This observation was also supported by the study of Beadling *et al* (36), who reported a higher incidence of *KIT* mutations in melanomas of the vulva, anorectum and vagina when compared with melanomas occurring at the head and neck sites. In addition, Schoenewolf *et al* (37) identified a high frequency of *KIT* mutations (45%) in vulvovaginal melanomas and no mutation in 12 sinonasal melanomas. On the basis of these previous findings, the frequency of *KIT* mutations in head and neck melanomas does not appear to be consistent, and this may be associated with differences in the sample quantity and the geographical region of patients. The two novel mutations detected by sequencing in the present study, namely p.L589M of *KIT* and p.A59Tof *NRAS*, have not been reported in primary mucosa melanoma. These results indicate that oral mucosa melanoma has different characteristics as compared with other mucosa melanomas. Although the significance of these novel mutations has yet to be revealed, the newly identified mutated sites may suggest a potential path to examine the mechanism underlying OMM pathogenesis.

In the present study cohort, 1 patient with a *KIT* mutation was observed, as well as 1 patient with an *NRAS* mutation and 1 patient with a *BRAF* mutation. The mutation rate of all the 3 genes was 1/9 (11.1%), and this result was consistent with previous OMM studies identified in the systematic

review (5,6,14,28,29), indicating that mutations of the *KIT*, *NRAS* and *BRAF* are associated with the occurrence of OMM. When compared with cutaneous melanoma, the distribution of *KIT* and *BRAF* mutations is markedly different in OMM (15,18). In contrast to its role in cutaneous melanoma, the mutated *KIT* serves a more significant role in comparison with the *BRAF* gene in the occurrence of OMM (13,31). However, on the basis of these results, the mutation rates of all the 3 genes are not sufficiently enough to support a definite relevance to OMM pathogenesis, as the highest rate was 26.7% for *KIT* (28). Therefore, it appears that none of the three mutations were the main genetic factors leading to OMM.

In order to reveal other factors involved in the pathogenesis of OMM, exome sequencing of candidate genes was performed in the current study, and the *FMNL2* gene was considered as a possible key molecule. This gene, as an encoding formin-associated protein gene, is essential in morphogenesis, cytokines is and cell polarity. The protein is strongly expressed in the central nervous system and numerous epithelia, whereas a lack of expression may cause weak immunoreactivity in certain mesenchymal cell types (38). Furthermore, it has been reported that the gene is associated with melanoma and colorectal tumors (38,39). In cultured melanoma cells, FMNL-2 co-localizes with F-actin at the tips of cellular protrusions, which supports the hypothesis that the gene is associated with the formation of actin filament in cellular protrusions during cellular migration. In consequence, the *FMNL2* mutation may be regarded as a potential cause of the oral melanoma in these patients.

In conclusion, despite a small cohort and a lack of extensive experimental repeat, the present study also revealed that *KIT*, *NRAS* and *BRAF* mutations may be associated with the occurrence of OMM, and that the *FMNL2* mutations may be regarded as a potential cause of OMM. The present study may be of benefit for elucidating the underlying mechanism of OMM, however, further studies are required to validate these conclusions.

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References

1. Balch CM, Gershenwald JE, Soong SJ, Thompson JF, Atkins MB, Byrd DR, Buzaid AC, Cochran AJ, Coit DG, Ding S, *et al*: Final version of 2009 AJCC melanoma staging and classification. J ClinOncol 27: 6199-6206, 2009.
2. Nikolaou V and Stratigos AJ: Emerging trends in the epidemiology of melanoma. Br J Dermatol 170: 11-19, 2014.
3. Gandini S, Sera F, Cattaruzza MS, Pasquini P, Picconi O, Boyle P and Melchi CF: Meta-analysis of risk factors for cutaneous melanoma: II. Sun exposure. Eur J Cancer 41: 45-60, 2005.
4. Mihajlovic M, Vlajkovic S, Jovanovic P and Stefanovic V: Primary mucosal melanomas: A comprehensive review. Int J Clin Exp Pathol 5: 739-753, 2012.
5. Soma PF, Pettinato A, Agnone AM, Donia C, Improta G and Fraggetta F: Oral malignant melanoma: A report of two cases with BRAF molecular analysis. Oncol Lett 8: 1283-1286, 2014.
6. Buery RR, Siar CH, Katase N, Gunduz M, Lefeuvre M, Fujii M, Inoue M, Setsu K and Nagatsuka H: NRAS and BRAF mutation frequency in primary oral mucosal melanoma. Oncol Rep 26: 783-787, 2011.
7. Meleti M, Leemans CR, Mooi WJ, Vescovi P and van der Waal I: Oral malignant melanoma: A review of the literature. Oral Oncol 43: 116-121, 2007.
8. Rapini RP, Golitz LE, Greer RO Jr, Krekorian EA and Poulson T: Primary malignant melanoma of the oral cavity. A review of 177 cases. Cancer 55: 1543-1551, 1985.
9. Gu GM, Epstein JB and Morton TH Jr: Intraoral melanoma: Long-term follow-up and implication for dental clinicians. A case report and literature review. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 96: 404-413, 2003.
10. Eggermont AM and Robert C: Melanoma: Smart therapeutic strategies in immuno-oncology. Nat Rev Clin Oncol 11: 181-182, 2014.
11. Sabbatino F, Wang Y, Wang X, Ferrone S and Ferrone CR: Emerging BRAF inhibitors for melanoma. Expert Opin Emerg Drugs 18: 431-443, 2013.
12. Romano E, Schwartz GK, Chapman PB, Wolchock JD and Carvajal RD: Treatment implications of the emerging molecular classification system for melanoma. Lancet Oncol 12: 913-922, 2011.
13. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, Teague J, Woffendin H, Garnett MJ, Bottomley W, *et al*: Mutations of the BRAF gene in human cancer. Nature 417: 949-954, 2002.
14. Colombino M, Lissia A, Franco R, Botti G, Ascierto PA, Manca A, Sini MC, Pisano M, Paliogiannis P, Tanda F, *et al*: Unexpected distribution of cKIT and BRAF mutations among southern italian patients with sinonasal melanoma. Dermatology 226: 279-284, 2013.
15. Wong CW, Fan YS, Chan TL, Chan AS, Ho LC, Ma TK, Yuen ST and Leung SY; Cancer Genome Project: BRAF and NRAS mutations are uncommon in melanomas arising in diverse internal organs. J Clin Pathol 58: 640-644, 2005.
16. Cohen Y, Rosenbaum E, Begum S, Goldenberg D, Esche C, Lavie O, Sidransky D and Westra WH: Exon 15 BRAF mutations are uncommon in melanomas arising in nonsun-exposed sites. Clin Cancer Res 10: 3444-3447, 2004.
17. Edwards RH, Ward MR, Wu H, Medina CA, Brose MS, Volpe P, Nussen-Lee S, Haupt HM, Martin AM, Herlyn M, *et al*: Absence of BRAF mutations in UV protected mucosal melanomas. J Med Genet 41: 270-272, 2004.
18. Uhara H, Ashida A, Koga H, Ogawa E, Uchiyama A, Uchiyama R, Hayashi K, Kiniwa Y and Okuyama R: NRAS mutations in primary and metastatic melanomas of Japanese patients. Int J Clin Oncol 19: 544-548, 2014.
19. Thompson L: World health organization classification of tumours: Pathology and genetics of head and neck tumours. Ear Nose Throat J 85: 74, 2006.
20. Curtin JA, Busam K, Pinkel D and Bastian BC: Somatic activation of KIT in distinct subtypes of melanoma. J Clin Oncol 24: 4340-4346, 2006.
21. Ellerhorst JA, Greene VR, Ekmekcioglu S, Warneke CL, Johnson MM, Cooke CP, Wang LE, Prieto VG, Gershenwald JE, Wei Q and Grimm EA: Clinical correlates of NRAS and BRAF mutations in primary human melanoma. Clin Cancer Res 17: 229-235, 2011.
22. Qu J, Yu F, Hong Y, Guo Y, Sun L, Li X, Zhang J, Zhang H, Shi R, Chen F and Li T: Underestimated PTCH1 mutation rate in sporadic keratocysticodontogenic tumors. Oral Oncol 51: 40-45, 2015.
23. Adzhubei I, Jordan DM and Sunyaev SR: Predicting functional effect of human missense mutations using polyphen-2. Curr Protoc Hum Genet: Chapter 7:Unit7.20, 2013.
24. Wang K, Li M and Hakonarson H: ANNOVAR: Functional annotation of genetic variants from next-generation sequencing data. Nucleic Acids Res 38: e164, 2010.
25. Si L, Kong Y, Xu X, Flaherty KT, Sheng X, Cui C, Chi Z, Li S, Mao L and Guo J: Prevalence of BRAF V600E mutation in Chinese melanoma patients: Large scale analysis of BRAF and NRAS mutations in a 432-case cohort. Eur J Cancer 48: 94-100, 2012.
26. Moher D, Liberati A, Tetzlaff J and Altman DG; PRISMA Group: Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. PLoS Med 6: e1000097, 2009.
27. Cohen Y, Goldenberg-Cohen N, Akrish S, Shani T, Amariglio N, Dratviman-Storobinsky O, Kaplan I, Barshack I and Hirshberg A: BRAF and GNAQ mutations in melanocytic tumors of the oral cavity. Oral Surg Oral Med Oral Pathol Oral Radiol 114: 778-784, 2012.

28. Rivera RS, Nagatsuka H, Gunduz M, Cengiz B, Gunduz E, Siar CH, Tsujigiwa H, Tamamura R, Han KN and Nagai N: C-kit protein expression correlated with activating mutations in KIT gene in oral mucosal melanoma. Virchows Arch 452: 27-32, 2008.
29. Antonescu CR, Viale A, Sarran L, Tschernyavsky SJ, Gonen M, Segal NH, Maki RG, Socci ND, DeMatteo RP and Besmer P: Gene expression in gastrointestinal stromal tumors is distinguished by KIT genotype and anatomic site. Clin Cancer Res 10: 3282-3290, 2004.
30. Peyssonnaux C and Eychène A: The Raf/MEK/ERK pathway: New concepts of activation. Biol Cell 93: 53-62, 2001.
31. Platz A, Egyhazi S, Ringborg U and Hansson J: Human cutaneous melanoma; a review of NRAS and BRAF mutation frequencies in relation to histogenetic subclass and body site. Mol Oncol 1: 395-405, 2008.
32. Malumbres M and Barbacid M: RAS oncogenes: The first 30 years. Nat Rev Cancer 3: 459-465, 2003.
33. Alexeev V and Yoon K: Distinctive role of the cKit receptor tyrosine kinase signaling in mammalian melanocytes. J Invest Dermatol 126: 1102-1110, 2006.
34. Lennartsson J, Jelacic T, Linnekin D and Shivakrupa R: Normal and oncogenic forms of the receptor tyrosine kinase kit. Stem Cells 23: 16-43, 2005.
35. Omholt K, Grafström E, Kanter-Lewensohn L, Hansson J and Ragnarsson-Olding BK: KIT pathway alterations in mucosal melanomas of the vulva and other sites. Clin Cancer Res 17: 3933-3942, 2011.
36. Beadling C, Jacobson-Dunlop E, Hodi FS, Le C, Warrick A, Patterson J, Town A, Harlow A, Cruz F III, Azar S, *et al*: KIT gene mutations and copy number in melanoma subtypes. Clin Cancer Res 14: 6821-6828, 2008.
37. Schoenewolf NL, Bull C, Belloni B, Holzmann D, Tonolla S, Lang R, Mihic-Probst D, Andres C and Dummer R: Sinonasal, genital and acrolentiginous melanomas show distinct characteristics of KIT expression and mutations. Eur J Cancer 48: 1842-1852, 2012.
38. Gardberg M, Talvinen K, Kaipio K, Iljin K, Kampf C, Uhlen M and Carpén O: Characterization of diaphanous-related formin FMNL2 in human tissues. BMC Cell Biol 11: 55, 2010.
39. Zhu XL, Zeng YF, Guan J, Li YF, Deng YJ, Bian XW, Ding YQ and Liang L: FMNL2 is a positive regulator of cell motility and metastasis in colorectal carcinoma. J Pathol 224: 377-388, 2011.