Coexisting overexpression of STOML1 and STOML2 proteins may be associated with pathology of oral squamous cell carcinoma

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Objective. The present study aimed to investigate the expression and co-localization of stomatin-like protein-1 (STOML1) and stomatin-like protein-2 (STOML2) in oral squamous cell carcinoma (OSCC) tissues in situ and evaluate their pathologic roles in OSCC.

Study Design. STOML1 and STOML2 in human OSCC tissues (n = 109) and normal oral/paracancerous tissues (n = 19) were detected by using multiple immunohistochemistry (IHC) staining. Positive staining scores and clinicopathologic features during the OSCC process were analyzed.

Results. STOML1 and STOML2 were significantly overexpressed in OSCC tissues compared with normal oral tissue/paracancerous tissues ($P < .0001$ and $P < .0001$, respectively). Furthermore, both STOML1 and STOML2 were positively associated with pathologic tumor (T) stages. Positive signals of both STOML1 and STOML2 were mainly localized to the cell membrane and the cytoplasm, whereas those of STOML1 were also expressed in the cell nucleus.

Conclusions. Our results indicated that overexpression of STOML1 and STOML2 was significantly associated with T1 and T2 stages of OSCC. STOML1 and STOML2 were mainly co-localized at the cell membrane and the cytoplasm. These findings suggested that either STOML1 or STOML2 may play critical roles in OSCC development and may serve as potential diagnostic biomarkers and therapeutic targets. (Oral Surg Oral Med Oral Pathol Oral Radiol 2020;129:591−599)

Oral squamous cell carcinoma (OSCC) is one of the most common malignancies worldwide. It is characterized by a high rate of incidence, recurrence, deterioration, and death.[1-4] Despite improvements in procedures associated with clinical diagnoses and treatment, the 5-year survival rate of patients with OSCC remains close to 50%.[5] In patients with OSCC, the rate of recurrence is much higher at the late tumor−node−metastasis (TNM) stages but remains lower at the early TNM stages.[6,7] However, specialized, sensitive biomarkers and effective therapeutics that may be utilized for early diagnosis and treatment of OSCC seem to be lacking. Thus, it is important to identify novel biomarkers that may act as useful tools that can assist in the diagnosis and treatment of OSCC.

Stomatin-like protein-1 (STOML1) and stomatin-like protein-2 (STOML2) belong to the stomatin superfamily.[8] There are 5 members in this family, namely, stomatin, STOML1, STOML2, stomatin-like protein-3 (STOML3), and podocin, which all exhibit a highly conserved characteristic structure, the core stomatin domain.[9] Members of the stomatin superfamily of proteins are commonly found in mammals, plants, and bacteria.[10] STOML1 is a cell membrane protein that modulates acid sensing in ion channels.[8] Significantly high levels of STOML1 expression have been detected in the frontal lobe, cerebral cortex, hippocampus, and other basal ganglia.[11] Bioinformatic analyses conducted previously by our team indicated that mutation of the *STOML1* gene may be significantly associated with OSCC development.[12] However, very few studies have investigated the mechanisms underlying the association between STOML1 expression and OSCC development. However, many studies have reported that STOML2 may be a key protein that facilitates the proliferation of different cancer cells, such as esophageal squamous cell carcinoma,[13] rectal carcinoma,[14] epithelial ovarian carcinoma,[15] endometrial adenocarcinoma,[16] and papillary thyroid carcinoma,[17] among others.[18,19] Moreover, immunohistochemistry (IHC) results have shown that STOML3 expression was increased in the mesenchymal tumor areas of

Statement of Clinical Significance

The expression of the stomatin-like protein-1 (STOML1) and stomatin-like protein-2 (STOML2) in oral squamous cell carcinoma (OSCC) pathology is little known. Here, we provide data about the expression of the STOML1 and STOML2 proteins in OSCC tissues in situ, and the results of our evaluation of their relationship to co-localization and their possible roles in OSCC.

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gliosarcomas,[20] indicating that STOML3 may be associated with the mesenchymal component of gliosarcoma. Thus, these studies have indicated key roles for stomatin family members in carcinogenesis. The present study investigated the correlation between the expression levels of STOML1 and STOML2 and the pathologic stages of OSCC in situ by using multiple IHC staining of tissue microarrays. Furthermore, the co-localization effects of STOML1 and STOML2 on OSCC tissues and cell lines were evaluated.

MATERIALS AND METHODS

Tissue samples

Two commercial paraffin-embedded tissue microarrays were obtained from human oral cavity tissues (Biomax, Houston, TX): One array (OR208; n = 69) included 60 OSCC tissues and 9 normal oral tissues, where each tissue case was sectioned in triplicate; the other array (OR601 b; n = 60) included 50 OSCC tissues and 10 normal oral tissues, where each case was sectioned once. Individual clinical parameters, such as gender, TNM classification, clinical stage, and pathologic grade, per tissue section, are listed in Table I. Thus, 110 OSCC and 19 normal oral tissue sections were examined. One OSCC tissue section (ID: 601 b A3) split off during STOML2 antibody staining with the use of the multiple stain−destain−restain procedure (described below). All of the 110 OSCC tissues were examined for STOML1 expression, and 109 were examined for STOML2 expression.

Table I. Clinical characteristics of study patients with oral squamous cell carcinoma (OSCC)

Parameters	*Case numbers (n)*	*%*
Age (years)		
≤ 55	57	52.29
> 55	52	47.71
Gender		
Female	41	37.61
Male	68	62.39
Histologic differentiation		
Well-differentiated	65	59.63
Moderately differentiated	33	30.28
Poorly differentiated	8	7.34
Unknown	3	2.75
Clinical stage		
Ⅰ	44	40.37
Ⅱ	41	37.61
Ⅲ-Ⅳ	23	21.11
Unknown	1	0.92
Tumor (T) classification		
T_1	45	41.28
T_2	42	38.53
T_{3-4}	20	18.35
Unknown	2	1.83
Anatomic site		
Tongue	67	61.47
Lip	13	11.93
Gingiva	7	6.42
Cheek	5	4.59
Maxillary sinus	4	3.70
Lower jaw	4	3.70
Upper jaw	3	2.75
Oral cavity	3	2.75
Palate	1	0.92
Parotid gland	1	0.92
Mandible	1	0.92

Immunofluorescent staining

Tca8113 cells were grown on coverslips and fixed with 2% paraformaldehyde in phosphate-buffered saline (PBS) for 15 minutes at 37°C. After blocking nonspecific signals by using 3% bovine serum albumin (BSA) in PBS for 30 minutes at room temperature, cells were incubated with primary anti-STOML1 (1:200) and anti-STOML2 (1:1000) antibodies for 1 hour and with corresponding secondary antibodies for 1 hour at room temperature, followed by immunofluorescent staining. Cells were observed under an Olympus FV1000 microscope with a × 60 oil-immersion lens, and positive fluorescent puncta of STOML1 and STOMlL2 were recorded.

Multiple IHC staining

Multiplex IHC staining,[21] via a stain−destain−restain procedure, was used to detect several antigens. Tissue microarrays were deparaffinized with xylene and rehydrated with an alcohol series in a decreasing gradient. Tissue sections containing antigens were retrieved in 10 mM citrate buffer (pH 6.0) for 30 minutes, following incubation with 3% hydrogen peroxide for 30 minutes. After these tissue sections were washed with PBS, they were incubated with appropriate concentrations of primary anti-STOML1 antibodies (rabbit, 1:100) or anti-STOML2 antibodies (mouse, 1:200; Proteintech, Rosemont, IL) at 4°C, overnight. Next, the tissue sections were incubated with secondary antibodies (goat antirabbit immunoglobulin G [IgG]−horseradish peroxidase [HRP]; Beyotime, China) for 60 minutes at room temperature and stained by using a 3-amino-9-ethylcarbazole kit (AEC kit; GBI Labs, Bothell, WA) to detect positive signals. Hematoxylin was used for nuclear counterstaining. PBS was used as the negative control in place of primary antibodies. Anti-Ki67 antibodies (rabbit, 1:2000; Proteintech, Rosemont, IL), were used as a positive control, in place of primary antibodies, to check the staining process. Special anti-STOML1 or anti-STOML2 antibody-positive tissue sections were selected (e.g., colorectal cancer tissue sections), and immunostaining positive controls were set up against specific antigens.

Cell culture

OSCC cell lines, including Tca-8113, HOEC, DOK, and NOMC, were seeded at a density of $5 \times 10^4/cm^2$ in

collagen-coated 6-well plates with RPMI-1640 medium or DMEM/Ham's F12 medium supplemented by 10% fetal bovine serum and incubated at 37°C under a humidified atmosphere of 5% carbon dioxide.

Western blot

Total proteins were collected from incubating cells with RIPA lysis buffer (Beyotime, China) containing a protease cocktail (Roche, Germany) for 30 minutes at 4°C. Following centrifugation at × 14,000*g* at 4°C for 10 minutes, each lysate was transferred to a fresh 1.5-mL centrifuge tube on ice, and the protein concentration was determined by using a BCA assay (Thermo Fisher Scientific, Waltham, MA). For Western blot analysis, an equal amount of total protein was loaded on sodium dodecyl sulfate (SDS) polyacrylamide gel for electrophoresis purposes and transferred to a nitrocellulose membrane (Whatman, Germany). The nitrocellulose membrane was then blocked by using tris-buffered saline with Tween 20 (TBST) with 5% BSA for 30 minutes at room temperature. Candidate proteins were identified by using specific antibodies, followed by HRP-conjugated secondary antibodies. Bands were visualized by using an enhanced chemiluminescent substrate kit (ECL; Pierce Biotechnology, Waltham, MA) and a Tanon analysis system (Tanon, China).

Immunostaining quantification

Images were recorded by using a scanner with a microscope (Aperio CS2/EM AC20; Leica, Wetzlar, Germany). Five fields from each section were randomly chosen under × 20 magnification. Special immune responses were analyzed by 3 independent examiners, who used intensity scores based on the German immunoreactive scoring (IRS) system.[22,23] The degree of positive signal intensity was determined by using the following scale: 0 = none; 1 = weak; 2 = moderate; 3 = intense; and 4 = strongly intense. The percentage of cells that showed positive signaling was recorded as follows: 0 = 0%; 1 = 1-25%; 2 = 26%−50%; 3 = 51%−75%; and 4 = 76%−100%. The final staining score was obtained by multiplying the 2 scores stated above. A score of 2 or less was considered "negative", a score greater than 2 was considered "positive", and these categories were used in the subsequent statistical analyses. Sections categorized as "positive" with a score of 3 or less was considered "low", a score greater than 3 was considered "high", and these score categories were used for statistical analyses.

Statistical analysis

Positive signals and percentages were measured by using ImageJ (National Institutes of Health, Bethesda, MD). Statistical analyses of the χ^2 test, Fisher's exact test, 1-way analysis of variance (ANOVA), and Student *t* test were performed with Graphpad prism 6 (Graphpad Software, Inc., San Jose, CA) and Excel (Microsoft Corp., Redmond, WA). All tests were 2-tailed, and statistical significance was set at $P < .05$.

RESULTS

Images of multiplex IHC staining showed the overexpression of STOML1 and STOML2 in OSCC tissues. To investigate STOML1 and STOML2 expressions in OSCC tissue sections, multiplex IHC staining was performed on 109 OSCC specimens and on 19 specimens of normal oral/paracancerous tissues in situ. Positive signals were detected in 84 STOML1 specimens and 93 STOML2 specimens. Up to 77.1% and 85.3% (84 positive STOML1 antigen and 93 positive STOML2 antigen) of the 109 OSCC cases exhibited strong positive signals representing STOML1 and STOML2 expressions, compared with only 15.8% and 10.5% (3 positive STOML1 antigen and 2 positive STOML2 antigen) of the 19 normal oral tissue cases (score ≥ 2). Positive signals of STOML1 were detected in the membrane, cytoplasm, and nuclei of squamous epithelial cells in OSCC (Figure 1A), whereas positive signals of STOML2 were detected only in the cell membrane and the cytoplasm of cancerous squamous epithelia (Figure 1B). Image data indicated that both STOML1 and STOML2 were significantly overexpressed in OSCC lesions compared with those in normal tissues (see Figures 1A and 1B; Table II) ($P < .001$). These results indicated that STOML1 and STOML2 showed potential as biomarkers for assisting in the diagnosis of OSCC.

Positive correlation of STOML1 and STOML2 with T stages in the TNM classification

To investigate the effect of the coexisting overexpression of STOML1 and STOML2 in OSCC, expression levels of STOML1 and STOML2 in OSCC tissues were analyzed in relation to clinical stage, pathologic differentiation, and TNM stage. The association between STOML1 and STOML2 expressions and clinicopathologic factors are described in Table III. Images with high and low scores were divided into 2 OSCC groups, a score of 3 or less was considered "low" and a score greater than 3 was considered "high". High-score and low-score groups were then analyzed in relation to clinicopathologic factors to determine if abnormal overexpression levels and co-localization of STOML1 and STOML2 were significantly correlated with OSCC development. Overexpression levels of both STOML1 and STOML2 were higher in early or advanced T stages in the TNM classification compared with those in normal tissues ($P < .0001$) (Figure 2). χ^2 and Fisher's exact tests yielded similar results ($P < .0001$) when the respective STOML1 and STOML2

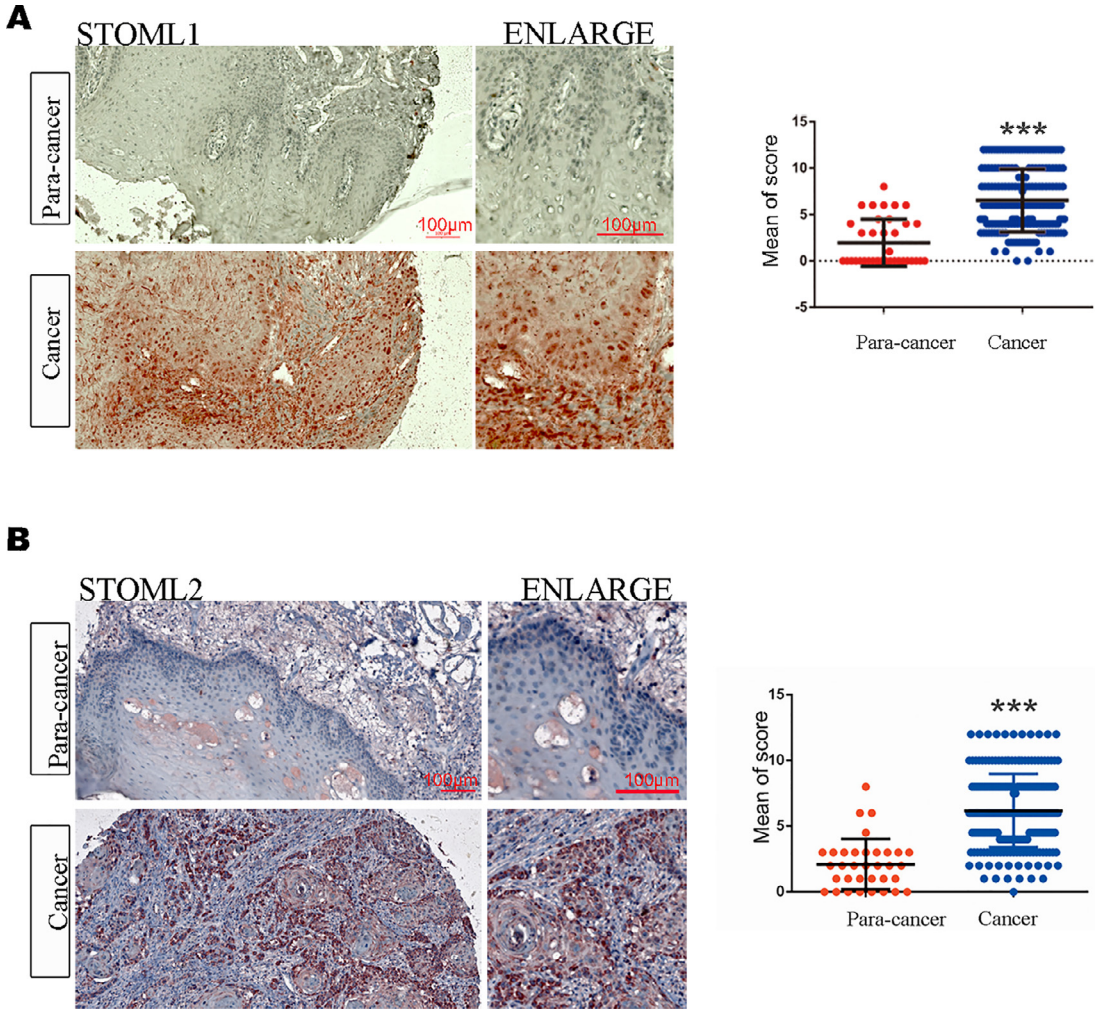

Fig. 1. Expression of STOML1 and STOML2 in oral squamous cell carcinoma (OSCC) tissues and normal oral/paracancerous tissues stained using multiplex immunohistochemistry (IHC) staining. A, *Left,* Higher STOML1 expression in OSCC tissues (n = 109) and lower STOML1 expression in normal oral/paracancerous tissues (n = 19) was observed (× 200 magnification). Enlarged regions are shown at higher magnification (scale bars: 100 μm). *Right,* Analysis of STOML1 expression in 109 OSCC tissues and 19 normal oral/paracancerous tissues indicating STOML1 upregulation in OSCC tissues. B, *Left,* Higher expression of STOML2 protein in OSCC tissues (n = 109) and lower expression of STOML2 in normal oral/paracancerous tissues (n = 19) are shown (× 200 magnification). The enlarged regions are shown at higher magnification (scale bars: 100 μm). *Right,* Analysis of STOML2 expression in 109 OSCC tissues and 19 normal oral/paracancerous tissues indicating STOML2 upregulation in OSCC tissues. *Note:* OSCC tissue cases: n = 109; normal oral tissue cases: n = 19. Red color dots depict positive signals. *** = $P < .0001$ (Student t test).

Table II. Differences in STOML1 and STOML2 expression between oral squamous cell carcinoma (OSCC) tissues and normal oral tissues by multiplex immunohistochemistry (IHC) staining

	STOML1 expression			*STOML2 expression*		
	Case numbers (n)	*Overexpression (n)/rate (%)*	*P value*	*Case numbers (n)*	*Overexpression (n)/rate (%)*	*P value*
Normal tissue	19	3/18.8%	< **.0001***	19	2/10.5%	< **.0001***
Cancer tissue	109	84/77.1%		109	93/85.3%	

P value were calculated by χ^2 test.
* Indicates a statistically significant difference ($P < .05$).

Table III. Association with overexpression of STOML1/STOML2 and clinicopathologic characteristics in oral squamous cell carcinoma (OSCC) tissues

Clinicopathologic characteristics	*STOML1 expression*			*STOML2 expression*		
	Low (n)	*High (n)*	*P value*	*Low (n)*	*High (n)*	*P value*
Age(years)						
≤ 55	9	41	.1020	5	44	.1164
> 55	17	43		11	49	
Gender						
Female	2	39	.0002*	6	35	.0305*
Male	24	45		25	58	
Histologic differentiation						
Well-differentiated	15	66	.0822	11	70	.1775
Moderately/poorly differentiated	6	12		4	14	
Clinical stage						
I	5	15	.0863	2	17	.4920
II-IV	3	26		3	26	
Tumor (T) classification						
T_{1-2}	19	69	< .0001**	11	76	< .0001**
T_{3-4}	5	15		3	17	

P values were calculated by using the χ^2 test.
*Indicates a statistically significant difference ($P < .05$).
**Indicates a statistically significant difference ($P < .0001$).

expression levels of stages T1 and T2 as well as T3 and T4 were compared (see Table III). The percentages of $STOML1^{High}$ and $STOML2^{High}$ were up to 47.3% and 52.9% at the early stages (T1, T2) and up to 40% and 45% at advanced stages (T3, T4) in OSCC cases. Although a significant difference was observed for another clinical factor, gender ($P < .005$), it was not considered for further investigation because the gender factor in this study was not normally distributed in a big population. As we collected samples and obtained information from tissue microarrays, female patients with OSCC were much fewer than male patients in this small group.

Co-localization of STOML1 and STOML2 in OSCC tissues and cells

Multiplex IHC positive signals were observed and recorded, and these results were analyzed via ImageJ software to show the intensity and distribution of STOML1 and STOML2 in cancer tissues and cells. The images showed that both STOML1 and STOML2 were located at the cell membranes and cytoplasm of OSCC, with similar staining distribution and intensity patterns (Figures 3A and 3B). The differences between staining distribution and intensity in cell nuclei were not measured because STOML2 was not located in the nucleus. Furthermore, to select appropriate cell lines for further testing in vitro, STOML1 and STOML2 expression levels in OSCC cell lines, including Tca-8113, HOEC, DOK, and Tca-8113, were detected by using Western blot. The results indicated that expression levels of both STOML1 and STOML2 in Tca-8113 cells were higher than those in other tested cell lines, and the detected levels of these proteins were significantly higher in all OSCC cell lines than in the normal oral epithelial cell line (HOEC) (Figure 3C). Immunofluorescent staining was used to confirm the co-localization of STOML1 and STOML2 in Tca-8113 cells in vitro. Both STOML1 and STOML2 protein signals via immunofluorescent staining in the cell membrane and the cytoplasm overlapped, but only the STOML1 signals were observed in the cell nucleus (Figure 3D).

DISCUSSION

Approximately 500,000 patients with OSCC are newly diagnosed each year, and two-thirds of these patients are from South East Asian Countries.[1,24] Therefore, identification of novel biomarkers that show potential as diagnostic and therapeutic targets of OSCC should be considered important for improving the cure and survival rates of patients.[25] Furthermore, a comprehensive understanding of molecular mechanisms underlying OSCC development may contribute toward the early diagnosis of OSCC as well as the initiation of appropriate therapy.

A few studies have been conducted on STOML1, a member of the mammalian stomatin superfamily. Reportedly, high levels of STOML1 expression in the human brain are associated with Alzheimer disease.[11,26] STOML1, which is commonly located at the plasma membrane, interacts with stomatin, thereby affecting late endosome localization.[27] However, the association between STOML1

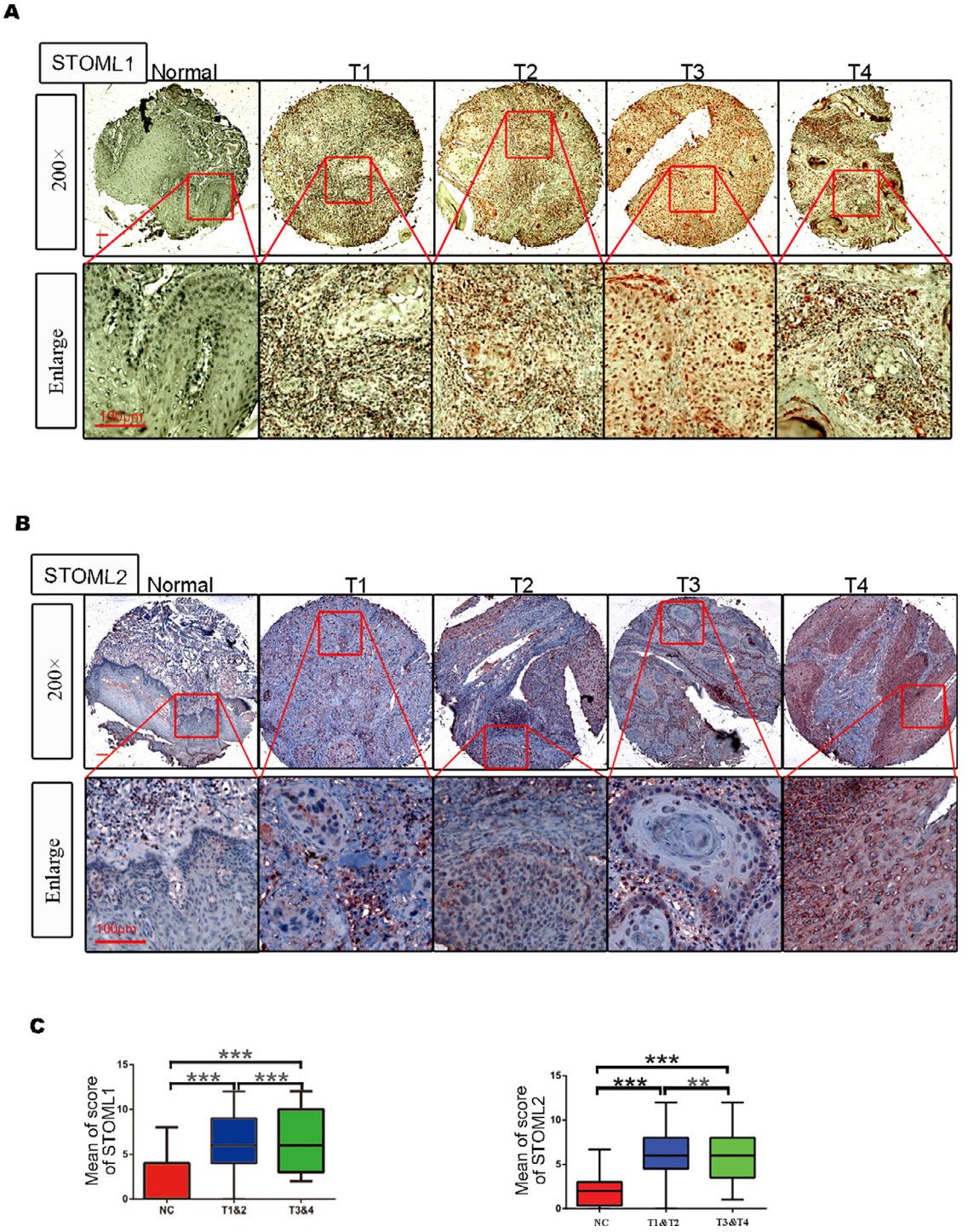

Fig. 2. Images of multiplex immunohistochemistry (IHC) staining indicated that overexpression of STOML1 and STOML2 was associated with different TNM (tumor−node−metastasis) stages in oral squamous cell carcinoma (OSCC) tissues. A, Overexpression of STOML1 in OSCC tissues was detected in different T stages compared with that in normal oral/paracancerous tissue cells (× 20 magnification). The enlarged regions are shown at higher magnification (scale bars: 100 μm). Red color dots depict positive signals. B, Overexpression of STOML2 in OSCC tissues detected in different TNM stages, compared with normal oral/paracancerous tissue cells (20 × magnification). Enlarged regions are shown at higher magnification (scale bars: 100 μm). Red color blots depict positive signals. C, The means of the intensity scores of STOML1 and STOML2 were higher at each TNM stage of OSCC, compared with those of normal oral/paracancerous tissue cells. *** = $P < .0001$ (1-way analysis of variance [ANOVA]; Student t test).

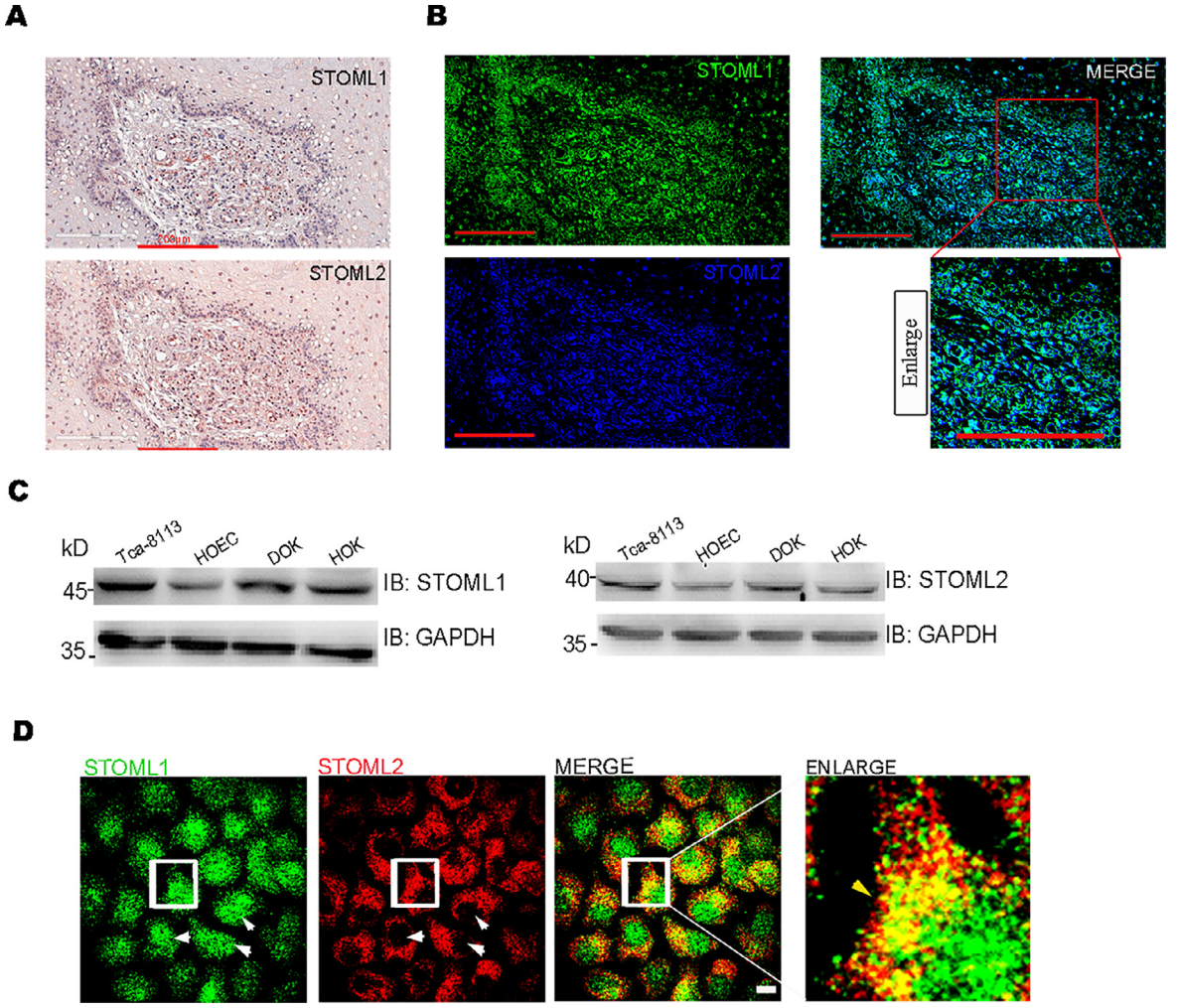

Fig. 3. Distribution between STOML1 and STOML2 proteins in oral squamous cell carcinoma (OSCC) tissues and cells. A, Images showing high positive numbers of STOML1 and STOML2 preferentially co-localized at the cytoplasm of OSCC cells by immunofluorescent staining (original magnification: × 200; scale bars: 100 μm). B, Co-localization analysis of STOML1 and STOML2 showing image of (A) on ImageJ software analysis. C, STOML1 and STOML2 expression levels in a panel of OSCC cells measured via Western blot. D, STOML1 and STOML2 co-localization at cell the membrane and cytoplasm of Tca-8113 cells. Double immunofluorescent staining of STOML1 *(green)* and STOML2 *(red)*; merged images are shown on the right. White arrows indicate STOML1 positive signals mainly localized in the cell nucleus, whereas STOML2 signals were not localized in the cell nucleus but were localized in the cell membrane and cytoplasm only. The lower enlarged regions are shown at higher magnification (× 600). Yellow arrowheads indicate co-localization of STOML1 and STOML2 at the cell membranes of Tca-8113 cells (original magnification: × 600; scale bars: 5 μm).

overexpression and the pathogenesis and pathophysiology of OSCC, as well as that of other human tumors, remains unclear.

STOML2, a mitochondrial protein located at the mitochondrial membrane,[28] is reportedly overexpressed in different cancers. Qu et al. evaluated the potential of STOML2 as a novel prognostic biomarker and suggested that STOML2 may promote head and neck squamous cell carcinoma by activating the interleukin (IL6)/STAT3 pathway.[29] In gallbladder cancer, high levels of STOML2 expression were associated with reduced 5-year survival rates.[30] Furthermore, STOML2 expression may regulate survivin expression via the β-catenin pathway in non−small cell lung cancer.[31,32] STOML2 suppression may decrease the growth of human cervical cancer cells, and increase cisplatin-induced apoptosis by activating MEK/ERK signaling and suppressing the mitochondrial pathway.[33] In addition, STOML2 may play a role in hepatocellular carcinoma by regulating cell proliferation, migration, and epithelial−mesenchymal transition.[34]

The results of the present study indicated that STOML1 and STOML2 were remarkably increased in OSCC tissues and cells. Importantly,

immunofluorescent staining demonstrated that expression levels of both STOML1 and STOML2 were higher at OSCC stages T1-T1 or T3-T4 compared with oral/paracancerous stages. Furthermore, STOML1 and STOML2 expressions were localized in the cellular membranes and the cytoplasm, with more co-localization in OSCC cells in vivo and in vitro compared with normal oral /paracancerous tissues. These findings suggest that detection of STOML1 and STOML2 expression, either separately or in combination, may have prognostic implications. They may also have potential as novel diagnostic markers and therapeutic targets.

Interestingly, the co-localization of STOML1 and STOML2 was not completely coincidental. STOML1 and STOML2 were mostly localized in the cell membrane and the cytoplasm. However, STOML1 was also localized in the cell nucleus, which had no distribution of STOML2. These findings suggested that STOML1 and STOML2 may work together to modulate the OSCC process and that STOML1 many also have different role(s) in the regulation of OSCC by some transcriptional function because of its localization in the cell nucleus. Further studies will be required for an in-depth understanding of the regulative role played by STOML1 and STOML2 in OSCC and other cancers, in addition to the effect of co-localization of STOML1 and STOML2 on OSCC cells.

CONCLUSIONS

Both STOML1 and STOML2 exhibited overexpression in OSCC tissues and in cell lines, suggesting that they may potentially serve as markers for assisting early diagnosis and evaluating medical treatments of this disease. Furthermore, while STOML1 and STOML2 were co-expressed in OSCC cell membrane and cytoplasm, only STOML1 was found within the nucleus. This suggests that the colocalization of STOML1 and STOML2 may play a role in OSCC development.

FUNDINGS

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Supplementary Table 1. Histopathologic diagnosis and immunohistochemistry score of STOML1 and STOML2 in OSCC and normal oral/paracancerous tissues

Case	*Age*	*Sex*	*Oragan/Anatomic site*	*TNM*	*Grade*	*Stage*	*STOML1*	*STOML2*
1	64	M	Lip	T1N0M0	1	I	12	12
2	42	M	Lip	T1N0M0	1	I	3	4
3	59	M	Tongue	T1N0M0	1	I	4.5	4.5
4	67	M	Palate	T4N0M0	1	IVA	2	1
5	50	M	Tongue	T3N0M0	1	III	4.5	4.5
6	75	F	Gingiva	T1N0M0	1	I	8	8
7	61	M	Tongue	T2N0M0	1	II	10	10
8	62	M	Tongue	T1N0M0	1	I	4.5	4.5
9	54	M	Tongue	T1N0M0	1	I	4.5	4
10	41	M	Lip	T2N0M0	1	II	6	4.5
11	51	M	Upper jaw	T4N0M0	1	IVA	10	12
12	66	F	Lip	T1N0M0	1	I	10	10
13	70	M	Lip	T1N0M0	1	I	2	2
14	41	F	Upper jaw	T4N0M0	1	IVA	4.5	3
15	36	F	Mandible	T4N0M0	1	IVA	4.5	4
16	57	F	Tongue	T2N0M0	1	I	6	8
17	51	M	Lip	T1N0M0	1	I	8	8
18	43	M	Maxillary sinus	T4N0M0	1	IVA	8	8
19	39	M	Gingiva	T4N0M0	1	IVA	3	6
20	57	M	Upper jaw	T4N0M0	1	II	2	3
21	52	F	Lip	T1N0M0	1	I	8	6
22	69	M	Gingiva	T3N0M0	1	III	12	12
23	50	F	Tongue	T2N0M0	1	II	10	10
24	50	F	Cheek	T1N0M0	1	I	10	10
25	60	M	Lip	T2N0M0	1	II	4.5	6
26	51	M	Oral cavity	T4N0M0	1	IVA	8	8
27	57	F	Cheek	T4N0M0	1	IVA	10	10
28	76	M	Tongue	T1N0M0	1	I	8	7.5
29	63	M	Gingiva	T1N0M0	1	I	6	8
30	59	M	Lip	T2N0M0	1	II	4.5	2
31	55	M	Tongue	T1N0M0	1	I	4.5	6
32	82	F	Lip	T1N0M0	1	I	8	8
33	67	F	Tongue	T3N0M0	-	III	12	12
34	47	F	Tongue	T2N0M0	1	II	6	8
35	61	M	Lip	T1N0M0	1	I	4.5	4.5
36	72	M	Lip	T1N0M0	1	I	3	3
37	62	F	Tongue	T1N0M0	2	I	4.5	4.5
38	51	M	Tongue	T1N0M0	1	I	10	12
39	66	F	Tongue	T1N0M0	1	I	6	6
40	55	F	Cheek	T1N0M0	1	I	3	3
41	73	M	Lower jaw	T2N0M0	1	II	2	4
42	61	M	Lower jaw	T1N0M0	1	I	3	8
43	75	M	Oral cavity	T2N0M0	1	II	8	10
44	40	F	Maxillary sinus	T2N0M0	1	II	8	8
45	55	M	Maxillary sinus	T3N0M0	1	III	3	4.5
46	70	F	Oral cavity	T3N0M0	1	III	3	3
47	56	M	Gingiva	T2N0M0	1	II	0	3
48	51	F	Tongue	T3N0M0	1	IVA	4.5	8
49	38	M	Lip	T1N0M0	1	I	8	8
50	45	M	Cheek	T2N0M0	1	II	4	6
51	52	F	Lower jaw	T2N0M0	2	I	4	6
52	64	M	Tongue	T1N0M0	2	I	3	6
53	55	M	Maxillary sinus	T1N0M0	2	I	4.5	8
54	50	M	Parotid gland	T3N0M0	2	III	10	10
55	49	M	Tongue	T2N0M0	2	II	3	6
56	42	M	Gingiva	T4N0M0	2	IVA	3	6
57	50	M	Tongue	T2N0M0	#	II	0	3
58	43	M	Lower jaw	T2N0M0	2	II	2	6
59	50	M	Gingiva	T2N0M0	3	II	3	3

(*continued*)

Supplementary Table 1. Continued

Case	*Age*	*Sex*	*Oragan/Anatomic site*	*TNM*	*Grade*	*Stage*	*STOML1*	*STOML2*
60	56	F	Cheek	T4N0M0	3	IVA	8	10
61	78	M	Tongue	T2N0M0	1	II	4	3
62	51	M	Tongue	T4N0M0	1	IVA	6	10
63	75	F	Tongue	T2N0M0	1	II	-	-
64	69	M	Tongue	T3N0M0	1	III	6	8
65	56	F	Tongue	T2N0M0	1	II	4	8
66	35	F	Tongue	T2N0M0	1	II	4	4
67	39	F	Tongue	T1N0M0	1	I	4	8
68	64	M	Tongue	T1N0M0	1	I	3	4.5
69	63	M	Tongue	T1N0M0	1	I	0	6
70	77	F	Tongue	T1N0M0	2	I	4	3
71	41	F	Tongue	T2N0M0	1	II	6	8
72	53	M	Tongue	T2N0M0	1	II	3	2
73	50	M	Tongue	T3N0M0	1	III	6	10
74	36	F	Tongue	T1N0M0	2	I	8	1
75	58	M	Tongue	T1N0M0	1	I	3	10
76	63	F	Tongue	T1N0M0	1	I	10	8
77	55	F	Tongue	T2N0M0	2	II	4	3
78	76	M	Tongue	T2N0M0	1	I	6	8
79	50	F	Tongue	T2N0M0	1	II	8	6
80	44	M	Tongue	T2N0M0	1	III	8	8
81	53	F	Tongue	T1N0M0	1	I	10	8
82	67	F	Tongue	T2N0M0	1	II	10	4.5
83	60	M	Tongue	T1N0M0	-	I	3	10
85	55	M	Tongue	T1N0M0	1	I	7.5	8
86	61	M	Tongue	T1N0M0	1	I	12	10
87	55	M	Tongue	T1N0M0	1	I	6	6
88	59	M	Tongue	T2N0M0	1	II	6	12
89	46	F	Tongue	T2N0M0	1	II	6	10
90	45	F	Tongue	T2N0M0	1	II	10	6
91	61	M	Tongue	T2N0M0	1	II	6	8
92	48	F	Tongue	T2N0M0	1	II	4.5	4
93	52	F	Tongue	T1N0M0	1	I	6	4
94	64	M	Tongue	T2N0M0	1	II	6	6
95	46	F	Tongue	T2N0M0	1	II	6	8
96	48	F	Tongue	T1N0M0	1	I	4	6
97	80	M	Tongue	T1N0M0	1	I	6	8
98	49	M	Tongue	T1N0M0	-	I	3	
99	60	M	Tongue	T2N0M0	1	II	4	6
100	57	M	Tongue	T1N0M0	1	I	8	8
101	45	M	Tongue	T2N0M0	1	II	6	10
102	47	F	Tongue	T2N0M0	1	II	8	6
103	37	M	Tongue	T2N0M0	1	III	4.5	6
104	60	M	Tongue	T2N0M0	2	II	10	10
105	40	F	Tongue	T2N0M0	3	II	10	10
106	49	M	Tongue	T1N0M0	1-2	I	6	10
107	50	M	Tongue	T2N0M0	3	II	10	10
108	60	M	Tongue	T1N0M0	3	I	4	10
109	56	F	Tongue	T2N0M0	3	II	12	10
110	77	M	Tongue	T2N0M0	3	II	3	6
111	56	M	Tongue	T2N1M0	2	III	3	12
112	50	M	Tongue	-	-	-	2	3
113	46	M	Tongue	-	-	-	0	1
114	19	M	Tongue	-	-	-	0	3
115	21Day	M	Tongue	-	-	-	0	1
116	21	F	Tongue	-	-	-	0	6
117	21	F	Tongue	-	-	-	2	3
118	21	F	Tongue	-	-	-	0	0
119	15	F	Tongue	-	-	-	0	0
120	16	M	Tongue	-	-	-	0	2

(*continued*)

Supplementary Table 1. Continued

Case	*Age*	*Sex*	*Oragan/Anatomic site*	*TNM*	*Grade*	*Stage*	*STOML1*	*STOML2*
121	76	M	Tongue	-	-	-	4.5	2
122	38	M	Tongue	-	-	-	0	0
123	51	F	Tongue	-	–	-	3	4.5
124	30	M	Tongue	-	-	-	3	0
125	50	M	Tongue	-	-	-	4.5	1
126	2Mon	M	Tongue	-	-	-	3	3
127	49	M	Tongue	-	-	-	4.5	0
128	40	M	Tongue	-	-	-	2	2
128	25	M	Tongue	-	-	-	3	3

"-" means no applicable or negative in IHC markers.
TNM grading:
T - Primary tumor
Tx - Primary tumor cannot be assessed
T0 - No evidence of primary tumor
Tis - Carcinoma in situ; intraepithelial or invasion of lamina propria
T1 - Tumor invades submucosa
T2 - Tumor invades muscularis propria
T3 - Tumor invades through muscularis propria into subserosa or into non-peritonealized pericolic or perirectal tissues.
T4 - Tumor directly invades other organs or structures and/or perforate visceral peritoneum
N - Regional lymph nodes
Nx - Regional lymph nodes cannot be assessed
N0 - No regional lymph node metastasis
N1 - Metastasis in 1 to 3 regional lymph nodes
N2 - Metastasis in 4 or more regional lymph nodes
M - Distant metastasis
Mx - Distant metastasis cannot be assessed
M0 - No distant metastasis
M1 - Distant metastasis