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Studies of the serum HER-2/*neu* and squamous cell carcinoma-related antigen expression in patients with oral squamous cell carcinoma

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BACKGROUND: Over-expression of HER-2/*neu* has been associated with advanced disease, metastasis, and poor clinical outcome. Squamous cell carcinoma (SCC)-related antigen (SCCA) is used as a tumor marker in a variety of SCC. This study was performed to investigate the pre- and post-treatment values of HER-2/*neu* and SCCA in patients with oral SCC.

MATERIALS: Patients with OSCC were enrolled between 2002 and 2004. Serum samples were obtained before treatment and at 1 month following treatment. HER-2/*neu* levels and SCCA levels were determined by enzyme-linked immunosorbent assay and radioimmunoassay, respectively.

RESULTS: A significant correlation was found between HER-2/*neu* and lymph node metastasis ($P = 0.0252$). SCCA correlated with tumor size ($P = 0.0042$). Both HER-2/*neu* and SCCA mean levels were reduced significantly after treatment.

CONCLUSION: The combination of HER-2/*neu* and SCCA serum levels may be a useful marker in evaluating therapeutic effects and in monitoring the recurrence rate of OSCC and survival rate of patients.

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Keywords: HER-2/*neu*; OSCC; SCCA

Introduction

Squamous cell carcinoma-associated antigen (SCCS) is a subfraction of TA-4, and it therefore shares common antigenicity with TA-4. Originally reported by Kato and Torigoe (1) as an SCCS of uterine cancer, TA-4 has a molecular weight of 48 kDa (1, 2). Serum TA-4 has been found to be markedly elevated in squamous cell carcinoma of the uterus (USCC). TA-4 can be used as a marker in monitoring USCC (3). Approximately 90% of oral malignancies are squamous cell carcinoma (OSCC). Elevated SCCA in patients with head and neck cancer has been reported (4–6).

HER-2/*neu* is a 185-kDa transmembrane glycoprotein, possessing activity of tyrosine kinase activity and sharing a structural homology and function similar to the epidermal growth factor receptor (EGFR) (7). Over-expression of HER-2/*neu* in breast, ovary, pancrease, prostate, salivary gland, endometrium, lung, bone, stomach, and oral cavity cancers has a strong correlation with poor prognosis (8–18). Soluble HER-2/*neu* oncoprotein in the sera of patients with ovarian (14) and breast cancers (10, 19) has been reported.

Recently, we reported elevated serum levels of HER-2/*neu* oncoprotein product in OSCC and breast cancer patients (19). In this study, we investigated the effect of treatment on the levels of SCCA and HER-2/*neu* detected, respectively, by radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) in the sera of patients with OSCC.

Materials and methods

Subjects

The protocol was approved by the institutional review board and informed consent was obtained before venipuncture. Patients with OSCC from the Department of Oral Maxillofacial Surgery, Chung-Ho Memorial Hospital, Taiwan were enrolled in the study between September 2002 and January 2004. Patients with other therapies pre-surgically or with adjuvant setting were excluded from this study.

Serum HER-2/*neu* levels were measured in 46 OSCC patients and serum SCCA levels were measured in 37 OSCC patients (Table 1). Serum samples were taken after histologic diagnosis and 1 month after surgical treatment. The reference value was based on the sera of eight healthy subjects who attended our clinic for routine physical examination.

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Table 1 Characteristics of patients with oral cancer

Characteristic	*HER-2/neu n (%)*	*SCCA n (%)*
Total	46 (100)	37 (100)
Age (years)		
≤40	6 (13)	6 (16)
41–60	32 (70)	23 (62)
>60	8 (17)	8 (22)
Habits		
Alcohol drinking	36 (78)	29 (78)
Betel quid chewing	42 (91)	34 (92)
Cigarette smoking	41 (89)	33 (89)
Cancer site		
Tongue	15 (33)	12 (32)
Buccal mucosa	21 (46)	17 (46)
Other	10 (22)	8 (22)
Cancer histology		
SCC (W-D)	36 (78)	29 (78)
SCC (M-D)	8 (17)	6 (16)
SCC (P-D)	2 (4)	2 (5)
Cancer histological grade		
Grade 1	30 (65)	23 (62)
Grade 2	12 (26)	10 (27)
Grade 3	4 (9)	4 (11)
Type of surgery		
WE	12 (26)	11 (30)
WE + SND	24 (52)	18 (49)
WE + RND	9 (20)	8 (22)

SCCA, squamous cell carcinoma-related antigen; W-D, well-differentiated; M-D, moderately differentiated; P-D, poorly differentiated; WE, wide excision; SND, selective neck dissection; RND, radical neck dissection.

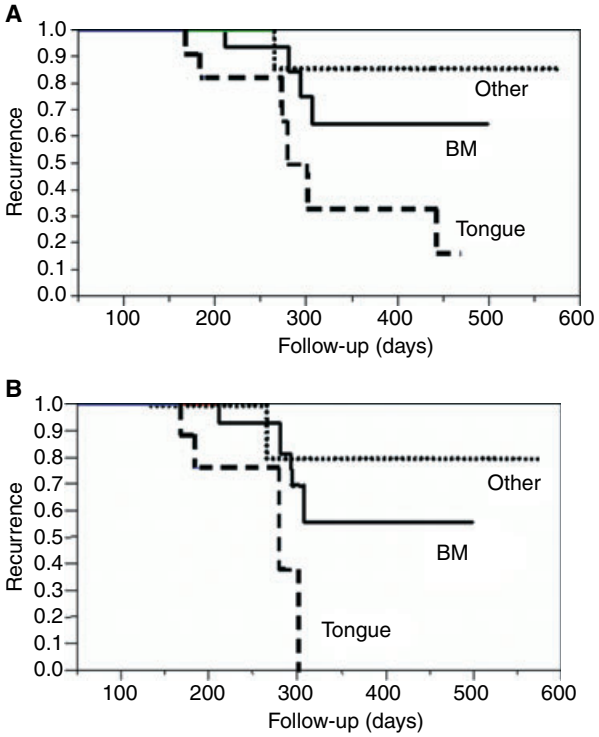

Figure 1 Recurrent curves for Her-2/*neu* group (A) ($\chi^2 = 7.2902$, $P = 0.0261$) and for SCCA group (B) ($\chi^2 = 7.4092$, $P = 0.0246$) OSCC patients presenting tumor at the primary site: buccal mucosa (BM), tongue, other.

Methods

HER-2/*neu* oncoprotein levels were measured by ELISA using the *neu* oncoprotein ELISA kit (QIA 10 *c-erb*B-2/1*cneu*, Rapid Format ELISA; Oncogene Research Products, Boston, MA, USA) and SCCA levels were quantified using radioimmunoassay (ABBOTT SCC RIABEAD; ABBOTT Diagnostic Division, Abbott Park, IL, USA).

Statistical analysis

ANOVA was used for comparing HER-2/*neu* and SCCA levels on lymph node, tumor size, and stage. Fisher's exact test was used to investigate the relationship between the post-surgical serum HER-2/*neu* level and neck recurrence. For the analysis of recurrence rates and survival rates the Kaplan–Meier survival curves as well as the log-rank tests were adopted. Furthermore, Cox regression models were conducted to investigate the effects from pre- and post-surgical SCCA and HER-2/*neu*, separately (univariate) and together (multivariate), on the recurrence rates and survival rates while adjusting for lymph node and tumor size. Statistical analyses were conducted by using SAS version 8 (SAS Institute, Cary, NC, USA).

Results

Characteristics of patients

A majority of our OSCC patients were 40–60 years old, and over 90% were betel quid chewers. Tumors were mainly located on the buccal mucosa (BM), histopathologically well-differentiated, and of grade 1 (Table 1). These characteristics are similar to that observed in many studies of OSCC in Taiwan (19, 20). Most patients received wide excision and selective neck dissection. Patients with primary site tumor of the tongue had a higher recurrent rate ($P < 0.05$) than those with primary site tumors of the BM or other (Fig. 1A,B).

*HER-2/*neu *and SCCA serum levels in normal and OSCC patients*

The HER-2/*neu* mean serum value for normal controls ($n = 8$) was 1.42 ± 0.36 ng/ml (Table 2). The SCCA mean value for normal controls was 1.5 ng/ml (Table 2). 65% of the OSCC patients' HER-2/*neu* (p185) was

Table 2 HER-2/*neu* and SCCA serum levels of 46 OSCC patients

HER-2/neu group		*SCCA group*	
Value (ng/ml)	*n (%)*	*Value (ng/ml)*	*n (%)*
≥1.76	30 (65)		
≥2.14	18 (39)	≥1.5	6 (16)
<2.14	28 (61)	<1.5	31 (84)

SCCA, squamous cell carcinoma-related antigen. ≥1.76 ng/ml: the upper limit for normal representing the 95th percentile in a group of normal controls ($n = 8$). HER-2/*neu* cut-off value: normal control mean + 2 × SD (1.42 + 2 × 0.36). SCCA cut-off value: 1.5 ng/ml.

≥1.76 ng/ml (the upper limit for normal, representing the 95th percentile in a group of the normal controls) (Table 2), while 39% of the OSCC patients' HER-2/*neu* was ≥2.14 ng/ml (normal control mean + 2 standard deviations), therefore: HER-2/*neu* was over-expressed in 65–39% of the OSCC patients. Six of thirty seven (16%) of the patients' serum SCCA was over-expressed (≥1.5 ng/ml) (Table 2).

Relationship of pre-surgical serum HER-2/neu and SCCA levels with TNM

The serum level of HER-2/*neu* (but not SCCA) was significantly associated with lymph node metastasis ($P = 0.0252$) (Table 3). The serum SCCA level was associated with T (tumor size) ($P = 0.0042$) (Table 3). No relationship between the serum level of either HER-2/*neu* or SCCA and patients' age, tumor primary site, histopathological classification and histopathological stage was observed (data not shown).

Difference in pre- and post-surgical serum levels of HER-2/neu and SCCA

The elevated post-surgical serum HER-2/*neu* level had a borderline relationship with neck lymph node recurrence ($P = 0.095$) (Table 4). Both serum HER-2/*neu* and SCCA levels were significantly different between pre- and post-surgery ($P < 0.005$) (Table 5).

Association of serum HER-2/neu and SCCA levels with tumor recurrence rate and patient survival rate

Only the mean post-surgical serum level of SCCA was associated ($P < 0.0001$) with tumor recurrence rate (Fig. 2) and patient survival rate ($P < 0.0001$) (Fig. 3). However, the combination of post-surgical HER-2/*neu* and SCCA levels better predicted the tumor recurrence rate (Table 6) than any one level alone. The combination of pre-surgical (Table 6) or the post-surgical (Table 6) serum levels of HER-2/*neu* and SCCA had better predictive values for survival rate than any one level alone.

Table 3 Relationships between lymph node metastasis (N)/tumor stages (I–IV) and HER-2/*neu* level, and between tumor size and SCCA level in oral SCCS

	HER-2/neu level (ng/ml)				*Serum SCCA level (ng/ml)*			
	n	*Mean*	*SD*	*ANOVA P-value*	*n*	*Mean*	*SD*	*ANOVA P-value*
Total	46	2.21	0.91		37	1.18	0.71	
Tumor size								
T1	11	1.99	0.47	0.4985	10	0.93	0.07	0.0042
T2	24	2.19	0.78		19	1.01	0.22	
T3 + T4	11	2.46	1.42		8	1.88	1.33	
N								
N0	4	1.46	0.24	0.0252	4	1.45	0.54	0.2698
N1	29	2.08	0.67		22	1.02	0.23	
N2	13	2.70	1.25		11	1.39	1.22	
Stage								
I + II	4	2.02	0.61	0.1839	4	1.00	0.20	0.1405
III	32	2.08	0.64		27	1.09	0.33	
IV	10	2.68	1.53		6	1.70	1.63	

SCCA, squamous cell carcinoma-related antigen.

Table 4 Post-surgical serum HER-2/*neu* levels vs. neck lymph node tumor recurrence

	n	*Neck recurrence (%)*	*No neck recurrence (%)*	*P-value*[a]
Post-surgical HER-2/*neu* (+)	14	4 (29)	10 (71)	0.0953
Post-surgical HER-2/*neu* (−)	32	3 (9)	29 (91)	

(+) = serum HER-2/*neu* level higher than pre-surgical level. (−) = serum HER-2/*neu* level lower than pre-surgical level.
[a]Fisher's exact test.

Table 5 Difference in pre-surgical and post-surgical HER-2/*neu* and SCCA serum levels in OSCC patients

	HER-2/neu (ng/ml)			*SCCA (ng/ml)*		
	Mean (SD)	*Lower 95%*	*Upper 95%*	*Mean (SD)*	*Lower 95%*	*Upper 95%*
Pre-surgical	2.21 (0.91)	1.93	2.48	1.18 (0.71)	0.94	1.41
Post-surgical	1.83 (0.67)	1.63	2.02	0.99 (0.45)	0.84	1.14

SCCA, squamous cell carcinoma-related antigen; OSCC, oral SCC. Paired *t*-test $P < 0.005$.

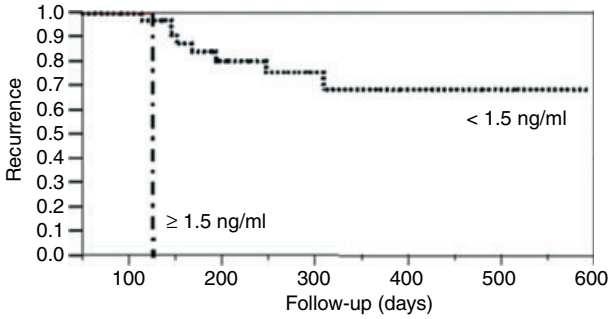

Figure 2 Recurrent curves for OSCC patients expressing post-surgical serum SCCA levels (< 1.15 ng/ml vs. ≥1.5 ng/ml, $\chi^2 = 15.76$, $P < 0.0001$).

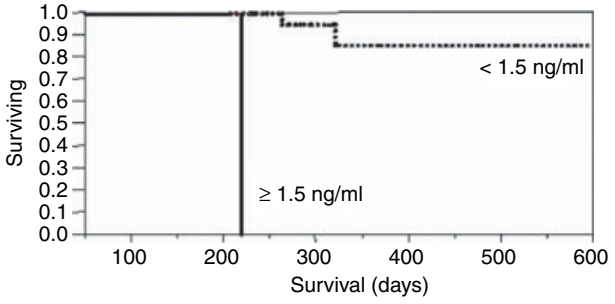

Figure 3 Survival curves for OSCC patients expressing post-surgical serum SCCA levels (< 1.5 ng/ml vs. ≥1.5 ng/ml, $\chi^2 = 24.00$, $P < 0.0001$).

Discussion

Previously, we had reported that the mean serum levels of HER-2/*neu* oncoprotein (p185) in 15 normal healthy individuals and in 84 OSCC patients were 8.64 ± 1.29 and 13.1 ± 4.56 ng/ml, respectively (19). 70.2% of those OSCC patients had HER-2/*neu* over-expressed, i.e., higher than the 95% cut-off value of healthy controls (19). In this investigation, we examined the serum levels of OSCC patients before and 1 month after the surgical removal of tumor to understand their relationship with patient survival rate patients and recurrent rate of OSCC. At the same time, we also evaluated the serum SCCA value to see whether its combination with HER-2/*neu* had a higher predictive power regarding the survival rate and recurrent rate of OSCC than any value alone would.

In the present study the mean serum levels of HER-2/*neu* in eight normal healthy individuals and 46 OSCC patients were 1.42 ± 0.36 and 2.21 ± 0.19 ng/ml, respectively. Both groups had about one-sixth the HER-2/*neu* levels of subjects in the previous report (19), however, the over-expression rate (65%) of OSCC was very similar to the previously reported rate (70.2%). In a further analysis, using a more critical standard, i.e., mean of normal controls plus 2 standard deviation as the cut-off value (2.14 ng/ml), the over-expression rate was 39%. This is very similar to the result (36%) reported in Xia et al. (21), who used immunohistochemistry to examine the expression levels of HER-2/*neu*. The pre-surgical serum levels of HER-2/*neu* seemed to have no significant correlation with the tumor stages ($P > 0.05$) (Table 3). They were very significantly correlated with node stages ($P = 0.0252$) (Table 3). This is in agreement with the findings of Xia et al. (21). The mean post-surgical serum level of HER-2/*neu* was significantly lower than the level before surgery ($P < 0.005$) (Table 5). The increased serum HER-2/neu after surgical treatment had a positive relationship with recurrent neck tumor ($P = 0.0953$) (Table 4). Although the over-expression of the pre-surgical mean serum level of SCCA was only 16%, it was associated with tumor size ($P = 0.0042$) (Table 3). This differs from the studies which reported that 63.3% (38/60) of OSCC patients' SCCA values were above the cut-off value of the normal serum SCCA value but had no association with tumor size (22), and that 50% of SCCA serum levels in stage I OSCC were greater than the normal control value (23). In the study by Miche et al. (24) 10–15% of patients with head and neck SCC had elevated pre-treatment serum SCCA levels that were above the cut-off level. They concluded that SCCA is probably of low value for tumor diagnosis and follow-up. However, Yasumatsu et al. (25) examined SCCA expression in 86 SCC cases of the tongue and reported that 17 of 86 cases (20%) showed evaluated serum SCCA levels greater than the upper limit (1.5 ng/ml).

Table 6 Results of univariate and multivariate analyses using the Cox regression analysis model

	Univariate[a]		*Multivariate*[b]		
	χ^2	*P-value*	*Combination of two factors*	χ^2	*P-value*
Post-surgical SCCA, HER-2/*neu* & recurrence					
SCCA	3.679	0.0551	SCCA + HER-2	6.817	0.0331
HER-2	0.003	0.9578			
Pre-surgical SCCA, HER-2/*neu* & survival					
SCCA	3.226	0.0725	SCCA + HER-2	10.780	0.0046
HER-2	0.152	0.6966			
Post-surgical SCCA, HER-2/*neu* & survival					
SCCA	1.824	0.1768	SCCA + HER-2	16.719	0.0002
HER-2	0.059	0.6966			

[a]Addition of single oncoprotein to Cox regression analyses, while adjusting for tumor size and lymph nodes.
[b]Multivariate addition of oncoproteins to Cox regression analysis, while adjusting for tumor size and lymph nodes.

The incidence of elevated serum SCCA level is related to TN classification and the clinical stage of tongue cancer (25). In spite of significant differences ($P = 0.0045$) in pre- and post-surgical serum SCCA levels, elevated post-surgical serum SCCA level was not associated with tumor recurrence ($P = 0.4$) (data not shown).

Cancer is not caused by only one oncogene: it is a multifactorial disease involving the interactions of many oncoproteins. Therefore, the power of using a single oncoprotein for the evaluation (or prediction) of cancer could be lower than using more than one. In this study, only the combination of post-surgical serum HER-2/*neu* and SCCA oncoprotein levels was significant for the prediction of the recurrence of OSCC. Similarly, only the combination of the HER-2/*neu* and SCCA serum levels (pre- or post-surgical) correlated with the survival rate of OSCC patients. In this context, Xia et al. (21) reported that a combination of EGFR, HER-2/*neu*, and HER-3 is a stronger predictor for the outcome of OSCC than any individual EGFR family member.

In conclusion, analysis of the combination of HER-2/*neu* and SCCA oncoproteins serum levels could be valuable in evaluating therapeutic effects and monitoring the disease status of OSCC.

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