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Original

Wnt5a Overexpression in Thick Primary Oral Mucosal Melanomas: Implications for its Role in Tumor Progression

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Abstract:Wnt genes encode a large family of secreted cysteine-rich signaling molecules involved in cell growth, differentiation and tumorigenesis. Wnt5a, a non-transforming member of the Wnt family behaves as a putative oncogene in many cancers including melanomas. The aim of our study was to determine Wnt5a expression in primary oral mucosal melanomas (OMM) and correlate it with tumor thickness. Archival tissues from 18 OMM cases were subjected to immunohistochemical detection of Wnt5a by the streptavidin-biotin method. These were categorized into tumors of <4 mm (thin and intermediate thickness lesions) and >4 mm (thick lesions) thickness. Most OMM cases (17/18; 94.4%) stained positive for Wnt5a, though heterogeneously. Seven thick (7/11; 64%) and one intermediate thickness (1/7, 14%) OMM demonstrated strongly positive Wnt5a staining ($P<0.05$). The only Wnt5a-negative case was a thick OMM without local recurrence after treatment. Strong Wnt5a expression at tumor advancing sites suggests a role in local tumor spread. Identification of pleomorphic epithelioid and spindle cells as melanoma cell populations with the most pronounced Wnt5a staining suggests that Wnt5a overexpression influences cellular phenotype. These results taken together suggest that Wnt5a is up-regulated in OMM and may play a role in tumor progression.

Key words: Wnt5a, Oral mucosal melanoma, Immunohistochemistry, Tumor thickness

Introduction

Oral mucosal melanoma (OMM) is an extremely rare but highly aggressive neoplasm accounting for 0.5% of all oral malignancies and 0.2-12.4% of all melanomas[1-3]. The relative rarity of this tumor meant that comparatively little is known about its etiology and pathobiology which are believed to be distinctive from its cutaneous counterpart[4-8].

The Wnt (the name is derived from mouse Int-1 and *Drosophilia* wingless) gene family, one of the major families of developmental signaling molecules, has 19 members identified in mammals to date. These Wnt gene members encode 38-45kDa secreted cysteine-rich protein which modulates diverse processes such as cell fate, proliferation, migration, polarity, tissue architecture and organogenesis during embryonic development[9-14] and homeostasis of hematopoiesis[15], osteogenesis[16], angiogenesis[17] and adipogenesis[18] during normal adult growth. Wnt signaling is complex and can display several distinct characteristics depending on the Wnt and cell type[19]. Wnt5a, located on chromosome 3p21-p14, is a non-transforming member of the Wnt gene family[12, 20]. It mediates cell to cell signaling via a paracrine mechanism during development and ontogeny by binding to members of the frizzled family of G-coupled receptors[21]. When a normal wnt gene expression is disrupted, there is the potential for abnormal cell proliferation and tumor formation[13, 22-24]. In tumorigenesis, high level of Wnt5a has been found in a number of cancer types including lungs, breast, and prostate carcinomas and in melanomas

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Table 1. Wnt5a expression level and clinicopathological characteristics of 18 patients with primary oral mucosal melanoma

	Age (yr)	Gender	Location	Thickness (mm)	WESTOP Classification	Tumor cell type	Pigmentation	Wnt5a
1	67	F	Palate	2	Invasive with *in situ*	Spindle	Melanotic	(+)
2	75	M	Palate	8	Invasive	Mixed	Melanotic	(+++)
3	73	M	Gingiva (U)	1.2	Invasive with *in situ*	Mixed	Amelanotic	(++)
4	68	M	Gingiva (U), palate	10	Invasive with *in situ*	Mixed	Melanotic	(+)
5	79	F	Gingiva (U), palate	5	Invasive with *in situ*	Epithelioid	Amelanotic	(-)
6	58	M	Palate	10	Invasive with *in situ*	Mixed	Melanotic	(+++)
7	61	F	Gingiva (U), palate	7	Invasive with *in situ*	Mixed	Melanotic	(+++)
8	29	M	Gingiva (L)	4	Invasive with *in situ*	Mixed	Melanotic	(+)
9	51	M	Gingiva (L)	4	Invasive with *in situ*	Spindle	Melanotic	(+++)
10	24	F	Gingiva (L)	10	Invasive	Epithelioid	Melanotic	(+++)
11	50	F	Gingiva (U)	7	Invasive with *in situ*	Mixed	Melanotic	(++)
12	43	F	Gingiva (L)	4	Invasive	Mixed	Melanotic	(+)
13	59	M	Palate	4	Invasive	Mixed	Melanotic	(++)
14	82	M	Buccal mucosa	6	Invasive with *in situ*	Epithelioid	Melanotic	(++)
15	71	F	Gingiva (L)	5	Invasive	Mixed	Amelanotic	(+++)
16	30	M	Palate	4	Invasive with *in situ*	Mixed	Melanotic	(++)
17	56	M	Palate	5	Invasive with *in situ*	Mixed	Amelanotic	(+++)
18	64	M	Gingiva (L)	6	Invasive with *in situ*	Epithelioid	Melanotic	(+++)

(U): Upper, (L): Lower, (-): negative, (+): weakly positive, (++): moderately positive, (+++): strongly positive

[20, 21]. In the latter, Wnt5a is of especial importance because microarray analysis has identified it as a gene that separates highly aggressive melanomas from their less invasive counterparts[25]. Furthermore, a fivefold increase in Wnt5a mRNA level is seen in up to 50% of primary malignant melanomas [20] while transfection of Wnt5a cDNA into non-invasive melanoma cells results in increased invasive potential[19]. All these studies implicate that Wnt5a behaves as a putative oncogene in melanoma.

While molecular and other in vitro studies have contributed substantial knowledge and understanding on the oncogenic function of Wnt5a in melanomas[19, 20, 25], less is known about the immunohistochemical distribution of this molecule in human melanoma[26, 27]. A review of the English language literature disclosed that Wnt5a expression in OMM has not been previously investigated. The focus of this study was to examine by immunohistochemistry 18 cases of primary OMM for Wnt5a expression and to evaluate its correlation with tumor thickness. A possible role of Wnt5a in the neoplastic progression of OMM was also speculated.

Materials and Methods

Tissue samples

The tissue samples in this study were from the surgical pathology files of the Department of Oral Pathology, Graduate School of Medicine and Dentistry, Okayama University, Japan, and Unit of Stomatology, Cancer Research Center, Institute for Medical Research, Kuala Lumpur, Malaysia. Clinical database variables including age, gender and tumor location of these cases were recorded. Information on tumor thickness (measured in millimeters from the most superficial layer of the oral stratified squamous epithelium to the deepest tumor cell) was extracted from pathology reports and clinical case notes. OMM cases were sorted into two categories with breakpoints set at ≤4 mm level (thin primaries: <1mm; and intermediate thickness lesions: 1.01 – 4 mm) and >4 mm level (thick lesions) for correlative studies with Wnt5a expression level[28].

Archival formalin-fixed, paraffin-embedded tissue blocks of 18 cases of histologically confirmed primary OMM were retrieved. New 3-ìm thick sections were prepared for staining with hematoxylin-eosin, and for immunohistochemistry with Wnt5a antibody [staining with melanoma markers including Masson-Fontana, S-100 protein, HMB-45 and Melan-A were performed previously for purposes of histological diagnosis of these tumors, but these data are not included here][5-8].

Immunohistochemistry

The streptavidin-biotin method was used for the immunohistochemical detection of Wnt5a. Briefly, deparaffinized sections of 3-ìm thickness were pretreated for antigen retrieval by autoclave heating (132°C, 6 min) in 10 nM of citrate buffer

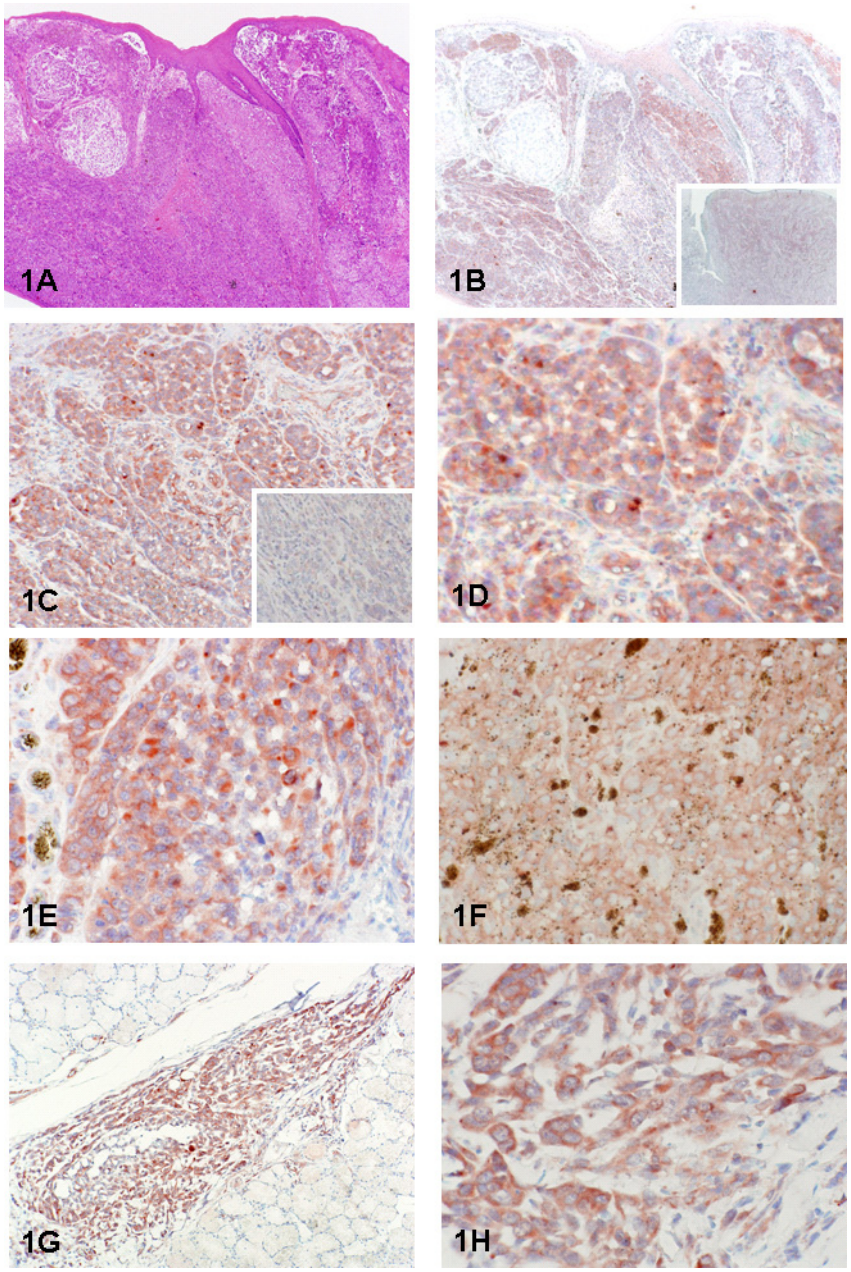

Figure 1. Advanced invasive OMM (A). Representative sections showing strong Wnt5a staining at advancing tumor sites: in and around epithelioid tumor nests (B-D), interconnecting cords of pleomorphic epithelioid cells(B, C), irregular islands (E) and sheets of pleomorphic epithelioid cells (F), and tumor prolongations composed of loosely cohesive spindle cells at the deep advancing front in the submucosa (G, H). Note that Wnt5a expression is localized at the cell membrane and cytoplasm of these melanoma cells (H). Insets depict OMM cases that stained weakly positive for Wnt5a (B, C). (A, H&E; B-H, Wnt5a; A,B, inset, x 40; c, inset, x200; D-F, H, x400; G, x 100).

(pH 3.3, 5 min). These sections were then immersed in 0.3% methanol containing 1% hydrogen peroxide for 30 min, to block endogenous peroxidase, and rinsed in 0.05 M Tris-buffered saline (TBS) (5 min, three times) before immersing in blocking solution (Funakoshi, Japan) for 10 min at room temperature. Then the sections were covered with primary antibody (goat polyclonal anti-mouse Wnt5a at 1:25 dilution; R&D Systems Inc., Minneapolis, MN, USA) and incubated overnight at 4°C. Immunoreactions were performed using Vectastain Elite ABC Kit (Vector Laboratories Inc., Burlingame, CA, USA). The antigenic sites were visualized using 3-amino-9-ethylcarbazole (AEC) substrate chromogen (Dako, Carpinteria, CA, USA) and counterstained with Mayer's hematoxylin. For negative control, sections were treated as above but without the primary antibody. All the control sections were negative. Positive staining control was also included and, where present in the specimens, internal staining controls were also checked for appropriate reactions with the primary antibody.

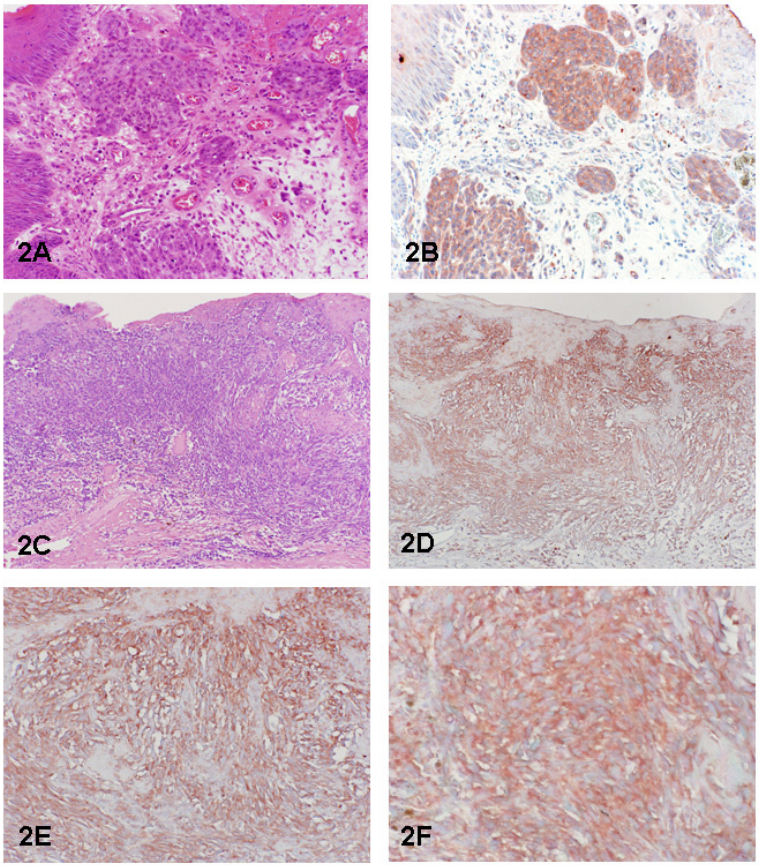

Figure 2. Invasive OMM with a predominant radial growth pattern (A,C). Representative sections showing strong Wnt5a staining in melanoma tumor nests (B) and diffuse sheets of spindle cells (D-F). (A, C, H&E; B, D-F, Wnt5a; A, B, E, x 200; C, D, x100; F, x400).

Immunohistochemical scoring

The tumors were analyzed subjectively according to the proportion of immunoreactive tumor cells and intensity of Wnt5a staining, and were categorized as follows: negative, no staining of tumor; weakly positive, staining is faintly present in focal areas (<25%); moderately positive, staining is evident in large parts of tumor (25-50%); and strongly positive, pronounced staining is present in large parts of the tumor (>50%).

Statistical analysis

The Kruskal-Wallis and Mann-Whitney tests were performed to compare the expression level of Wnt5a with the clinical data of OMM including tumor thickness. $P<0.05$ was considered significant.

Results

Clinicopathological findings

The clinical variables of the 18 OMM cases are summarized in Table 1. There were 11 (61%) male and seven (39%) female patients. Their average age was 57.8 years (age range: 24 -82 years). The tumor sites of origin in decreasing order of frequency were the upper gingival and palatal mucosa in 11 cases (61%) followed by the lower gingival mucosa in six cases (33%) and buccal mucosa in one case (6%).

According to the WESTOP classification of OMM (29), the sample studied comprised 18 invasive OMM, 13 with in-situ components. Fourteen (78%) OMM in this series were melanotic, the remaining amelanotic (Table1). Histologically, the tumor cell morphology was predominantly mixed (spindle and pleomorphic epithelioid) in 12 (67%) tumors, pleomorphic epithelioid in four (22%) tumors, and spindle in two (11%) tumors.

Immunohistochemical findings

Wnt5a staining in OMM was heterogeneous: one tumor was negative, four were weakly positive (Fig. 1B, C inset), five displayed moderately positive staining and eight had large areas of strongly positive staining (Fig. 1A-H). In the latter two groups, the expression pattern was especially marked around pleomorphic epithelioid tumor nests (Fig. 1B-D), interlacing cords of pleomorphic epithelioid cells (Fig. 1B, C), islands (Fig. 1E) and diffuse sheets of pleomorphic epithelioid cells (Fig. 1F), and tumor prolongations composed of loosely cohesive spindle-shaped cells at the deep advancing front in the submucosa (Fig 1G, H). Positive immunoreactivity for Wnt5a was distinctly identified at both the

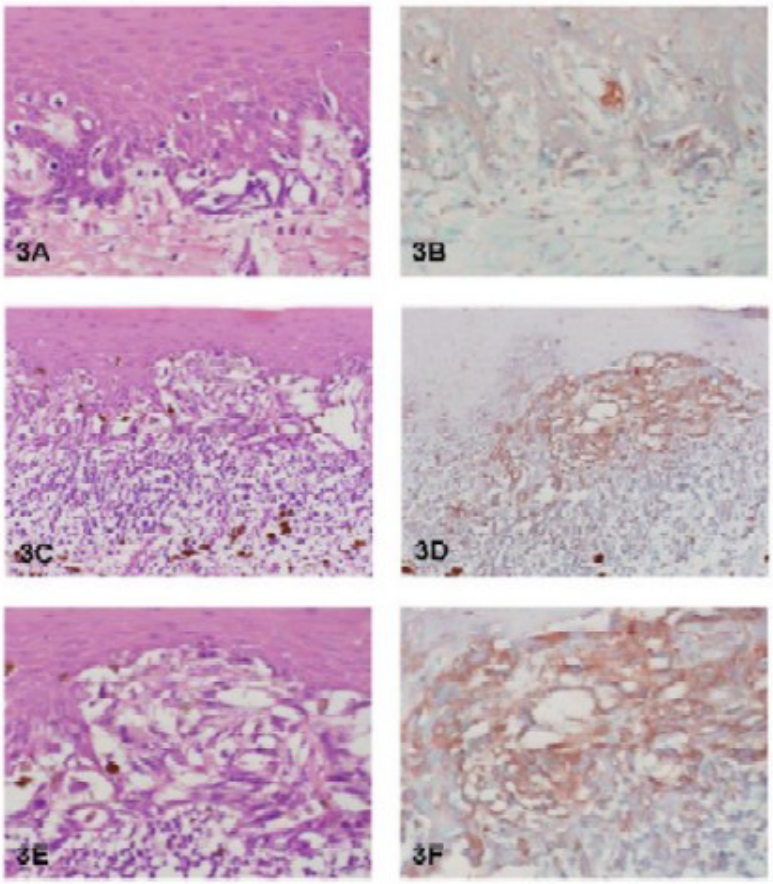

Figure 3. In-situ OMM (A, C, E). Representative sections showing strong Wnt5a staining in single (b) and scattered nests/clusters of large, pleomorphic and atypical melanocytes at the epithelial-connective tissue junction (D, F). (A, C, E, H&E; B, D,F, Wnt5a; A, B, E, F, x 400; C, D, x200).

cell membrane and within the cytoplasm of these melanoma cells (Fig. 1H). In these Wnt5a positive tumor areas, sometimes concomitant mild to heavy melanin pigment deposits may be present and these may mask the cellular details.

In those tumors that showed moderately to strongly positive Wnt5a staining, this distribution pattern extended inconstantly to the peripheral regions of the specimens where a predominant radial growth pattern was observed (Fig. 2A-F), as well as into those areas where the neoplasm was limited to the oral epithelium and epithelial-connective tissue interface (*in-situ* OMM components) (Fig. 3A-F). In the former, tumor nests (Fig. 2B, C) and diffuse spindle cell tumor sheets (Fig. 2D-F) displayed strongly positive staining for Wnt5a.

In *in-situ* OMM, Wnt5a expression was also heterogeneous: negative in some areas and positive in others. The Wnt5a-positive areas were usually found in single (Fig. 3 A, B) or scattered nests/clusters of large, pleomorphic and atypical melanocytes present at the epithelial-connective tissue junction (Fig. 3C-F).

Correlation between Wnt5a expression level and tumor thickness

The results of Wnt5a expression level and tumor thickness are summarized in Table 2 and shown in Fig. 4. There were 11 (61%) thick and seven (39%) intermediate thickness OMM but no thin primaries. Their overall mean tumor thickness was 5.76 mm (range: 1.2 - 10 mm). A statistically significant association was observed between the expression level of Wnt5a and OMM tumor thickness. Seven thick OMM cases (7/11; 64%) compared with one intermediate thickness OMM (1/7, 14%) demonstrated strongly positive Wnt5a staining ($P<0.05$) (Fig. 4).

No statistically significant correlation was found between Wnt5a expression and the other clinicopathological variables of OMM (Table 2).

Discussion

This study highlights for the first time the expression pattern of Wnt5a in primary OMM. Our results demonstrated that Wnt5a expression was up-regulated in 17 out of 18 cases (94.4%) of OMM. In the current literature, aberrant levels of Wnt5a has been reported in 10 primary melanomas[27] and 24 melanomas[26] but it is not known whether any of these tumors evaluated were of oral

Table 2 Wnt5a expression and clinicopathological characteristics of 18 patients with primary oral mucosal melanomas

	Intensity of Wnt5a staining				Total	P value
	-	+	++	+++		
Age						
≤58 years	0	2	2	4	8	>0.05
>58 years	1	2	3	4	10	
Gender						
Male	0	2	4	5	11	>0.05
Female	1	2	1	3	7	
Tumor thickness						
≤4 mm	0	3	3	1	7	>0.05<0.05
>4 mm	1	1	2	7	11	
Tumor location*						
Palate	1	2	2	4	9	>0.05
Gingiva	1	3	2	5	11	
Others	0	0	1	0	1	
Tumor subset						
Melanotic	0	4	4	6	14	>0.05
Amelanotic	1	0	1	2	4	

(-): negative, (+): weakly positive, (++): moderately positive, (+++): strongly positive; * In three cases, the tumor involved both the palatal and gingival mucosa

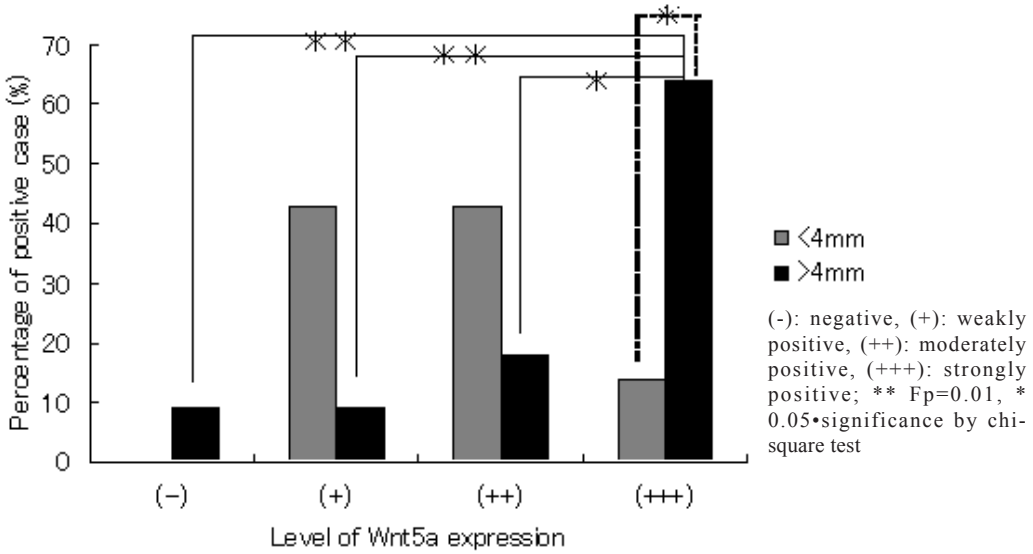

Figure 4. Level of Wnt5a expression correlates with oral melanoma thickness of progression

mucosal origin. Wnt5a overexpression has also been reported in other cancer types including those from the lungs, prostate and breast[20, 21], and in head and neck squamous carcinoma cell lines (30). Despite the observation of Wnt5a in many cancer types, no direct information is available on its expression in human OMM until now.

In this study, to assess the association between levels of Wnt5a expression and tumor thickness, OMM cases were sorted into two categories: tumors of ≤4 mm (thin primaries: <1 mm, and intermediate thickness lesions: 1.01 – 4 mm) and >4 mm (thick lesions) thickness. There were 11 (61%) thick and seven (39%) intermediate thickness lesions, but no thin primaries. Our observations suggest a significant correlation between Wnt5a expression level and tumor thickness. Seven thick (7/11; 64%) compared with one intermediate thickness tumor (1/7, 14%) demonstrated strongly positive Wnt5a staining (P<0.05). This finding suggests that very high levels of Wnt5a activity may frequently be acquired late in OMM development, thereby implicating this molecule in tumor progression. In some cancers including melanomas, early expression of Wnt5a may result in suppression of tumorigenesis, whereas if it is expressed at a later stage, it becomes a potent inducer of cancer cell migration and motility[19]. As only a limited number of OMM were evaluated here, studies on larger series including thin primaries are needed

to clarify the clinical relevance of this observation more fully.

The heterogeneous pattern of Wnt5a staining in OMM as observed in this study correlated well with those in primary melanomas from other sites [26, 27]. Reportedly, melanomas tend to show a wider gamut of staining for Wnt5a compared to nevi[27], and have a more variable expression level[26]. This inconsistent staining pattern reflects the heterogeneity of Wnt5a activity in different parts of a given tumor[25, 27]. In cancers of lungs, prostate and breast the immunohistochemical pattern of Wnt5a expression is unclear[20, 21].

In melanomas, cell populations with strong Wnt5a expression become more pronounced in higher-grade tumors[27]. In our series, 17/18 OMM were considered high-grade tumors based upon an integrated assessment of their histomorphological features in HE stained sections, their clinical course and Wnt5a expression level. Clinically, all cases presented with progressive disease manifested as regional lymph node metastases, and all but one case (Wnt5a-negative tumor) developed local recurrence after primary treatment. This single Wnt5a-negative OMM was considered low-grade. It has also been suggested that Wnt5a expression level correlates with both the survival and time to the development of metastases[27]. We have no follow-up data of our present series to confirm this contention.

There are conflicting reports concerning correlation between intensity of Wnt5a expression and morphological forms of melanoma cells. One study demonstrated that melanoma cells with giant cell and sarcomatoid morphology tend to stain strongly positive for Wnt5a compared to epithelioid cell forms, and suggested that the two former cell types are highly malignant cellular phenotypes associated with aggressive melanomas[27]. Another report found that Wnt5a expression was strongly positive in melanomas characterized by small uniform melanoma cells, and weakly positive in those showing large pleomorphic melanoma cells[26]. The one hundred month survival for melanomas with small epithelioid cells is 49%[31]. In the present study, the main tumor cell morphology encountered were pleomorphic epithelioid and spindle cells. Parallel microscopic examination of HE stained and Wnt5a stained sections of each tumor was methodically performed in an attempt to relate Wnt5a-positive areas with the cell types observed. We found that in Wnt5a-positive OMM, both pleomorphic epithelioid and spindle cells showed comparable Wnt5a staining within a given tumor. These observations suggest that in OMM, these two cells most probably represent melanoma cell populations associated with tumors of a more aggressive behavior. Furthermore, the observation that these Wnt5a-positive cells occurred at sites of the tumor corresponding to the radial and vertical growth phases of OMM suggest that Wnt5a activity may relate to both growth phases. The disparity in findings between our study and previous reports suggests that there may be some fundamental differences governing the pathobiology of OMM and melanoma at other sites.

In summary, the results of our study presented herein support the following key observations: 1. A high proportion of OMM tend to show up-regulation of Wnt5a expression, though heterogeneously; 2. Wnt5a activity is most frequently detected in advanced OMM where it may play a role in tumor progression; 3. expression level of Wnt5a in OMM correlates with tumor grade, thickness and cellular morphology; and 4. further investigations are needed to explore the potential value of Wnt5a as a therapeutic target in OMM.

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References

1. Hicks MJ and Flaitz CM. Oral mucosal melanoma: epidemiology and pathobiology. Oral Oncol 36: 152–169, 2000
2. Rapidis AD, Apostolidis C, Vilos G, Valsamis S. Primary malignant melanoma of the oral mucosa. J Oral Maxillofac Surg 61: 1132–1139, 2003
3. Umeda M, Komatsubara H, Shibuya Y, Yokoo S, Komori T. Premalignant melanocytic dysplasia and malignant melanoma of the oral mucosa. Oral Oncol 7: 714–722, 2002
4. Garzino-Demo P, Fasolis M, Maggiore GM, Pagano M, Berrone S. Oral mucosal melanoma: a series of case reports. J Craniomaxillofac Surg 32: 251–257, 2004
5. Lee YJ, Nagai N, Siar CH, et al. Angioarchitecture of primary oral malignant melanomas. J Histochem Cytochem 50: 1555–1562, 2002
6. Lee YJ, Nagaoka N, Nagatsuka H, et al. Pheomelanin and eumelanin of malignant melanomas and melanosis in oral mucosa. J Hard Tissue Biol 10: 89-95, 2001
7. Nagai N, Lee YJ, Nagaoka N, et al. Elemental sulphur and alkali elutable melanin detected in oral melanosis and malignant melanoma by energy-filtering transmission electron microscopy. J Oral Pathol Med 31: 481-487, 2000
8. Nagatsuka H, Rivera RS, Gunduz M, et al. Immunolocalization and distribution patterns of type IV collagen chains in oral mucosal melanoma. Virchows Arch 447: 710-716, 2005
9. Li C, Hu L, Xiao J, Chen H, et al. Wnt5a regulates Shh and Fgf10 signaling during lung development. Dev Biol 287: 86-97, 2005
10. Li C, Xiao J, Hormi K, Borok Z, Minoo P. Wnt5a participates in distal lung morphogenesis. Dev Biol 248: 68-81, 2002
11. Logan CY and Nusse R. The Wnt signaling pathway in development and disease. Annu Rev Cell Dev Biol 20: 781–810, 2004
12. Olson DJ and Gibo DM. Antisense wnt-5a mimics wnt-1 mediated C57MG mammary epithelial cell transformation. Exp Cell Res 241: 134-141, 1998

13. Smalley MJ, Dale TC. Wnt signaling in mammalian development and cancer. Cancer Metastasis Rev 18: 215-230, 1999
14. Wodarz A, Nusse R. Mechanisms of Wnt signaling in development. Annu Rev Cell Dev Biol 14: 59–88, 1998
15. Austin TW, Solar GP, Ziegler FC, Liem l, Matthews W. A role for Wnt gene family in hematopoiesis:expansion of multilineage progenitor cells. Blood 89: 3624-3635, 1997
16. Gaur T, Lengner CJ, Hovhannisyan H, et al. Canonical WNT signaling promotes osteogenesis by directly stimulating Runx2 gene expression. J Biol Chem 280: 33132-33140, 2005
17. Goodwin AM, D'Amore PA. Wnt signaling in the vasculature. Angiogenesis 5:1-9, 2002
18. Ross SE, Hemati N, Longo KA, et al. Inhibition of adipogenesis by Wnt signaling. Science 289: 950-953, 2000
19. Weeraratna AT. A Wnt-er Wonderland – The complexity of Wnt signaling in melanoma. Cancer Metastasis Rev 24:237-250, 2005
20. Izzo RV, Eichstetter I, Danielson KG. Aberrant expression of the growth factor Wnt-5A in human malignancy. Cancer Res 55:3495-3499, 1995
21. Leris AC, Roberts TR, Jiang WG, Newbold RF, Mokbel K. WNT5A expression in human breast cancer. Anticancer Res 25:731-734, 2005
22. Polakis P. Wnt signaling and cancer. Genes Dev 14:1837-1851, 2000
23. Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 1:1–24, 2003
24. Lustig B, Behrens J. The Wnt signaling pathway and its role in tumor development. J Cancer Res Clin Oncol 129:199–221, 2003
25. Bittner M, Meltzer P, Chen Y. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature 406: 536-540, 2000
26. Pham K, Milovanovic T, Barr RJ, Truong T, Holcombe RF. Wnt ligand expression in malignant melanoma: Pilot study indicating correlation with histopathological features. Mol Pathol 56: 280–285, 2003
27. Weeraratna AT, Jiang Y, Hostetter G, et al. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. Cancer Cell 1: 279–288, 2002
28. Balch CM, Buzaid AC, Atkins MB, et al. A new American joint committee on cancer staging system for cutaneous melanoma. Cancer 88:1484-1491, 2000
29. 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 Endo 83: 672–679, 1997
30. Rhee CS, Sen M, Lu D, et al. Wnt and frizzled receptors as potential targets for immunotherapy in head and neck squamous cell carcinomas. Oncogene 21:6598-6605, 2002
31. Day CL Jr, Harrist TJ, Lew RA, Mihm MC Jr. Classification of malignant melanomas according to the histologic morphology of melanoma nodules.J Dermatol Surg Oncol 8:874-875, 1982