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Expression of hepatocyte growth factor and c-met protein is significantly associated with the progression of oral squamous cell carcinoma in Taiwan

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BACKGROUND: Hepatocyte growth factor (HGF) is a pleotropic growth factor that regulates cell proliferation, migration, survival, tumor angiogenesis, and tumor cell invasion and metastasis. Its diverse biological effects are mediated through its interaction with its receptor, c-met protein.
METHODS: In this study, we examined the expression of HGF and c-met protein in 93 specimens of oral squamous cell carcinoma (OSCC), 10 specimens of oral epithelial dysplasia (OED), 14 specimens of oral epithelial hyperkeratosis (OEH), and 16 specimens of normal oral mucosa (NOM) by immunohistochemistry. The HGF and c-met labeling indices (LIs) in OSCC, OED, OEH, and NOM groups were calculated and compared between groups. The correlation between the expression of HGF or c-met in OSCCs and clinicopathological parameters, or survival of OSCC patients was analyzed statistically to investigate the possible influence of HGF or c-met on the progression and prognosis of OSCCs in Taiwan.
RESULTS: Positive HGF or c-met staining was mainly cytoplasmic. The mean HGF LI increased significantly from NOM (3.1 ± 5.1%) through OEH (32.5 ± 19.8%) and OED (52.0 ± 19.3%) to OSCC (71.9 ± 28.6%; $P = 0.000$). The mean c-met LI also increased significantly from NOM (25.8 ± 30.8%) and OEH (34.4 ± 19.3%) through OED (53.0 ± 20.0%) to OSCC (73.0 ± 29.4%; $P = 0.000$). Statistical analysis showed that the c-met LI in either the tumor center or invasion front was significantly associated with T status, N status, and clinical staging of OSCC. However, only the HGF LI in the tumor invasion front was significantly correlated with N status and clinical staging of OSCC.
CONCLUSION: Our results suggest that the expression of HGF and c-met protein is an early event in oral carcinogenesis in Taiwan. The HGF LI in the tumor invasion front and the c-met LI in either the tumor center or invasion front can predict the progression of OSCCs in Taiwan.
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Introduction

Oral cancer is the fifth most common cancer in the world (1). In Taiwan, oral cancers rank as the seventh most prevalent cancer in both sexes, and account for the fourth most common cancers in males (2). Areca quid (AQ) chewing, cigarette smoking, and alcohol drinking are the three major risk factors responsible for the development of oral squamous cell carcinomas (OSCCs). Furthermore, there are two million people who habitually chew AQs (3); approximately 80% of all oral cancer deaths are associated with this habit (4).

Hepatocyte growth factor (HGF), also known as scatter factor (SF), has been shown to induce epithelial cell proliferation, migration, survival, tumor angiogenesis, and tumor cell invasion and metastasis (5–20). These diverse biological effects of HGF are mediated via interaction with a transmembrane tyrosine kinase receptor, c-met protein. The production of HGF and c-met is modulated by cytokines, such as interleukin (IL)-1, tumor necrotic factor-α (TNF-α), and IL-6 (21–23), wild-type p53 protein (24), and HGF itself (25, 26). HGF can induce tumor growth through activation of extracellular signal-related kinase (ERK), the phosphatidyl inositol 3′-kinase (PI3K)/Akt, or nuclear factor κB (NFκB) signaling pathway (5). HGF can promote tumor angiogenesis and inhibit suspension-induced apoptosis (anoikis), which in turn, can increase not only the tumor growth, but also tumor invasion and metastasis (5, 11–14). HGF also induces the production of matrix

metalloproteinases (MMPs) that may promote cancer metastasis (19, 20). High expression of HGF or c-met has been shown to correlate with poor survival in patients with nasopharyngeal (27), esophageal (28), cervical (29), or breast carcinoma (30, 31).

Recently, overexpression of HGF and/or c-met has been demonstrated in carcinomas arising from breast (30–32), thyroid (33), pancreas (34), prostate (35), uterine cervix (29), esophagus (28), larynx (36), nasopharynx (27), and oral cavity (37). Results from previous studies suggest that HGF and c-met may be biomarkers for epithelial malignancies and may play an important role in human carcinogenesis. However, the expression of HGF and c-met in OSCCs in Taiwan has not been studied. In this study, we investigated the expression of HGF and c-met protein in specimens of OSCC, oral epithelial dysplasia (OED), oral epithelial hyperkeratosis (OEH), and normal oral mucosa (NOM) by immunohistochemistry. The HGF and c-met labeling indices (LIs) in OSCC, OED, OEH, and NOM groups were calculated and compared between groups. The correlation between the expression of HGF or c-met protein in OSCCs and the clinicopathological parameters or survival of the OSCC patients was analyzed statistically to evaluate the possible influence of HGF and c-met protein on the progression and prognosis of OSCCs in Taiwan.

Materials and methods

Formalin-fixed, paraffin-embedded tissue blocks were obtained from 93 patients (48 men and 45 women; mean age 53.8 years, range 31–82 years) with OSCC, 10 patients (9 men and 1 woman; mean age 48 years, range 42–62 years) with OED, and 14 patients (13 men and 1 woman; mean age 52.4 years, range 28–78 years) with OEH. Diagnosis of OSCC, OED, and OEH was based on histological examination of hematoxylin and eosin-stained tissue sections. All patients received total surgical excision of their lesions of OSCC, OED, and OEH at the Department of Oral and Maxillofacial Surgery, National Taiwan University Hospital, Taipei, Taiwan during the period from 1990 to 2002. Specimens were obtained from total surgical excision of the lesions. If lymph nodes were diagnosed as positive for OSCC, neck dissection and postoperative radiation therapy were also included in the treatment protocol. Of the 93 cases of OSCC, 60 (64.5%) were tongue, 20 (21.5%) buccal, 7 (7.5%) gingival, 5 (5.4%) palatal, and 1 (1.1%) lip cancers. The majority of our OSCC cases were tongue cancers because this study intended to include 34 tongue cancer cases from 34 female patients without any oral habit (OH) in order to assess whether there was a difference in the expression of HGF or c-met protein between male and female OSCC cases, as well as between OH-associated and non-OH-associated OSCC cases. The T-primary tumor; N-regional lymph nodes; M-distant metastasis (TNM) status and clinical stages of OSCCs at initial presentation were determined according to the International Union Against Cancer (UICC) convention (38). None of the patients had received any form of tumor-specific therapy before initial biopsies.

Details of the patients' OHs, including daily/weekly consumption of AQ, cigarette, and alcohol, as well as the duration of these habits, were recorded. Patients with OSCC were defined as AQ chewers when they chewed two or more AQs daily for at least 1 year, cigarette smokers when they smoked every day for at least 1 year and consumed more than 50 packs of cigarettes per year, and alcohol drinkers when they drank more than 4 days and consumed more than 20 g of pure alcohol per week for at least 1 year. According to these definitions, 46 OSCC patients (38 men and 8 women) were AQ chewers, 49 (40 men and 9 women) were smokers, and 42 (35 men and 7 women) were alcohol drinkers. Histological features of OSCC were further classified into three different types (well-, moderately, and poorly differentiated SCC). Of the 93 OSCC cases, there were 75 (80.6%) well- and 18 (19.4%) moderately differentiated OSCCs. None of our OSCCs belonged to poorly differentiated OSCC. The OED cases included six mild, two moderate, and two severe OED. The OEH cases usually showed both hyperkeratosis and epithelial hyperplasia with a mild-to-severe chronic inflammatory cell infiltrate in the lamina propria.

Sixteen biopsy specimens of NOM were obtained from 16 subjects (8 men and 8 women; mean age 24.2 years, range 18–38 years) during extraction of impacted permanent lower third molars after obtaining informed consent, and were used as the controls.

All the specimens for immunohistochemical staining were fixed in 10% neutral formalin, embedded in paraffin, and cut in serial sections of 4 μm. Immunohistochemical staining was performed using a peroxidase-labeled streptavidin–biotin technique. Briefly, tissue sections were deparaffinized, rehydrated, and treated with 3% H_2O_2 in methanol for 10 min to quench endogenous peroxidase activity. After washing in 10 mM Tris-buffered saline (TBS; pH 7.6), sections were incubated with 10% normal goat serum to block non-specific binding. Sections were then incubated overnight at 4°C with 1 : 20 dilution of anti-HGF (H-55, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) or 1 : 100 dilution of anti-c-met (C-28, Santa Cruz Biotechnology, Inc.) primary polyclonal rabbit antibodies. After washing in TBS, sections were treated with biotinylated goat anti-rabbit IgG and subsequently with a streptavidin–peroxidase conjugate (Zymed Laboratories, San Francisco, CA, USA). The 0.02% diaminobenzidine hydrochloride (DAB; Zymed) containing 0.03% H_2O_2 is used as chromogen to visualize the peroxidase activity. The preparations were lightly counterstained with hematoxylin, mounted with Permount, and examined by light microscopy.

Because HGF was basically a cytoplasmic protein and c-met a membranous and cytoplasmic protein, only epithelial or cancer cells exhibiting a brown cytoplasmic and/or membranous staining were counted as positive for HGF or c-met protein. Although positive nuclear staining for HGF or c-met was also found in our tissue sections of OSCC, OED, and OEH lesions, it was not assessed in this study. The sections were initially scanned at low power; at least three high-power fields were then chosen randomly, and 1000 cells were counted for each case. The HGF and c-met LIs were counted as a ratio of immunostaining positive cells to the total number of cells counted. An eyepiece graticule was used to ensure that all cells were evaluated once only. The HGF and c-met LIs in both the tumor center and invasion front of OSCC were calculated and compared to assess

whether the LI in the tumor invasion front was a better biomarker for prediction of the progression of OSCC and of patients' survival than the LI in the tumor center. Each of these assessments was independently carried out by two investigators. The sections with an interobserver variation of more than 10% were reassessed by using a double-headed light microscope to achieve consensus.

The mean HGF or c-met LIs for OSCC, OED, OEH, and NOM samples were compared among groups using analysis of variance (ANOVA) and multiple comparison. The correlation between HGF or c-met LI in OSCCs and clinicohistological parameters of OSCC patients was analyzed by ANOVA or Student's *t*-test, where appropriate. Cumulative survival was analyzed with the Kaplan–Meier product-limit method. The duration of survival was measured from the beginning of treatment to the time of death or the last follow-up. Comparison of cumulative survival between groups was performed using the log-rank test with the STATISTICA program (StatSoft Inc., USA). A *P*-value of less than 0.05 was considered statistically significant.

Results

Hepatocyte growth factor staining was mainly cytoplasmic and less commonly nuclear; it could be found in both OSCC and epithelial cells of OED and OEH lesions, but was rarely noted in the epitheial cells of NOM (Fig. 1A–F). HGF staining was also detected in some of the fibroblasts, endothelial cells, and inflammatory cells in either the stroma of OSCC or the lamina propria of OED or OEH lesions (Fig. 1B–E). The mean HGF LI increased significantly from NOM (3.1 ± 5.1%) through OEH (32.5 ± 19.8%) and OED (52.0 ± 19.3%) to OSCC (71.9 ± 28.6%; $P = 0.000$; Table 1). A significant difference in the mean HGF LI was found between OSCC and OED ($P < 0.05$),

Table 1 The mean HGF and c-met LIs in OSCC, OED, OEH, and NOM samples

Groups	*Mean HGF LI ± SD (%)*	*Mean c-met LI ± SD (%)*
OSCC ($n = 93$)	71.9 ± 28.6	73.0 ± 29.4
OED ($n = 10$)	52.0 ± 19.3	53.0 ± 20.0
OEH ($n = 14$)	32.5 ± 19.8	34.4 ± 19.3
NOM ($n = 16$)	3.1 ± 5.1	25.8 ± 30.8

A significant difference in the mean HGF or c-met LI was found among OSCC, OED, OEH, and NOM groups ($P = 0.000$). The following comparisons were statistically significant for the mean HGF LI: OSCC vs. OED, $P < 0.05$; OSCC vs. OEH or NOM, $P = 0.000$; OED vs. OEH, $P < 0.05$; OED or OEH vs. NOM, $P = 0.000$. A significant difference in the mean c-met LI was found as follows: OSCC vs. OED, $P < 0.05$; OSCC vs. OEH or NOM, $P = 0.000$; OED vs. OEH or NOM, $P < 0.05$.

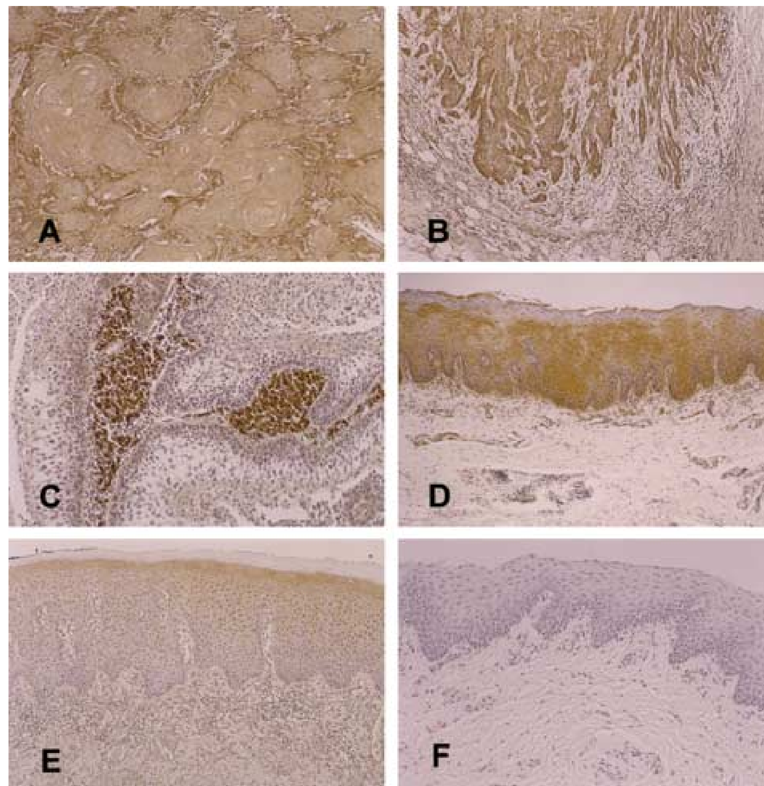

Figure 1 Immunohistochemical staining for HGF. (A) A well-differentiated OSCC showing positive HGF staining in the cytoplasm of all tumor cells. The peripheral cells of the tumor nests are stained more densely than the central cells of the tumor nests. (B) Tumor nests in the invasion front of a well-differentiated OSCC demonstrating dense cytoplasmic staining in tumor cells. Some of the fibroblasts, endothelial cells, and inflammatory cells are also positive for HGF. (C) A well-differentiated OSCC exhibiting a weak cytoplasmic staining in cancer cells of the tumor nests and a dense cytoplasmic staining in the stromal inflammatory cells among tumor nests. (D) OED demonstrating positive cytoplasmic HGF staining in nearly all the epithelial cells. (E) OEH showing positive cytoplasmic HGF staining in the spinous cells. (F) NOM exhibiting negative HGF staining in epithelial cells (A, B, D, and E: 120×; C and F: 240×).

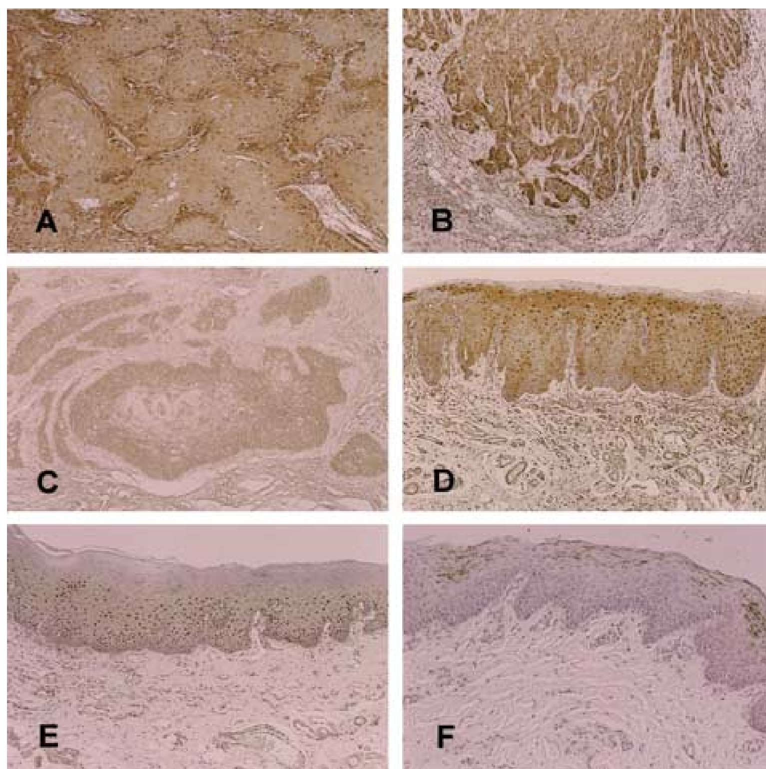

Figure 2 Immunohistochemical staining for c-met protein. (A) A well-differentiated OSCC showing positive c-met staining in the cytoplasm of all tumor cells and in the nuclei of some tumor cells. The peripheral cells of the tumor nests are stained more densely than the central cells of the tumor nests. (B) Tumor nests in the invasion front of a well-differentiated OSCC demonstrating dense cytoplasmic and nuclear staining in tumor cells. Some of the fibroblasts, endothelial cells, and inflammatory cells are also positive for c-met. (C) A well-differentiated OSCC exhibiting a weak cytoplasmic and membranous c-met staining in cancer cells of the tumor nests. (D) OED demonstrating positive cytoplasmic staining in nearly all the epithelial cells and positive nuclear staining in some epithelial cells. The endothelial cells lining the small capillaries in the lamina propria are also positive for c-met. (E) OEH showing positive c-met staining in the cytoplasm and nuclei of the spinous cells. (F) NOM exhibiting positive cytoplasmic c-met staining in a few superficial spinous cells (A, B, D, and E: 120×; C and F: 240×).

OEH ($P = 0.000$), or NOM ($P = 0.000$); between OED and OEH ($P < 0.05$) or NOM ($P = 0.000$); and between OEH and NOM ($P = 0.000$; Table 1).

The c-met staining was also mainly cytoplasmic (Fig. 2A–F). However, nuclear staining was found frequently, and membranous stain was detected occasionally in cancer or epithelial cells (Fig. 2A–E). Positive c-met staining could also be found in fibroblasts, endothelial cells, and inflammatory cells in either the stroma of OSCC or the lamina propria of OED and OEH lesions (Fig. 2B,D,E). The mean c-met LI increased significantly from NOM (25.8 ± 30.8%) and OEH (34.4 ± 19.3%) through OED (53.0 ± 20.0%) to OSCC (73.0 ± 29.4%; $P = 0.000$; Table 1). A significant difference in the mean c-met LI was noted between OSCC and OED ($P < 0.05$), OEH ($P = 0.000$), or NOM ($P = 0.000$), and between OED and OEH ($P < 0.05$) or NOM ($P < 0.05$). However, no significant difference in the mean c-met LI was noted between OEH and NOM samples ($P > 0.05$; Table 1).

Correlation between HGF or c-met LI in the tumor center of OSCC samples and clinicopathological parameters of OSCC patients is shown in Table 2. The c-met LI in the tumor center was significantly correlated with T status ($P = 0.01$), N status ($P = 0.003$), and clinical staging ($P = 0.000$) (Table 2). OSCC patients with larger tumor size (T3 and T4), reginal lymph node metastasis (N1, N2, and N3), or more advanced clinical stages (stages 3 and 4) were prone to have higher c-met LI in the tumor center. However, the HGF LI in the tumor center was not significantly related to any item of clinicopathological parameters. In addition, the c-met LI in the tumor center was not significantly associated with patients' age and gender, cancer location, recurrence, histological differentiation of OSCC, and OH (Table 2).

Correlation between HGF or c-met LI in the tumor invasion front of OSCC samples and clinicopathological parameters of OSCC patients is shown in Table 3. The HGF LI in the tumor invasion front was significantly correlated with N status ($P = 0.013$) and clinical staging ($P = 0.005$). Moreover, the c-met LI in the tumor invasion front was significantly correlated with T status ($P = 0.022$), N status ($P = 0.002$), and clinical staging ($P = 0.000$; Table 2). OSCC patients with larger tumor size (T3 and T4), reginal lymph node metastasis (N1, N2, and N3), and more advanced clinical stages (stages 3 and 4) were prone to have higher HGF or c-met LI in the tumor invasion front. However, the HGF or c-met LI in the tumor invasion front was not significantly associated with patients' age and gender, cancer location, recurrence, histological differentiation of OSCC, and OH (Table 3).

Table 2 Correlation between HGF or c-met LI in the tumor center of OSCCs and clinicopathological parameters of OSCC patients

	Mean HGF LI ± SD (%)	*P-value*	*Mean c-met LI ± SD (%)*	*P-value*
Patients' age (year)	–	0.561	–	0.492
≤50 ($n = 39$)	67.8 ± 32.8	–	75.7 ± 29.0	–
>50 ($n = 54$)	71.6 ± 29.6	–	71.4 ± 30.1	–
Patients' sex	–	0.185	–	0.415
Men ($n = 48$)	75.7 ± 23.8	–	70.5 ± 29.8	–
Women ($n = 45$)	67.8 ± 32.8	–	75.5 ± 29.0	–
Cancer location	–	0.595		0.396
Tongue ($n = 59$)	70.7 ± 32.0	–	75.0 ± 31.2	–
Other sites ($n = 34$)	74.0 ± 21.9	–	69.6 ± 25.9	–
T status	–	0.433		0.01
T1 + T2 ($n = 72$)	70.6 ± 27.8	–	68.8 ± 30.6	–
T3 + T4 ($n = 21$)	74.4 ± 27.7	–	87.4 ± 19.0	–
N status	–	0.177		0.003
N0 ($n = 63$)	69.1 ± 29.7	–	66.9 ± 31.4	–
N1 + N2 + N3 ($n = 30$)	77.7 ± 25.8	–	85.8 ± 19.4	–
Clinical staging	–	0.076		0.000
Stage 1 + 2 ($n = 55$)	67.5 ± 29.3	–	63.6 ± 31.9	–
Stage 3 + 4 ($n = 38$)	78.2 ± 26.7	–	86.6 ± 18.4	–
Recurrence	–	0.367		0.07
With ($n = 43$)	74.8 ± 26.8	–	78.5 ± 26.3	–
Without ($n = 50$)	69.4 ± 30.1	–	68.3 ± 31.3	–
Histology of OSCC	–	1.0		0.215
WD OSCC ($n = 75$)	71.9 ± 28.6	–	74.9 ± 28.1	–
MD OSCC ($n = 18$)	71.9 ± 29.5	–	65.3 ± 34.1	–
OH	–	0.162		0.597
With at least one OH ($n = 55$)	75.1 ± 26.3	–	74.4 ± 28.5	–
None ($n = 38$)	67.2 ± 31.4	–	71.1 ± 30.9	–

Comparison between groups was performed by Student's *t*-test. WD, well differentiated; MD, moderately differentiated.

The correlation between HGF or c-met LI in OSCCs and survival of OSCC patients was analyzed with Kaplan–Meier product-limit method. No significant difference was found between HGF or c-met LI in either the tumor center or invasion front of OSCCs and survival of OSCC patients (data not shown). In this study, 45 OSCC patients were operated more than 5 years prior to the survival evaluation was completed. Kaplan–Meier analysis also showed no significant association between HGF or c-met LI in either the tumor center or invasion front of OSCCs and survival of these 45 OSCC patients (data not shown).

Discussion

This study showed that the mean HGF LI increased significantly from NOM (3.1 ± 5.1%) through OEH (32.5 ± 19.8%) and OED (52.0 ± 19.3%) to OSCC (71.9 ± 28.6%; $P = 0.000$; Table 1). Furthermore, the mean c-met LI increased significantly from NOM (25.8 ± 30.8%) and OEH (34.4 ± 19.3%) through OED (53.0 ± 20.0%) to OSCC (73.0 ± 29.4%; $P = 0.000$; Table 1). These results indicate that about one quarter of normal oral epithelial cells express c-met receptors, but normal oral epithelial cells do not regularly express HGF. A stepwise and significant increase in the expression of HGF or c-met was noted in the transformation from NOM to OED and from OED to OSCC. Jin et al. (32) examined the expression of HGF and c-met in benign and malignant breast tissues and discovered that either HGF or c-met staining score increased significantly from normal breast/benign hyperplasia through ductal carcinoma *in situ* (DCIS) to invasive carcinoma. In addition, both the HGF and c-met staining scores of DCIS were significantly higher than the corresponding scores of normal breast/benign hyperplasia (32). The significant elevation in the expression of HGF and c-met from NOM to OED and from normal breast to DCIS suggests that the expression of HGF and c-met may be an early event in both oral and breast carcinogenesis.

This study showed a significantly higher mean LI for both HGF (71.9 ± 28.6%) and c-met (73.0 ± 29.4%) in OSCCs than in NOM samples. Marshall & Kornberg (37) also demonstrated a significantly greater staining intensity for both HGF and c-met in OSCCs than in OED or NOM samples. However, no significant difference in the staining intensity for both HGF and c-met was found between OED and NOM specimens. Takada et al. (28) found a significant elevation of HGF concentration in esophageal SCC tissue, compared to that in normal esophageal mucosal tissue. Sawatsubashi et al. (36) showed the expression of c-met protein in 54.9% (45/82) laryngeal carcinomas. Qian et al. (27) demonstrated a higher c-met expression level in the nasopharygeal carcinoma cells than in normal nasopharygeal squamous epithelium. Furthermore, many other authors have demonstrated the expression/overexpression of HGF

Table 3 Correlation between HGF or c-met LI in the tumor invasion front of OSCCs and clinicopathological parameters of OSCC patients

	Mean HGF LI ± SD (%)	*P-value*	*Mean c-met LI ± SD (%)*	*P-value*
Patients' age (year)	–	0.796	–	0.541
≤50 ($n = 39$)	76.0 ± 26.9	–	77.4 ± 28.4	–
>50 ($n = 54$)	74.4 ± 30.9	–	73.6 ± 30.2	–
Patients' sex	–	0.061	–	0.504
Men ($n = 48$)	80.5 ± 23.1	–	73.2 ± 29.9	–
Women ($n = 45$)	69.2 ± 33.7	–	77.3 ± 28.9	–
Cancer location	–	0.537		0.207
Tongue ($n = 59$)	73.6 ± 32.8	–	78.1 ± 30.9	–
Other sites ($n = 34$)	77.5 ± 21.6	–	70.1 ± 26.1	–
T status	–	0.091	–	0.022
T1 + T2 ($n = 72$)	72.3 ± 29.4	–	71.5 ± 30.8	–
T3 + T4 ($n = 21$)	84.5 ± 26.6	–	88.1 ± 19.3	–
N status	–	0.013	–	0.002
N0 ($n = 63$)	69.9 ± 31.1	–	69.0 ± 31.7	–
N1 + N2 + N3 ($n = 30$)	85.8 ± 21.1	–	88.3 ± 18.0	–
Clinical staging	–	0.005	–	0.000
Stage 1 + 2 ($n = 55$)	68.1 ± 30.8	–	66.0 ± 32.5	–
Stage 3 + 4 ($n = 38$)	85.1 ± 23.5	–	88.6 ± 17.2	–
Recurrence	–	0.096	–	0.095
With ($n = 43$)	80.5 ± 25.0	–	80.7 ± 25.9	–
Without ($n = 50$)	70.4 ± 31.8	–	70.5 ± 31.5	–
Histology of OSCC	–	0.622	–	0.266
WD OSCC ($n = 75$)	74.3 ± 29.0	–	76.9 ± 27.8	–
MD OSCC ($n = 18$)	78.1 ± 30.4	–	68.3 ± 35.0	–
OH	–	0.136	–	0.786
With at least one OH ($n = 55$)	78.5 ± 27.4	–	75.9 ± 28.4	–
None ($n = 38$)	69.2 ± 31.9	–	74.2 ± 31.1	–

Comparison between groups was performed by Student's *t*-test. WD, well differentiated; MD, moderately differentiated.

and/or c-met in a number of different human carcinomas arising from breast (30–32), thyroid (33), pancreas (34), prostate (35), and uterine cervix (29). These results suggest that overexpression of HGF or c-met is commonly found in some human carcinomas. Thus, HGF and c-met may be biomarkers of certain types of human carcinomas and may play an important role in human carcinogenesis.

The augmented expression of HGF and c-met in oral precancers and cancers could be because of the stimulation from cytokines, wild-type p53 protein, and HGF itself. HGF appears to be partially regulated by tumor-induced cytokines, including IL-1 and TNF-α (21). Macrophages and lymphocytes in the lamina propria of OEH and OED lesions and in the OSCC stromal tissue may secrete IL-1 and TNF-α, respectively. Recombinant human IL-1α enhances the production of HGF in human gingival fibroblast cultures (22). In addition, Hasina et al. (17) found that a human OSCC cell line can secrete IL-1, which in turn, stimulates the fibroblasts to secrete HGF. HGF is able to induce its own production via enhancing the production of urokinase-type plasminogen activator that can generate increased levels of HGF via cleavage of the inactive precursor (25). Expression of c-met protein can be increased by a number of cytokines, including IL-1, IL-6, and TNF-α (23). These three soluble factors are produced primarily as a result of the non-specific inflammatory response present in the tumor microenvironment. Expression of *c-met* proto-oncogene is also regulated by wild-type p53 protein. RKO (a colon carcinoma cell line) cells treated with UV irridiation can express wild-type p53 protein, which in turn, increases the level of the endogenous c-met protein (24). Our previous study showed a high p53 expression rate of 58% and a low p53 mutation rate of 5.4% in OSCCs in Taiwan (39, 40). This result implies that OSCC cells probably express wild-type rather than mutated p53 protein. Interestingly, c-met expression may also be regulated by HGF itself. Boccaccio et al. (26) demonstrated that cultured epithelial cells treated with HGF show up-regulation of the c-met expression. The above findings suggest that the increased expression of HGF and c-met in OSCC may be regulated by complicated circuits involving the cytokines, wild-type p53 protein, and HGF itself.

This study showed a significant association of c-met LI in either the tumor center or invasion front with the tumor size of OSCC. The HGF LI in the tumor invasion front was higher in T3 and T4 OSCCs than in T1 and T2 OSCCs, although the difference was only marginally significant ($P = 0.091$). It is easy to appreciate the intimate relation between the HGF/c-met ligand/receptor pair and tumor growth, because HGF is a pleotropic growth factor that regulates cell proliferation, survival, and tumor angiogenesis (5, 9–14). Activation of HGF/c-met system can stimulate cancer cell proliferation, survival, and growth through the ERK, PI3K/Akt, and NFκB signaling pathways (5). OSCC cells can obtain a growth advantage via up-regulation of

either HGF or c-met or both. Stephens et al. (41) showed that oral mucosal fibroblasts can express both HGF and c-met receptor. Uchida et al. (16) demonstrated that human gingival fibroblasts can secrete a large amount of HGF *in vitro*, but cannot express c-met receptor. In contrast, OSCC cells can express c-met receptor (16, 20), but cannot secrete HGF *in vitro* (16). In this study, we found that OSCC cells expressed high amounts of both HGF and c-met, and some of the stromal fibroblasts, endothelial cells, and inflammatory cells also expressed HGF and c-met. These findings suggest that oral cancer cells can be stimulated to grow via both paracrine and autocrine mechanisms.

OSCC cells may also obtain a growth advantage and a metastatic potential from HGF-induced tumor angiogenesis. HGF has been shown to induce tumor angiogenesis, a process required for tumor growth and metastasis, through stimulating the cancer and stromal cells to secrete angiogenic factors, such as IL-8 and vascular endothelial growth factor (VEGF; 12, 42, 43). Dong et al. (13) found that HGF can promote expression of IL-8 and VEGF in head and neck SCC (HNSCC) cell lines through binding to c-met receptor and through both MEK- and PI3K-dependent pathways. Michi et al. (14) showed that human OSCC cell lines promote angiogenesis via expression of VEGF and up-regulation of KDR/flk-1 expression in endothelial cells. These results indicate that HGF may promote cancer growth and dissemination through the induction of IL-8 and VEGF production.

HGF is also a potent antiapoptotic factor (9, 10). Fan et al. (9) found that HGF protects various epithelial and carcinoma cell types against apoptosis induced by DNA-damaging agents and UV light. HGF helps to maintain the level of the survival-promoting protein Bcl-X_L (9). Gao et al. (10) reported that HGF can prevent mitochondrial apoptosis by blocking the activation of multiple caspases. Zeng et al. (5, 11) found that HGF provides anoikis resistance for HNSCC cells. Anoikis resistance plays an important role in tumor progression and metastasis. These results suggest that HGF can prolong the cancer cell survival and promote metastasis by inhibition of apoptosis.

In this study, the expression of HGF in the tumor invasion front and the expression of c-met in either the tumor center or invasion front was significantly correlated with regional lymph node metastasis. Uchida et al. (16) found that in type I collagen matrix, HGF significantly enhances the invasive growth of the cancer cells. Furthermore, HGF markedly enhances the migration of OSCC cells in a Transwell invasion chamber. HGF concentrations in metastatic cancer tissues are significantly higher than those in non-metastatic cancer tissues and normal gingiva (16). Hasina et al. (17) found that a human OSCC cell line can secrete IL-1, which in turn, stimulates the fibroblasts to secrete HGF. HGF strongly enhances the *in vitro* invasion of OSCC cells. Moreover, Matsumoto et al. (7) discovered that HGF induces tyrosine phosphorylation of focal adhesion kinase, and promotes the migration and invasion by OSCC cells. Morello et al. (44) demonstrated that c-met receptor is over-expressed but not mutated in OSCCs, and that c-met oncogene is involved in progression of OSCC toward an invasive-metastatic behavior. In addition, Shimabukuro et al. (18) found that HGF produced by stromal cells influences the mode of stromal invasion of squamous cervical cancer by selectively decreasing the expression of both E-cadherin and actin. These results suggest that HGF can promote the cancer cell invasion and metastasis via complicated mechanisms that involve enhancement of cell migration, cytokine production, tyrosine phosphorylation of focal adhesion kinase, and inhibition of expression of E-cadherin and actin.

HGF may also promote cancer cell invasion and metastasis by induction of secretion of MMPs (19, 20). Hanzawa et al. (19) found that HGF can induce expression of the E1AF transcription factor gene whose product in turn activates MMP genes and leads to oral cancer cell invasion. Bennett et al. (20) found that HGF can induce MMP-2 and/or MMP-9 expression in OSCC cell lines. Because MMP-2 and MMP-9 are type IV collagenases, high production of these two MMPs by OSCC cells may increase the invasive and metastatic potential of OSCC cells (20).

This study showed a positive association of HGF and c-met overexpression with higher T- and N statuses in OSCCs. Because higher T- and N statuses always result in a more advanced clinical stage of OSCC, it is easy to explain why OSCC patients with higher expression of HGF and c-met in their tumors are prone to have the more advanced clinical stages of OSCC. This study did not demonstrate a significant correlation between the expression of HGF and c-met in OSCCs and survival of the OSCC patients. Furukawa et al. (34) also reported that HGF expression is not related to the survival of the patients with pancreatic cancer. However, previous studies have demonstrated that high expression of HGF or c-met correlates with poor survival in patients with nasopharyngeal (27), esophageal (28), cervical (29), or breast carcinoma (30, 31). Although the exact causes resulting in this discrepancy are unclear, HGF and c-met may play different roles in carcinomas from different regions of the body.

This study showed a significant elevation in HGF and c-met LIs from NOM to OED, suggesting that the expression of HGF and c-met is an early event in oral carcinogenesis in Taiwan. We also found that the HGF LI in the tumor invasion front was significantly correlated with N status and clinical staging of OSCC. Furthermore, the c-met LI in either the tumor center or invasion front was significantly associated with T status, N status, and clinical staging of OSCC. These results indicate that the calculation of HGF LI in the tumor invasion front and of c-met LI in either the tumor center or invasion front can predict the progression of OSCC. In addition, the HGF LI in the tumor invasion front is a better biomarker for prediction of the progression of OSCC than that in the tumor center.

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