Journal of
Oral Pathology & Medicine

doi: 10.1111/jop.12335

J Oral Pathol Med (2016) 45: 89–95

wileyonlinelibrary.com/journal/jop

Association of OCT4, SOX2, and NANOG expression with oral squamous cell carcinoma progression

Ting-Ying Fu[1,2], I-Chien Hsieh[1], Jiin-Tsuey Cheng[3], Meng-Han Tsai[4], Yu-Yi Hou[5,6], Jang-Hwa Lee[1], Huei-Han Liou[7], Sheng-Feng Huang[7], Hung-Chih Chen[2,8], Leing-Ming Yen[1], Hui-Hwa Tseng[1], Luo-Ping Ger[3,7]

[1]Department of Pathology and Laboratory Medicine, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; [2]Shu-Zen Junior College of Medicine and Mangement, Kaohsiung, Taiwan; [3]Department of Biological Sciences, National Sun Yat-Sen University, Kaohsiung, Taiwan; [4]Department of Neurology, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan; [5]Department of Otorhinolaryngology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; [6]Department of Nursing, Yuh-Ing Junior College of Health Care and Management, Kaohsiung, Taiwan; [7]Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan; [8]Department of Stomatology, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan

BACKGROUND: OCT4, SOX2, and NANOG are major transcription factors related to stem cell self-renewal and differentiation. The aim of this study was to examine the association of OCT4, SOX2, and NANOG expression levels with the development and prognosis of patients with oral squamous cell carcinoma (OSCC).

MATERIALS AND METHODS: Expression levels of OCT4, SOX2, and NANOG were evaluated by immunohistochemistry with tissue microarray slides of 436 OSCC, 362 corresponding tumor-adjacent normal (CTAN) tissues, and 71 normal uvula epithelium tissues. The clinicopathologic and follow-up data of the OSCC patients were recorded.

RESULTS: OCT4 expression was significantly higher in normal and CTAN tissues than in tumor tissue (both $P < 0.001$). SOX2 expression in CTAN tissue was significantly higher than that in normal ($P = 0.021$) and tumor tissues ($P < 0.001$). However, NANOG expression was significantly higher in CTAN ($P = 0.014$) and tumor tissues ($P = 0.009$) than in normal tissue. Higher OCT4 and SOX2 expressions were associated with earlier AJCC stage ($P = 0.002$ and $P < 0.001$), small tumor size ($P = 0.017$ and $P = 0.001$), and the absence of lymph node metastasis ($P = 0.015$ and $P = 0.025$). Higher levels of SOX2 expression were associated with better disease-specific survival ($P = 0.002$) even after adjustment for clinicopathologic factors.

DISCUSSION: OCT4 and SOX2 are biomarkers of tumorigenesis and early stage OSCC. SOX2 is an independent prognostic factor for OSCC.

J Oral Pathol Med (2016) **45**: 89–95

Keywords: NANOG; oral cancer; OCT4; SOX2; squamous cell carcinoma

Correspondence: Luo-Ping Ger, Department of Medical Education and Research, Kaohsiung Veterans General Hospital, Taiwan, and Institute of Biomedical Sciences, National Sun Yat-Sen University, 386, Dazhong 1st Rd., Zuoying Dist., Kaohsiung 81362, Taiwan. Tel: +886-7-3422121, Fax:+886-7-3422288, E-mail: lpger@vghks.gov.tw and Hui-Hwa Tseng Department of Pathology and Laboratory Medicine, Kaohsiung Veterans General Hospital, 386, Dazhong 1st Rd., Zuoying Dist., Kaohsiung 81362, Taiwan. Tel: +886-7-3422121, Fax:+886-7-3468317, E-mail: hhtseng@vghks.gov.tw
Accepted for publication May 7, 2015

Introduction

Oral cancer is the sixth most common malignancy worldwide, with a predilection for south Asian and southeast Asian populations (1). In Taiwan, oral cancer was the fourth leading cause of cancer death for males in 2012 and the most common cancer in young adult males aged 25–44 years old (2). The majority (>95%) of oral cancer cases are oral squamous cell carcinoma (OSCC), and the two most common locations are the tongue and buccal mucosa (3). In spite of surgical resection, the 5-year survival rate for patients with oral cancer remains unfavorable, at around 55–60%. For advanced-stage OSCC under standard therapy, the 3-year survival rate decreased to 30 from 50% (4–6). In addition, previous studies have suggested that 26–80% of patients with early-stage OSCC subsequently develop locoregional recurrence or distant metastases (7). Therefore, standard therapy and clinical TNM staging is insufficient to reliably predict the prognoses of patients with OSCC. Reliable biomarkers are needed for the development of more individualized treatment strategies for patients with OSCC.

SRY-related HMG-box gene 2 (SOX2), initially linked strongly with inhibition of neuronal differentiation, has been shown to act as an important transcription factor in maintaining the self-renewal capability of embryonic stem cells (8). Octamer-binding protein 4 (OCT4), known to bind

in partnership with SOX2, is also an essential regulator for pluripotency and self-renewal (9). Homeodomain protein NANOG is another transcription factor that plays a central role in sustaining pluripotency during embryonic development. NANOG protein is down-regulated during the differentiation of these pluripotent stem cells (10). Several studies have suggested that these lineage-survival transcriptional factors may have a role in human malignancy. SOX2 has been found frequently to be overexpressed in esophagus and lung SCC, acting as an indicator of a favorable prognosis (11, 12). In OSCC, copy number gain and overexpression of SOX2 has also been demonstrated (13, 14). In lung cancer, up-regulated SOX2 is associated with a favorable prognosis, whereas the effect of SOX2 expression on prognosis in head and neck SCC remains controversial (12, 14, 15).

Herein, we evaluated the association between OCT4, SOX2, and NANOG protein expression in tumor tissues, corresponding tumor adjacent normal tissues (CTAN), and normal uvula epithelium tissues by immunohistochemistry (IHC) assay. In addition, we examined the role of OCT4, SOX2, and NANOG expression levels in the prognosis of OSCC.

Methods

Tissue specimens

Tumor and CTAN specimens from 248 patients with primary oral tongue SCC and 188 patients with buccal mucosal SCC were obtained from the Department of Pathology at Kaohsiung Veterans General Hospital between 1993 and 2006. Survival time was estimated from the time of operation to October 2012. Disease-specific survival was measured from the time of initial resection of the primary tumor to the date of cancer-specific death or last follow-up. The median follow-up period was 48.5 months (range 1.6–236.3 months). TNM classification was determined at the time of the initial resection of the tumor in accordance with the guidelines of the 2002 American Joint Committee on Cancer (AJCC) system. Forty normal tongue specimens and 31 normal buccal specimens were obtained from patients who underwent uvulopalatopharyngoplasty for obstructive sleep apnea.

This study protocol was independently reviewed and approved by the institutional review board of Kaohsiung Veterans General Hospital (*VGHKS11-CT12-13*).

Tissue microarray construction

A tissue microarray (TMA) block was composed of 149 cores, 1.5 mm in diameter, including 48 trios, with each trio containing two cores from the tumor tissue and one core from the CTAN of the same patient. Five cores of normal uvula epithelium were available on each TMA block.

Immunohistochemistry

TMA sections (4 μm) were de-waxed in xylene and then rehydrated in graded alcohol. Antigen retrieval was facilitated by immersion in Tris-EDTA (10 mM, pH 9.0) for 10 min at 125°C in the pressure boiler. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol for 30 min. Slides were incubated with anti-OCT4 mouse monoclonal (dilution 1:50; Abcam, Cambridge, MA, USA), anti-SOX2 goat polyclonal (dilution 1:40; R&D, Minneapolis, MN, USA), and anti-NANOG rabbit monoclonal (dilution 1:400; Epitomics, Cambridge, MA, USA) antibodies in Tris-buffered saline (TBS) solution with 5% bovine serum albumin (BSA) overnight at 4°C in a wet chamber. Color was developed with a solution of 0.03% diaminobenzidine for two minutes at room temperature, and the sections were counterstained with hematoxylin.

Positive controls used seminoma and lung SCC sections. Substitution of the primary antibody with antibody dilution buffer served as negative controls.

Immunohistochemical analysis and scoring

Two pathologists independently reviewed the slides without access to information on clinical outcome. If disagreement occurred (intensity score discrepancy >1 or percentage level >10%), the slides were re-evaluated together to obtain a consensus diagnosis. The degree of immune reactivity was scored using a semi-quantitative approach based on staining intensity and percentage. In brief, the extent of positivity was scored as 0 when the percentage of positive cells was <5%; 1 when it was 5–24%; 2 when it was 25–49%; 3 when it was 50–74%; and 4 when it was >75%. The intensity was scored as 0 when no positive cells were identified; weak staining as 1; moderate as 2, and strong as 3 (Fig. 1). The extent and intensity scores were added to obtain a total score, which ranged from 0 to 7. Specimens were divided into two groups based on their overall scores: (i) lower expression, ≤1 point; (ii) high expression, ≥2 points.

Statistical analysis

The Kruskal–Wallis one-way ANOVA or Wilcoxon signed-rank test was used to evaluate protein expression differences in mean ranks between various tissue types (from normal to CTAN and to OSCC tissues) or various clinicopathologic parameters. The correlations between the three different reprogramming proteins in OSCC tissues were analyzed by Spearman's rank correlation test. The cumulative survival curves were estimated using the Kaplan–Meier method. The log-rank test was used to compare the survival curves. A Cox proportional hazards model was employed to evaluate the impact of protein expression on survival, using significant variables in univariate analysis as covariates. A value of $P < 0.05$ (2 sided) was considered significant.

Results

Four hundred and thirty-six patients with OSCC were studied, including 248 with tongue SCC and 188 with buccal mucosal SCC. Mean age at disease diagnosis was 51.81 years (range: 21–89 years), and there was a male predominance (402/436, 92.2%) (Table 1). AJCC staging and cell differentiation were listed in Table 1. One hundred and nineteen (27.2%) patients received postoperative radiotherapy, and 9 (2%) received postoperative chemotherapy. Mean follow-up period was 66.91 months (range, 1.6–236.3). The 5-year overall and disease-specific survival rates were 57.1 ± 2.4% and 60.5 ± 2.4, respectively. The 3-year disease-free survival rate was 56.7 ± 2.5, and it remained constant 3 years after diagnosis.

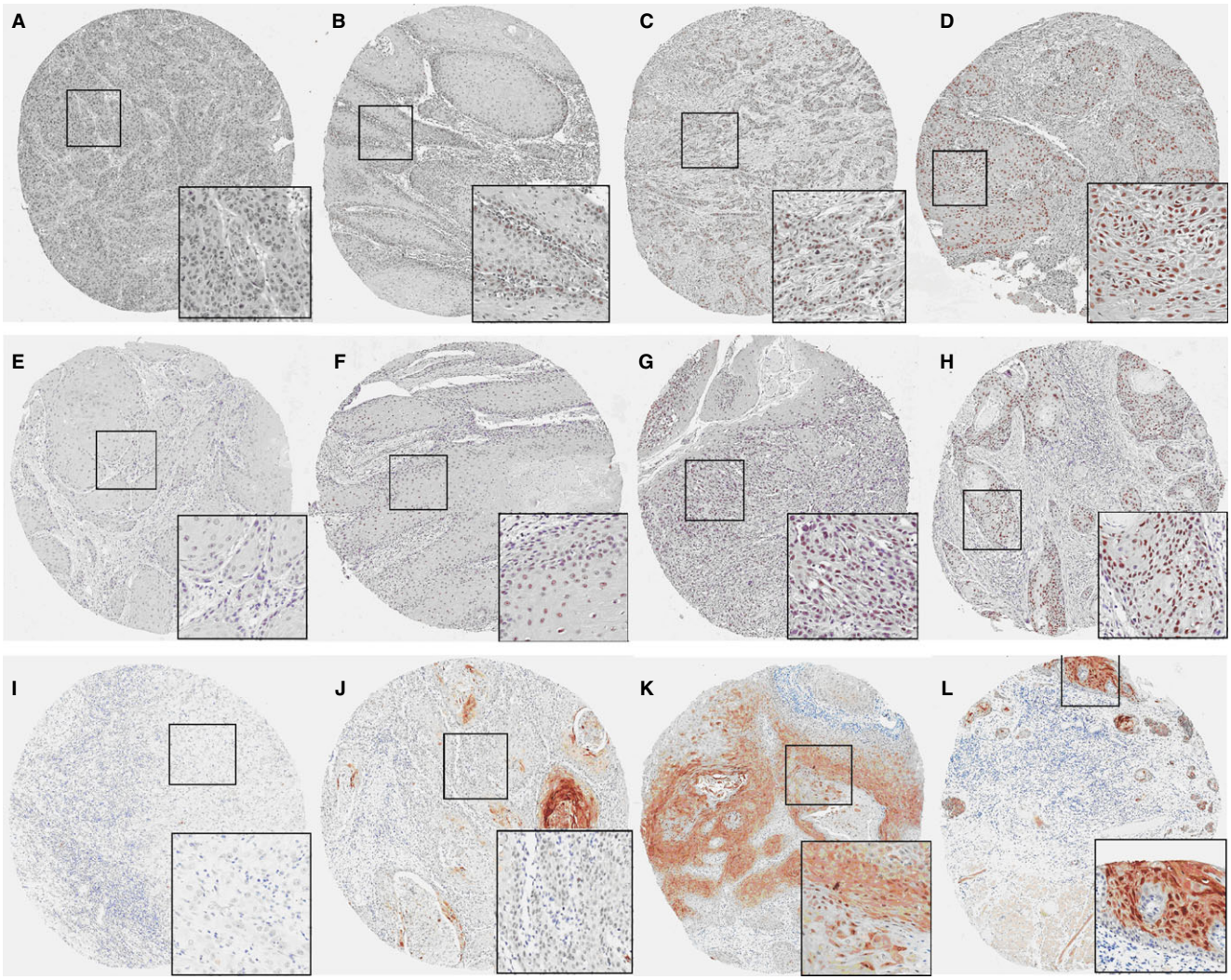

Figure 1 Immunoreactivity of OCT4, SOX2 and NANOG in OSCC. (A) OCT4 score 0, (B) OCT4 score 1, (C) OCT4 score 2, (D) OCT4 score 3, (E) SOX2 score 0, (F) SOX2 score 1, (G) SOX2 score 2, (H) SOX2 score 3, (I) NANOG score 0, (J) NANOG score 1, (K) NANOG score 2, (L) NANOG score 3.

OCT4, SOX2, and NANOG expression in tumor tissues, tumor adjacent normal tissues, and normal oral tissues

Our results showed that OCT4 and SOX2 were expressed only in the cell nucleus, whereas NANOG was observed in the cell nucleus and diffusely in the cytoplasm (data not shown). In OSCC tissues, OCT4 expression had a weak positive correlation with SOX2 ($r = 0.170$, $P < 0.001$), but no correlation with NANOG-N ($r = -0.002$, $P = 0.97$), and SOX2 had a weak correlation with NANOG-N ($r = 0.105$, $P = 0.029$).

There were significant differences in SOX2, OCT4, and NANOG-N protein expression between the tumor, CTAN, and normal tissues (Table 2). In the post hoc analysis, we found the following: OCT4 expression was significantly higher in CTAN ($P < 0.001$) and normal tissues ($P < 0.001$) than in tumor tissues. SOX2 expression was significantly higher in CTAN tissue than in tumor ($P < 0.001$) and normal ($P = 0.021$) tissues. In addition, NANOG-N expression was significantly higher in CTAN ($P = 0.009$) and tumor tissues ($P = 0.014$) than in normal tissues, but there was no significant difference in NANOG expression between tumor and CTAN tissues. Furthermore, the expression of OCT4 ($n = 348$, $P < 0.001$) and SOX2 ($n = 362$, $P < 0.001$) was significantly reduced in tumor tissues compared to paired CTAN tissues.

OCT4, SOX2, and NANOG expression and clinicopathological parameters

In OSCC (Table 1), a higher expression of OCT4 and SOX2 was associated with early AJCC stage ($P = 0.002$ and $P < 0.001$), small tumor size ($P = 0.017$ and $P = 0.001$), and the absence of lymph node metastasis ($P = 0.015$ and $P = 0.025$). NANOG expression was not correlated with any clinicopathological parameters, except location of OSCC. An increased expression of NANOG was found in the tongue compared to that in the buccal mucosa.

OCT4, SOX2, and NANOG expression and survival

Kaplan–Meier analyses (Fig. 2) showed that higher OCT4 and SOX2 expression was associated with better disease-specific survival (by log-rank test, $P = 0.002$ and $P < 0.001$, respectively) (Table 3). Cox regression analysis showed that high SOX2 expression was associated with better disease-specific survival (crude hazard ratio (CHR):

Table 1 Expressions of OCT4, SOX2, NANOG, and clinicopathologic outcomes in patients with OSCC

		OCT4			*SOX2*			*NANOG*		
Variable	*No. (%)*	*Mean ± SD*	*Median*	P-*value*	*Mean ± SD*	*Median*	P-*value*	*Mean ± SD*	*Median*	P-*value*
Sex										
Female	34 (7.8)	3.06 ± 2.06	3.00	0.698*	1.65 ± 1.84	1.00	0.741*	0.32 ± 1.12	0.00	0.100*
Male	402 (92.2)	3.12 ± 1.78	3.00		1.81 ± 2.04	1.00		0.68 ± 1.41	0.00	
Age, years										
≦40	72 (16.5)	3.49 ± 1.85	4.00	0.182†	1.96 ± 1.78	2.00	0.283†	0.89 ± 1.62	0.00	0.184†
41–50	139 (31.9)	3.04 ± 1.75	3.00		1.59 ± 2.03	0.00		0.57 ± 1.29	0.00	
51–60	125 (28.7)	2.89 ± 1.88	3.00		1.91 ± 2.07	2.00		0.76 ± 1.45	0.00	
>60	100 (22.9)	3.25 ± 1.68	3.00		1.83 ± 2.12	1.00		0.46 ± 1.27	0.00	
Subsite										
Buccal	188 (43.1)	3.10 ± 1.59	3.00	0.882*	1.87 ± 2.14	2.00	0.848*	0.39 ± 1.15	0.00	<0.001*
Tongue	248 (56.9)	3.13 ± 1.94	3.00		1.75 ± 1.93	1.00		0.85 ± 1.53	0.00	
Cell differentiation										
Well	78 (17.9)	3.27 ± 1.68	3.00	0.397†	2.21 ± 2.19	2.00	0.094†	0.50 ± 1.29	0.00	0.333†
Moderate	331 (75.9)	3.11 ± 1.80	3.00		1.75 ± 2.01	1.00		0.67 ± 1.42	0.00	
Poor	27 (6.2)	2.78 ± 2.08	2.00		1.19 ± 1.36	0.00		0.81 ± 1.36	0.00	
AJCC pathological stage										
I, II	282 (64.7)	3.32 ± 1.74	3.00	0.002*	2.07 ± 2.10	2.00	<0.001*	0.68 ± 1.43	0.00	0.705*
III, IV	154 (35.3)	2.75 ± 1.85	3.00		1.30 ± 1.76	0.00		0.60 ± 1.34	0.00	
T classification										
T1, T2	338 (77.5)	3.23 ± 1.76	3.00	0.017*	1.96 ± 2.08	2.00	0.001*	0.63 ± 1.39	0.00	0.429*
T3, T4	98 (22.5)	2.74 ± 1.88	3.00		1.22 ± 1.71	0.00		0.72 ± 1.43	0.00	
N classification										
N0	339 (77.8)	3.24 ± 1.79	3.00	0.015*	1.92 ± 2.09	2.00	0.025*	0.68 ± 1.43	0.00	0.578*
N1, N2	97 (22.2)	2.71 ± 1.78	3.00		1.37 ± 1.73	0.00		0.56 ± 1.27	0.00	

OCT4, octamer-binding protein 4; SOX2, sex-determining region Y (SRY)-related Box 2; *OSCC, oral squamous cell carcinoma; and* AJCC, American Joint Committee on Cancer.
**P*-values were estimated by *Mann–Whitney U-test.*
†*P*-values were estimated by Kruskal–Wallis one-way ANOVA test.

Table 2 The comparisons of OCT4, SOX2, and NANOG expression in the three different tissues of OSCC

	Normal tissue		*Tumor adjacent normal*		*Tumor*			
Variables	*Mean ± SD*	*Median*	*Mean ± SD*	*Median*	*Mean ± SD*	*Median*	χ^2	P-*value*
Independent samples*								
OCT4	(n = 71) 4.03 ± 2.12[a]	4.00	(n = 348) 3.87 ± 1.65[b]	4.00	(n = 436) 3.12 ± 1.80[a,b]	3.00	35.444	**<0.001**
SOX2	(n = 66) 2.38 ± 2.46[c]	2.00	(n = 362) 3.14 ± 2.49 [c,d]	3.00	(n = 436) 1.80 ± 2.02[d]	1.00	58.145	**<0.001**
NANOG	(n = 68) 0.19 ± 0.74[e,f]	0.00	(n = 362) 0.68 ± 1.47[e]	0.00	(n = 436) 0.65 ± 1.40[f]	0.00	6.738	**0.034**
Dependent samples†								
OCT4	–	–	(n = 348) 3.87 ± 1.65	4.00	(n = 348) 3.19 ± 1.83	3.00	−5.759	**<0.001**
SOX2	–	–	(n = 362) 3.14 ± 2.49	3.00	(n = 362) 1.79 ± 1.98	1.50	–9.641	**<0.001**
NANOG	–	–	(n = 362) 0.68 ± 1.47	0.00	(n = 362) 0.64 ± 1.38	0.00	−0.550	0.583

OCT4, octamer-binding protein 4; SOX2, sex-determining region Y (SRY)-related *Box 2;* OSCC, oral squamous cell carcinoma; and SD, standard deviation.
*Independent samples were estimated by Kruskal–Wallis one-way ANOVA test.
†Dependent samples were estimated by Wilcoxon signed-rank test.
[a]$P < 0.001$; [b]$P < 0.001$; [c]$P = 0.021$; [d]$P < 0.001$; [e]$P = 0.014$; [f]$P = 0.009$.

0.55, 95% CI: 0.41–0.73, $P < 0.001$), even after adjustment for cell differentiation (moderate or poor vs. well), AJCC pathological stage (stage II, III, IV vs. stage I), and postoperative radiotherapy (yes vs. none) (adjusted hazard ratio (AHR): 0.63, 95% CI: 0.47–0.84, $P = 0.002$). High OCT4 expression was associated with better disease-specific survival (CHR: 0.59, 95% CI: 0.42–0.38, $P = 0.002$), which was not found in multivariate analysis. Conversely, the expression level of NANOG was not associated with disease-specific survival of patients with OSCC. In addition, we found that OCT4, SOX2, and NANOG were not correlated with disease-free survival (data not shown).

Discussion

OCT4, SOX2, and NANOG constitute the core network of transcription factors that regulate downstream pluripotency circuitry and mediate embryonic stem cell self-renewal.

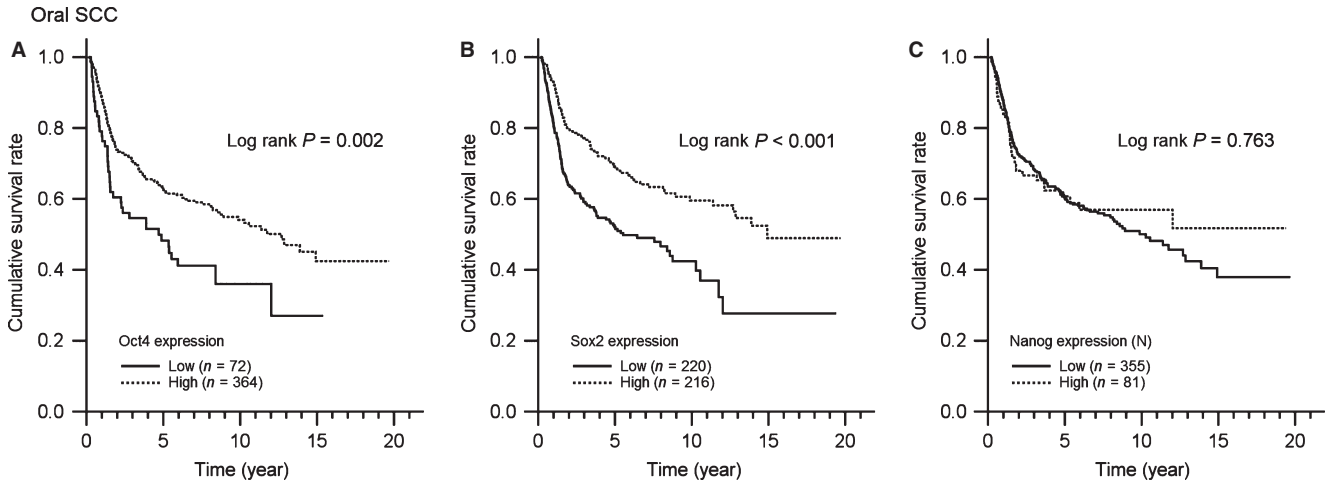

Figure 2 Kaplan-Meier survival curves. Kaplan-Meier curves of disease-specific survival rates according to (A) OCT4 expression status, (B) SOX2 expression status, (C) NANOG expression status in OSCC.

Table 3 The expression levels of OCT4, SOX2, and NANOG for disease-specific survival of patients with OSCC

	OSCC (n = *436*)				
Variable	*No (%)*	*CHR (95% CI)*	P-*value*	*AHR (95% CI)*	P-*value**
OCT4 expression					
Low	72 (16.5)	1.00		1.00	
High	364 (83.5)	**0.59 (0.42–0.83)**	**0.002**	0.79 (0.56–1.12)	0.189
SOX2 expression					
Low	220 (50.5)	1.00		1.00	
High	216 (49.5)	**0.55 (0.41–0.73)**	**<0.001**	**0.63 (0.47–0.84)**	**0.002**
NANOG expression					
Low	355 (81.4)	1.00		1.00	
High	81 (18.6)	0.95 (0.65–1.37)	0.763	0.85 (0.58–1.25)	0.413

OCT4, octamer-binding protein 4; SOX2, sex-determining region Y (SRY)-related Box 2; *OSCC, oral squamous cell carcinoma;* AJCC, American Joint Committee on Cancer; CHR, crude hazard ratio; CI, confidence interval; and AHR, adjusted hazard ratio.
**P*-value were adjusted for cell differentiation (moderate or poor vs. well), AJCC pathological stage (stage II, III, or IV vs. stage I), postoperative RT by multiple Cox's regression.

These genes are down-regulated via hypermethylation during differentiation in embryonic cells (16–18). Our study showed that the expression levels of both OCT4 and SOX2 in oral malignancy tissues were significantly lower than those in CTAN tissue or normal tissue. Li et al. also reported that SOX2 was strongly and moderately expressed in the nuclei of the gastric foveolar epithelium and intestinal metaplasia, respectively; the expression was much higher than that in gastric carcinomas (19). In pre-invasive lesions of the lung, elevated SOX2 expression has also been reported to occur in normal bronchial epithelium, alveolar bronchiolization, and squamous dysplasia (20). SOX2 overexpression and deregulation of its downstream target genes have been suggested to participate in early transformation during SCC carcinogenesis, which involves hyperplasia and dysplasia (21). SOX2 may protect malignant squamous cells from apoptosis all along the carcinogenesis sequence (12, 22). In addition, SOX2 was capable of effectively cooperating with FGFR2 to transform human bronchial epithelial cells into squamous cells *in vitro* (12, 22). Therefore, SOX2 expression is higher in CTAN tissue than in normal oral tissues, suggesting that cells adjacent to tumor tissues might harbor early molecular changes similar to pre-invasive lesions. Furthermore, 'normal squamous precursor cells', which need early overexpression of SOX2 and OCT4, would be more prevalent in CTAN tissues than in tumor tissues. However, the low expression of NANOG in normal oral epithelial tissues and high expression of NANOG in tumor tissue contradict this hypothesis.

In our study, NANOG expression, different from SOX2 and OCT4, was higher in tumor tissues and CTAN tissues than in normal tissues. The reason for the NANOG expression difference remains uncertain. Navarro et al. reported that NANOG activity is auto-repressive and OCT4/SOX2 independent. The influence of NANOG on OCT4 and SOX2 expression is minimal (18). We also found that NANOG expression had a weak positive correlation with SOX2 ($r = 0.105$, $P = 0.029$), but was not correlated with OCT4 expression ($r = -0.002$, $P = 0.97$) in OSCC tissue. Furthermore, Silva et al. has shown that NANOG is decisive for attaining a pluripotent ground state in the final phase of reprogramming when other key factors are already present (23). It is possible that NANOG proteins function differently from other reprogramming proteins in tumorigenesis; this has also been implicated in studies of patients with gastric cancer (24). The increased expression of

NANOG in tumor as well as CTAN tissues needs to be elucidated in further functional studies.

In our study, the expression of OCT4 and SOX2 was significantly associated with an early pathological stage of disease, small tumor size, and the absence of lymph node metastasis. Our data also showed that high SOX2 and OCT4 expression was significantly associated with a better prognosis for patients with OSCC. Previous studies of SCC at various locations and gastric carcinoma found an association of elevated SOX2 and OCT4 expression with favorable clinicopathological features and overall longer survival (11, 12, 21, 25, 26). In patients with early-stage OSCC, up-regulation of SOX2 was correlated with a lower incidence of lymph node metastasis (14). These findings are consistent with our study results and imply that higher SOX2 and OCT4 levels are associated with an early stage of OSCC and better prognosis. The down-regulation of SOX2 expression in advanced-stage OSCC in our study suggested that the early over-expression of SOX2 might be lessened gradually during OSCC progression. However, whether the molecular mechanisms accounting for SOX2 and OCT4 are associated with a favorable prognosis in OSCC is still unknown. More studies are needed to clarify the functional aspects of SOX2-/OCT4-related pathways in the progression of carcinogenesis.

Studies concerning the expression of SOX2/OCT4 in OSCC showed contradictory results. Other researchers have found that a high expression of SOX2 is significantly associated with a higher histological grade and poorer survival in esophageal and histologically node-negative OSCC patients (4, 27). They suggested that the expression of SOX2 may play a role in conferring a less-differentiated phenotype or inactivating the ability to differentiate. Chiou et al. also found that an elevated expression of OCT4 was associated with medium-to-poor differentiation and worse survival in 52 patients with OSCC (28). However, these studies were limited by relatively small sample sizes and the selection of certain pathological stages.

In conclusion, this study showed that OCT4 and SOX2 expression is correlated with tumorigenesis and an earlier stage of disease in patients with OSCC. SOX2 can be a useful prognostic predictor for OSCC located in both the buccal mucosa and tongue. Understanding the mechanisms of SOX2/OCT4 pathways in the initiation and progression of SCC development may reveal a potential target for further novel therapy.

References

1. Petersen PE. Strengthening the prevention of oral cancer: the WHO perspective. *Commun Dent Oral Epidemiol* 2005; **33**: 397–9.
2. Department of Health. Cancer Registry annual Report in Taiwan Area. Taipei: Department of Health, 2008. The Executive Yuan, Taiwan ROC 2009.
3. Kao SY, Chu YW, Chen YW, Chang KW, Liu TY. Detection and screening of oral cancer and pre-cancerous lesions. *J Chin Med Assoc* 2009; **72**: 227–33.
4. Du L, Yang Y, Xiao X, et al. Sox2 nuclear expression is closely associated with poor prognosis in patients with histologically node-negative oral tongue squamous cell carcinoma. *Oral Oncol* 2011; **47**: 709–13.
5. Chen YK, Huang HC, Lin LM, Lin CC. Primary oral squamous cell carcinoma: an analysis of 703 cases in southern Taiwan. *Oral Oncol* 1999; **35**: 173–9.
6. Fu TY, Hou YY, Chu ST, et al. Manganese superoxide dismutase and glutathione peroxidase as prognostic markers in patients with buccal mucosal squamous cell carcinomas. *Head Neck* 2011; **33**: 1606–15.
7. Chhetri DK, Rawnsley JD, Calcaterra TC. Carcinoma of the buccal mucosa. *Otolaryngol Head Neck Surg* 2000; **123**: 566–71.
8. Dalerba P, Cho RW, Clarke MF. Cancer stem cells: models and concepts. *Annu Rev Med* 2007; **58**: 267–84.
9. Schulenburg A, Ulrich-Pur H, Thurnher D, et al. Neoplastic stem cells: a novel therapeutic target in clinical oncology. *Cancer* 2006; **107**: 2512–20.
10. Chambers I, Colby D, Robertson M, et al. Functional expression cloning of Nanog, a pluripotency sustaining factor in embryonic stem cells. *Cell* 2003; **113**: 643–55.
11. Bass AJ, Watanabe H, Mermel CH, et al. SOX2 is an amplified lineage-survival oncogene in lung and esophageal squamous cell carcinomas. *Nat Genet* 2009; **41**: 1238–42.
12. Wilbertz T, Wagner P, Petersen K, et al. SOX2 gene amplification and protein overexpression are associated with better outcome in squamous cell lung cancer. *Mod Pathol* 2011; **24**: 944–53.
13. Freier K, Knoepfle K, Flechtenmacher C, et al. Recurrent copy number gain of transcription factor SOX2 and corresponding high protein expression in oral squamous cell carcinoma. *Genes Chromosom Cancer* 2010; **49**: 9–16.
14. Zullig L, Roessle M, Weber C, et al. High sex determining region Y-box 2 expression is a negative predictor of occult lymph node metastasis in early squamous cell carcinomas of the oral cavity. *Eur J Cancer* 2013; **49**: 1915–22.
15. Lee SH, Oh SY, Do SI, et al. SOX2 regulates self-renewal and tumorigenicity of stem-like cells of head and neck squamous cell carcinoma. *Br J Cancer* 2014; **111**: 2122–30.
16. Masui S, Nakatake Y, Toyoka Y, et al. Pluripotency governed by Sox2 via regulation of Oct3/4 expression in mouse embryonic stem cells. *Nat Cell Biol* 2007; **9**: 625–35.
17. Loh YH, Wu Q, Chew JL, et al. The Oct4 and Nanog transcription network regulates pluripotency in mouse embryonic stem cells. *Nat Genet* 2006; **38**: 431–40.
18. Navarro P, Festuccia N, Colby D, et al. OCT4/SOX2-independent Nanog autorepression modulates heterogeneous Nanog gene expression in mouse ES cells. *EMBO J* 2012; **31**: 4547–62.
19. Li XL, Eishi Y, Bai YQ, et al. Expression of the SRY-related HMG box protein SOX2 in human gastric carcinoma. *Int J Oncol* 2004; **24**: 257–63.
20. Yuan P, Kadara H, Behrens C, et al. Sex determining region Y-Box 2 (SOX2) is a potential cell-lineage gene highly expressed in the pathogenesis of squamous cell carcinomas of the lung. *PLoS ONE* 2010; **9**: e9112.
21. Lu YI, Futtner C, Rock JR, et al. Evidence that SOX2 overexpression is oncogenic in the lung. *PLoS ONE* 2010; **5**: e11022.
22. Hussenet T, du Manoir S. SOX2 in squamous cell carcinoma: amplifying a pleiotropic oncogene along carcinogenesis. *Cell Cycle* 2009; **9**: 1480–6.
23. Silva J, Nichols J, Theunissen TW, et al. Nanog is the gateway to the pluripotent ground state. *Cell* 2009; **138**: 722–37.
24. Matsuoka J, Yashiro M, Sakurai K, et al. Role of the stemness factors sox2, oct3/4, and nanog in gastric carcinoma. *J Surg Res* 2012; **174**: 130–5.
25. Ge N, Lin HX, Xiao XS, et al. Prognostic significance of Oct4 and Sox2 expression in hypopharyngeal squamous cell carcinoma. *J Transl Med* 2010; **8**: 94.

26. Zhang X, Yu H, Yang Y, et al. SOX2 in gastric carcinoma, but not Hath1, is related to patients' clinicopathological features and prognosis. *J Gastrointest Surg*. 2010; **14**: 1220–6.
27. Wang Q, He W, Lu C, et al. Oct3/4 and Sox2 are significantly associated with an unfavorable clinical outcome in human esophageal squamous cell carcinoma. *Anticancer Res* 2009; **29**: 1233–41.
28. Chiou SH, Yu CC, Huang CY, et al. Positive correlations of Oct-4 and Nanog in oral cancer stem-like cells and high-grade oral squamous cell carcinoma. *Clin Cancer Res* 2008; **14**: 4085–95.

Acknowledgements

This project was supported by grants from Veterans General Hospital Kaohsiung, Taiwan (Project No. VGHKS101-115, VGHKS102-002, and VGHKS103-106).

Conflict of interest

None of the authors have conflict of interest to declare