Clinical Trial/Experimental Study

Medicine

OPEN

Prognostic significance of RKIP, TGM2, and CMTM4 expression in oral squamous cell carcinoma

Tianyu Luo[a], Tao Xu, MD[b,*], Yurong Ou, MD[c], Hongfei Ci, MD[c], Junhui Sun[a]

Abstract

Background: The expression of RKIP, TGM2, and CMTM4 in oral squamous cell carcinoma (OSCC) and normal oral tissues was detected and their correlations were analyzed. The relationships between RKIP, TGM2, and CMTM4 and the clinicopathological parameters and prognosis of patients were analyzed.

Methods: Seventy cancerous and adjacent normal tissue samples were selected, recorded in the pathology department, and embedded in paraffin. Protein expression was detected by immunohistochemistry. Statistical software (SPSS 25.0, IBM Corporation) was used for the statistical analysis. The chi-squared (χ^2) test was used to analyze the expression of RKIP, TGM2, and CMTM4 proteins and their clinicopathological features. Differences in RKIP, TGM2, and CMTM4 protein levels between OSCC and normal tissues were compared using a χ^2 test. Survival analysis was performed using the Kaplan–Meier method, and differences between survival curves were determined using the log-rank test. The effects of RKIP, TGM2, and CMTM4 expression on patient prognosis were analyzed using a multivariate Cox proportional hazards regression model. $P < .05$ was considered statistically significant.

Results: The expression level of RKIP correlated with age and clinical stage ($P < .05$). TGM2 was associated with clinical stage and lymph node metastasis ($P < .05$). The expression of CMTM4 increased with a decrease in cancer differentiation. Kaplan–Meier survival analysis suggested that the positive expression of TGM2 and CMTM4 may predict poor prognosis in patients with OSCC. The multivariate Cox proportional hazards regression model suggested that TGM2 could be an independent prognostic factor for patients with OSCC.

Conclusion: Combined expression of TGM2 and CMTM4 can be used as an indicator to evaluate the risk of metastasis and prognosis of OSCC.

Abbreviations: CKLFSF = chemokine like factor super family, CSC = cancer stem cell, EMT = epithelial-mesenchymal transition, HNSCC = head and neck squamous cell carcinoma, IHC = immunohistochemistry, OSCC = oral squamous cell carcinoma, PDL1 = programmed death-ligand 1, PEBP = phosphatidylethanolamine-binding protein, RKIP = Raf kinase inhibitor protein, TGM2 = transglutaminase 2, TNM = tumor-node-metastasis, TSCC = tongue squamous cell carcinoma.

Keywords: CMTM4, immunohistochemistry, OSCC, prognosis, RKIP, TGM2

1. Introduction

Oral squamous cell carcinoma (OSCC) is the most common type of oral cancer in the world.[1] its occurrence and development are affected by both endogenous and exogenous factors. Owing to the rich blood supply in the oral and maxillofacial regions, OSCC is prone to invasion and metastasis, and its 5-year survival rate is as low as 50%.[2]

The Raf kinase inhibitor protein (RKIP) is a tumor suppressor in the PEBP family. It mainly plays a role by binding to Raf-1 to affect the MEK/ERK signaling pathway and regulate the G protein-coupled receptor (GPCR) and NF-κB signaling pathways.[3–5] transglutaminase2 (TGM2), a highly complex calcium-dependent multifunctional protein, is expressed to varying degrees in almost all body cells. It plays an essential role in the epithelial-mesenchymal transition, cell metastasis, apoptosis, and differentiation of cancer stem cells through non-covalent interactions with various cellular proteins.[6,7] CMTM4 is a member of the CMTM protein family. The original name of the CMTM family is the chemokine-like factor superfamily (CKLFSF), which was named CMTM l-8 by the International Committee on Human Gene Nomenclature (2005).[8] CMTM

The authors have no funding and conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

[a] Bengbu Medical University, Bengbu, China, [b] Department of Stomatology, The First Affiliated Hospital of Bengbu Medical University Bengbu, Anhui, China, [c] Department of Pathology, The First Affiliated Hospital of Bengbu Medical University Bengbu, Anhui China.

** Correspondence: Tao Xu, Department of Stomatology, The First Affiliated Hospital of Bengbu Medical University Bengbu, Anhui 233000, China (e-mail: xutao9297@126.com).*

How to cite this article: Luo T, Xu T, Ou Y, Ci H, Sun J. Prognostic significance of RKIP, TGM2, and CMTM4 expression in oral squamous cell carcinoma. Medicine 2024;103:7(e37278).

Received: 21 October 2023 / Received in final form: 19 January 2024 / Accepted: 25 January 2024

http://dx.doi.org/10.1097/MD.0000000000037278

family members contribute to the EMT[9–11] process and influence the expression of critical molecules. CMTM4 is widely expressed in various tissues.[6–8,12–14]

The above 3 proteins all play a role in EMT and are expressed as tumor suppressor genes or tumor promoting genes in a variety of cancers.[4,6,11] This study aimed to investigate the expression of RKIP, TGM2, and CMTM4 in OSCC, examine their relationships, and determine their potential as diagnostic and prognostic biomarkers.

2. Materials and methods

2.1. Materials

2.1.1. Reagent. Rabbit anti-human RPIK antibody was obtained from Wuhan Sanying Biotechnology Co. Ltd. Rabbit anti-human CMTM4 antibody was purchased from Abcam, and rabbit anti-human TGM2 antibody was purchased from Affinity Biosciences. Goat anti-rabbit IgG-horseradish peroxidase (HRP) secondary antibody was purchased from Fuzhou Maixin Biotechnology Development Co., Ltd.

2.1.2. Specimen. Seventy cases of oral squamous cell carcinoma tissues and adjacent normal tissues preserved in the Department of Pathology, the First Affiliated Hospital of Bengbu Medical College from 2015 to February 2018 were selected. Inclusion criteria: 1. Oral squamous cell carcinoma was diagnosed by pathological examination. 2. All the patients underwent initial surgery and did not receive preoperative radiotherapy or chemotherapy. 3. This study included patients with complete clinicopathological data and postoperative follow-up data. Exclusion criteria: 1. Patients with oral metastatic cancers originating from other sites. 2. Patients with oral leukoplakia, lichen planus, or other oral mucosal disease. 3. Patients who died during the perioperative period or discontinued treatment. 4. Patients with autoimmune diseases or other major systemic diseases. The purpose and procedures of this study were explained to the patients and their families and informed consent was obtained. This study was approved by the Ethics Committee of the First Affiliated Hospital of Bengbu Medical College (approval number: 2023YJS200).

2.1.3. Date of the case. This study collected data on patient sex, age (<60 or ≥ 60 years), pathological grade (grade I or II–III), lymphatic metastasis, clinical stage (stage I–II or III–IV), tumor size (3 cm or ≥3 cm), and tumor location (tongue, gingiva, floor of the mouth, or other sites). Follow-up surveys were conducted through outpatient review, telephone interviews, and other methods until March 1, 2023, to record the overall postoperative overall survival.

2.2. Methods

2.2.1. Immunohistochemical detection. OSCC and adjacent tissues were collected, frozen, sectioned, flattened, mounted on slides, numbered and baked. Four paraffin blocks with a thickness of 3 μm were prepared for each patient for use in the different staining and control groups. The tissue sections were deparaffinized and hydrated using xylene and alcohol. Tissue antigens were repaired using a citrate repair solution in a water bath. After incubation at room temperature, primary antibodies against RKIP, TGM2, and CMTM4 were added dropwise, overnight at 4 °C. After the addition of HRP-labeled goat anti-rabbit IgG, the incubation was continued. Subsequently, DAB chromophobe solution was applied and color development was observed under a microscope. Once a positive yellow color emerged, the tissue section was immersed in distilled water to halt the color development. Following counterstaining with hematoxylin, gradual dehydration using a series of alcohol solutions ranging from low to high gradients and xylene treatment were performed. Finally, the slides were sealed with neutral gum prior to microscopic observation and photography.

Five fields with positive staining were randomly selected for cell counting under a 400× microscope for each tissue section. The staining intensity (SI) was graded as follows: light yellow (1 point), yellow-brown (2 points), and tan (3 points). The percentage of positive cells (PP) determined the score:0 for PP < 5%, 1 for PP between 5% and 24%, 2 for PP between 25% and 49%, 3 for PP between 50% and 74%, and finally, a score of ≥75% received a value of 4. Two pathologists independently evaluated the slides using a double-blinded method to obtain the final results. Based on the immune response score (IRS) = SI × PP, the product represented the final score, where scores less than 3 indicated low expression (–), while scores equal to or greater than 3 indicated high expression (+). Generally, high expression of RKIP is defined as negative expression, and low expression of RKIP is defined as positive expression because RKIP is a tumor suppressor gene.

2.2.2. Statistical analysis. Statistical analyses were performed using SPSS version 25.0. The χ^2 test was used to examine the expression of RKIP, TGM2, and CMTM4 with clinicopathological features. Differences in RKIP, TGM2, and CMTM4 protein levels between OSCC and normal tissues were compared using a χ^2 test. Survival analysis was performed using the Kaplan–Meier method, while differences between survival curves were assessed using the log-rank test. The impact of RKIP, TGM2, and CMTM4 expression levels on patient prognosis was analyzed using a multivariate Cox proportional hazards regression model. Statistical significance was set at $P < .05$ was considered statistically significant.

3. Results

3.1. Patient information

Of the 70 patients included in this study, there were 52 males and 18 females, with ages ranging from 29 to 77 years and a median age of 59.5 years. The tumors were located in the tongue (27 cases), floor of mouth (11 cases), gingiva (17 cases), and other sites (15 cases, including 3 in the lip, 4 in the buccal, 5 in the palate, and 3 in the retromolar triangle). 47 cases had tumor size 3 cm and 23 cases had tumor size ≥ 3 cm. 25 patients had lymph node metastasis while 45 patients did not. There were 26 cases of pathological grade I and 44 cases of pathological grades II and III. The clinical stage was divided into stages I and II in 43 cases and stages III and IV in 27 cases. Of the 70 patients, 37 survived for 5 years, resulting in an overall 5-year survival rate of 52.9%.

3.2. Expression of RKIP, TGM2, and CMTM4 in OSCC and normal oral tissues

RKIP, TGM2, and CMTM4 exhibited cytoplasmic localization with brownish-yellow staining. Additionally, TGM2 and CMTM4 were expressed in the cell membrane, with positive expression levels in cancer tissues at rates of 77.1% (54/70) and 72.9% (51/70), respectively. Tgm2 and CMTM4 were negatively expressed in adjacent tissues, and the difference was statistically significant ($P < .001$). RKIP showed low expression in cancer tissues, with a positive expression rate of 62.9% (44/70). RKIP expression was higher in squamous epithelial tissues and significantly higher in normal and glandular tissues ($P < .001$) (Fig. 1 and Table 1).

3.3. RKIP, TGM2, and CMTM4 were correlated

There was no significant difference in the expression of RKIP, TGM2, and CMTM4 proteins between the 2 groups and OSCC, using the χ^2 test (Table 2).

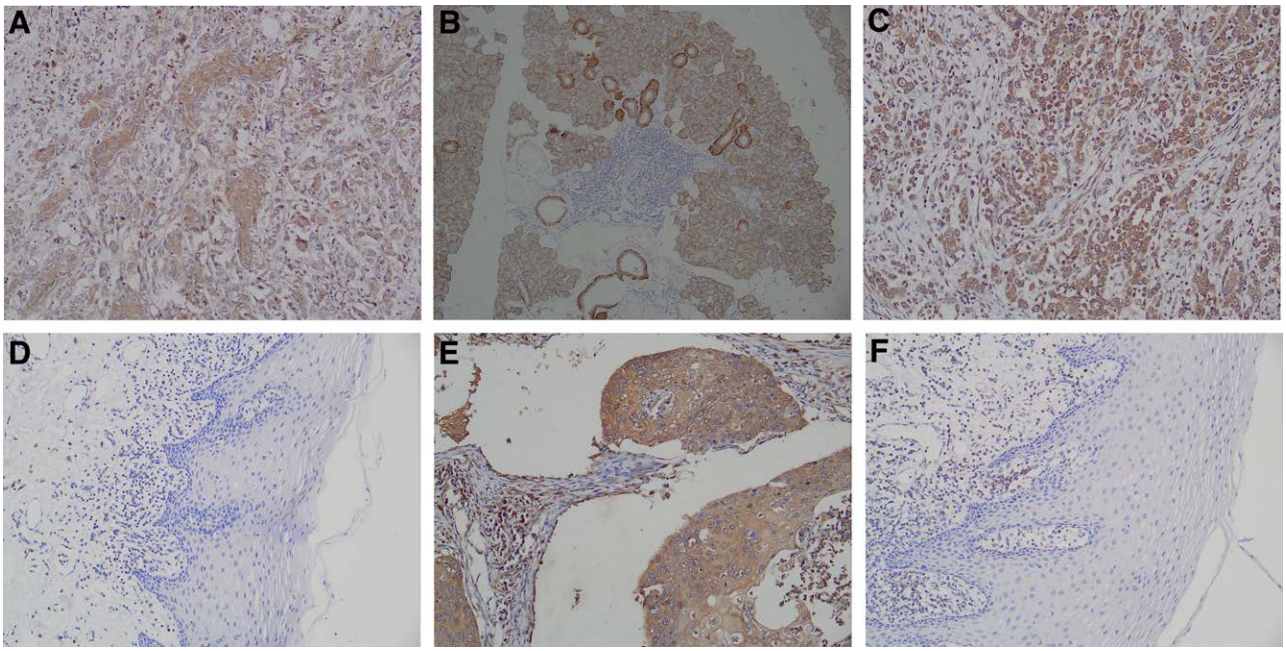

Figure 1. Immunohistochemical staining of RKIP, TGM2, and CMTM4 in OSCC (IHC × 200).(A) Expression of RKIP in OSCC. (B) RKIP expression in normal tissues. (C) Expression of TGM2 in OSCC. (D) TGM2 expression in normal tissues. (E) Expression of CMTM4 in OSCC. (F) CMTM4 expression in normal tissues. CMTM4 = protein CMTM4, OSCC = oral squamous cell carcinoma, RKIP = Raf kinase inhibitor protein, TGM2 = transglutaminase 2.

Table 1

Expression of RKIP, TGM2, and CMTM4 proteins in OSCC and normal tissues.

		Tumor	Normal	*P*
Number		70	70	
RKIP(%)	Negative	26 (37.1)	52 (74.3)	<.001
	Positive	44 (62.9)	18 (25.7)	
TGM2 (%)	Negative	16 (22.9)	61 (87.1)	<.001
	Positive	54 (77.1)	9 (12.9)	
CMTM4(%)	Negative	19 (27.1)	62 (88.6)	<.001
	Positive	51 (72.9)	8 (11.4)	

CMTM4 = protein CMTM4, OSCC = oral squamous cell carcinoma, RKIP = Raf kinase inhibitor protein, TGM2 = transglutaminase 2.

3.4. Correlations between RKIP, TGM2, CMTM4, and clinicopathological variables in oral squamous cell carcinoma

The results of univariate analysis comparing the clinicopathological features of OSCC patients with the expression of RKIP, TGM2, and CMTM4 are shown in Table 3. The expression level of RKIP was not significantly related to sex ($P = .061$), pathological grade ($P = .737$), tumor size ($P = .416$), and location ($P = .952$), but was related to age ($P = .048$), lymph node metastasis ($P = .017$), and clinical stage ($P = .041$). The Positive expression of RKIP was more obvious in OSCC patients older than 60 years (59.1%, 26/44), in patients without lymph node metastasis (56.8%, 25/44), and in those with clinical stage I–II (52.3%, 23/44). The positive expression of RKIP in OSCC indicates that the elderly patients are at an early stage. Positive expression of TGM2 was more obvious in patients without lymph node metastasis (61.1%, 33/54) and those with clinical stage I–II (53.7%, 29/54). There were no significant correlations between the TGM2 levels and other clinical factors. Positive CMTM4 expression was more evident in patients with pathological grades II–III (70.6%, 36/51). The expression intensity increased with a decrease in the degree of cancer differentiation. High expression levels may indicate a high degree of malignancy in OSCC patients.

3.5. Analysis of survival

Kaplan–Meier survival curves based on the expression status of RKIP, TGM2, and CMTM4 in 70 patients with OSCC are shown in Figure 2. Survival analysis suggested that the positive expression of TGM2 and CMTM4 may predict poor prognosis in patients with OSCC (Fig. 2B, $P = .01$, Fig. 2C, $P = .013$). The combined expression of RKIP and TGM2 and the combined expression of TGM2 and CMTM4 could be used as clinical reference indicators for poor prognosis in patients with OSCC (Fig. 2D, $P = .0079$, Fig. 2F, $P = .00049$). There was no significant correlation between the positive expression of RKIP and combined expression of RKIP and CMTM4 with overall survival (Fig. 2A, $P = .34$, Fig. 2E, $P = .14$).

3.6. The effect of RKIP, TGM2, and CMTM4 expression levels on the prognosis of OSCC patients was analyzed by multivariate analysis

The 5-year cumulative overall survival rate of patients with OSCC was used as the dependent variable, and sex, age, pathological grade, lymph node metastasis, clinical stage, tumor size, and location were used as covariates. A Cox regression model was used for multivariate analysis. The results of the protein expression analysis are presented in Table 4. TGM2 protein expression level was an independent prognostic factor affecting the overall survival of patients with OSCC, and the difference was statistically significant ($P = .046$). Patients with positive TGM2 expression had 3.55 times higher risk of death and recurrence than those with negative TGM2 expression. The results of protein joint expression analysis are presented in Table 5. The investigation revealed that the combined positive expression of TGM2 and CMTM4 proteins was a significant, independent prognostic factor that affected the overall survival of patients with OSCC ($P = .017$). Patients with copositive TGM2 and CMTM4 expression had 3.32

times higher risk of death and recurrence than those with negative expression.

4. Discussion

A total of 354,900 new cases and 177,400 deaths of patients with oral cancer occur worldwide each year, accounting for 2.0% and 1.9% of all tumors, respectively.[15] Oral squamous cell carcinoma commonly affects the tongue, buccal mucosa, gingiva, and other parts of the mouth, causing significant harm to the patients' quality of life and lifespan. In this study, 38.57% of the tumors were located in the tongue, which corresponds to the highest incidence of tongue squamous cell carcinoma (TSCC) among oral squamous cell carcinomas.[16] Functional and selective neck dissection and vascularized flap transplantation, alone or in combination with radiotherapy, chemotherapy, immunotherapy, or targeted therapy, can effectively improve the survival rate of patients.

In a prospective clinical trial conducted by Yokoyama J[17] et al, the 5-year overall survival (OS) rate of patients with early oral cancer was 83.3%. Early diagnosis of OSCC will positively impact the clinical treatment and prognosis of patients and correspondingly improve their survival rates. Salivary biomarkers,[18,19] metabolic reprogramming,[20] protein biomarkers,[21] indocyanine green (ICG),[17] and other early diagnosis methods combined with lab-on-a-chip, microfluidic technology, nano-diagnostic technology, liquid biopsy technology, omics technology, synthetic biology, near-infrared fluorescence imaging[22] diagnostic technology. It plays an important role in early diagnosis of oral squamous cell carcinoma.[23]

The expression of TGM2 is increased in a variety of cancer tissues, such as gastric cancer,[24] colorectal cancer,[25] esophageal cancer,[26] and papillary thyroid cancers (PTC).[27] Positive expression of TGM2 expression is associated with high cell invasiveness, low cell survival rate, and poor survival rate in cancer patients. TG2 expression induces the activation of FAK, Akt, cyclic AMP response element-binding protein, and NF-κB and downregulates the tumor suppressor protein PTEN.[7] Treatment with TGM2 knockdown or a TGM2 inhibitor reduced cancer cell invasion, migration, and tumor formation.[24–26] Down-regulation of TGM2 in tumors can be used to treat patients with cancer. However, the expression and function of TGM2 in OSCC remains unclear. Through the analysis of the expression of TGM2 in OSCC and the survival of patients in this study, we can speculate that the positive expression of TGM2 may predict the tendency of lymph node metastasis and poor prognosis in OSCC patients.

Lu[28] proposed that low RKIP expression in OSCC indicates a higher stage and a tendency for lymph node metastasis, which can be used as a clinical reference index for poor prognosis in OSCC patients. In this study, RKIP expression in cancer tissues was not significantly correlated with patient prognosis ($P = .407$). The prognostic significance of RKIP expression in patients with OSCC requires further study using statistical analysis with a large sample size.

TGM2expression induces the activation of FAK, Akt, cyclic AMP response element binding protein, and NF-κB and

Table 2

Intergroup expression of RKIP, TGM2, and CMTM4 proteins in OSCC.

		RKIP			CMTM4		
		Negative	Positive	*P*	Negative	Positive	*P*
Number		26	44		19	51	
TGM2	Negative	5 (19.2)	11 (25.0)	0.579	7 (36.8)	9 (17.6)	.089
	Positive	21 (80.8)	33 (75.0)		12 (63.2)	42 (82.4)	
CMTM4	Negative	8 (30.8)	11 (25.0)	0.600	–	–	–
	Positive	18 (69.2)	33 (75.0)		–	–	

CMTM4 = protein CMTM4, OSCC = oral squamous cell carcinoma, RKIP = Raf kinase inhibitor protein, TGM2 = transglutaminase 2.

Table 3

Correlations of RKIP, TGM2, CMTM4 protein expression status with clinical pathological characteristics of OSCC.

		RKIP			TGM2			CMTM4		
		Negative	Positive	*P*	Negative	Positive	*P*	Negative	Positive	*P*
Number		26	44		16	54		19	51	
Sex (%)	Female	10 (38.5)	8 (18.2)	.061	2 (12.5)	16 (29.6)	.169	5 (26.3)	13 (25.5)	.944
	Male	16 (61.5)	36 (81.8)		14 (87.5)	38 (70.4)		14 (73.7)	38 (74.5)	
Age (%)	<60	17 (65.4)	18 (40.9)	.048	8 (50.0)	27 (50.0)	1.000	13 (68.4)	22 (43.1)	.060
	≥60	9 (34.6)	26 (59.1)		8 (50.0)	27 (50.0)		6 (31.6)	29 (56.9)	
Pathologic grade (%)	I	9 (34.6)	17 (38.6)	.737	7 (43.8)	19 (35.2)	.533	11 (57.9)	15 (29.4)	.028
	II-III	17 (65.4)	27 (61.4)		9 (56.2)	35 (64.8)		8 (42.1)	36 (70.6)	
Lymph nodes metastasis (%)	N0	22 (84.6)	25 (56.8)	.017	14 (87.5)	33 (61.1)	.048	14 (73.7)	33 (64.7)	.477
	N1–3	4 (15.4)	19 (43.2)		2 (12.5)	21 (38.9)		5 (26.3)	18 (35.3)	
Clinical stage (%)	I-II	20 (76.9)	23 (52.3)	.041	14 (87.5)	29 (53.7)	.015	13 (68.4)	30 (58.8)	.463
	III-IV	6 (23.1)	21 (47.7)		2 (12.5)	25 (46.3)		6 (31.6)	21 (41.2)	
Tumor size (%)	<3	19 (73.1)	28 (63.6)	.416	11 (68.8)	36 (66.7)	.876	16 (84.2)	31 (60.8)	.064
	≥3	7 (26.9)	16 (36.4)		5 (31.2)	18 (33.3)		3 (15.8)	20 (39.2)	
Tumor location (%)	Tongue	9 (34.6)	18 (40.9)	.952	6 (37.5)	21 (38.9)	.966	9 (47.4)	18 (35.3)	.729
	Gingiva	7 (26.9)	10 (22.7)		4 (25.0)	13 (24.1)		3 (15.8)	14 (27.5)	
	Sublingual	4 (15.4)	7 (15.9)		2 (12.5)	9 (16.7)		3 (15.8)	8 (15.7)	
	Other	6 (23.1)	9 (20.5)		4 (25.0)	11 (20.4)		4 (21.1)	11 (21.6)	
Mortality (%)	Death	16 (61.5)	21 (47.7)	.263	13 (81.2)	24 (44.4)	.010	15 (78.9)	22 (43.1)	.008
	Survival	10 (38.5)	23 (52.3)		3 (18.8)	30 (55.6)		4 (21.1)	29 (56.9)	

CMTM4 = protein CMTM4, OSCC = oral squamous cell carcinoma, RKIP = Raf kinase inhibitor Protein, TGM2 = transglutaminase2.

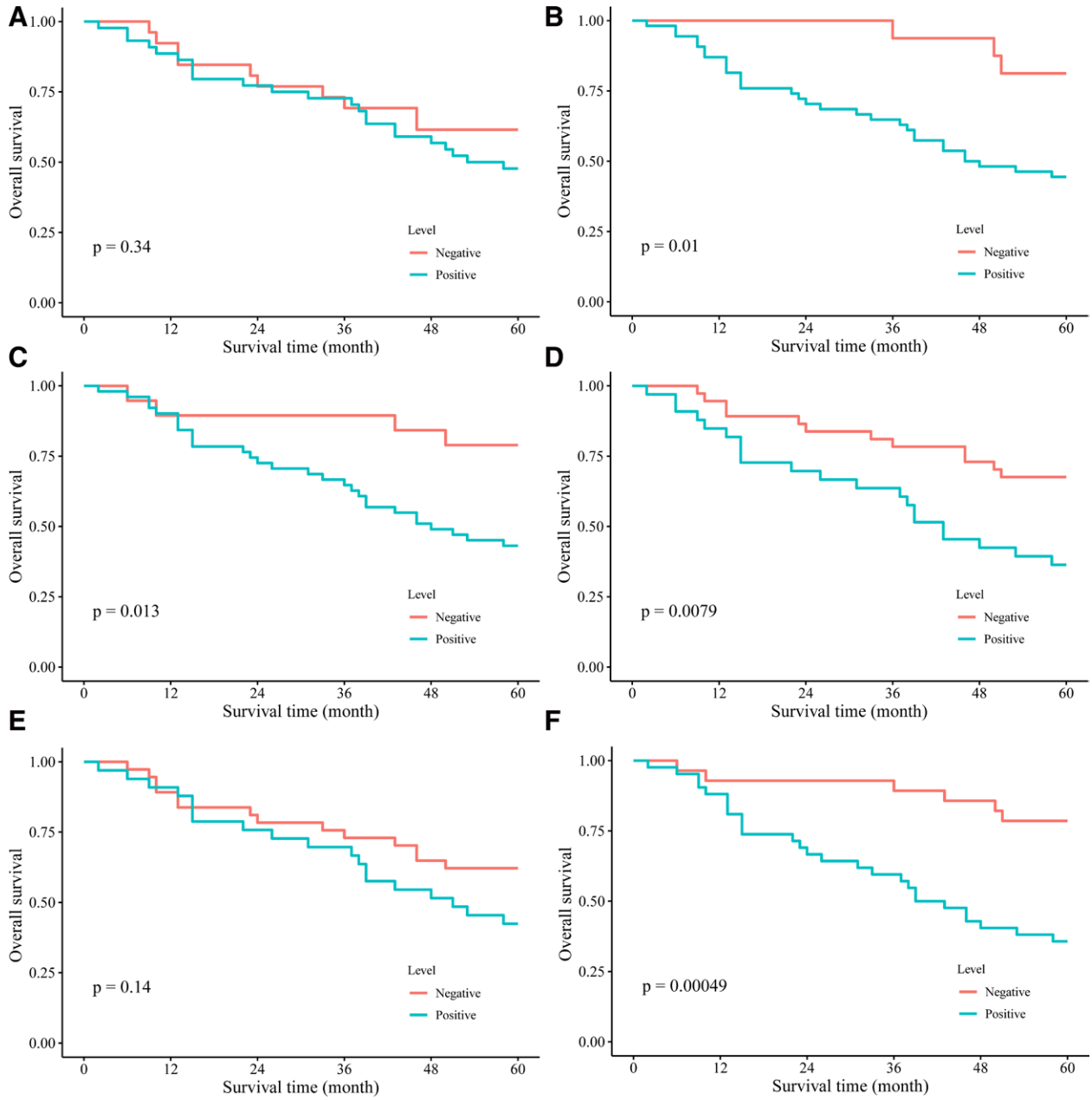

Figure 2. Kaplan–Meier analysis of the survival rate of OSCC patients. (A) Overall survival of all patients in relation to RKIP expression. (B) Overall survival of all patients in relation to TGM2 expression. (C) Overall survival of all patients in relation to CMTM4expression. (D) Overall survival of all patients in relation to the combined expression of RKIP and TGM2. (E) Overall survival of all patients in relation to the combined expression of RKIP and CMTM4. (F) Overall survival of all patients in relation to the combined expression of TGM2 and CMTM4. CMTM4 = protein CMTM4, OSCC = oral squamous cell carcinoma, RKIP = Raf kinase inhibitor protein, TGM2 = transglutaminase 2.

downregulates the tumor suppressor protein PTEN.[7] It plays a cancer-promoting role. NF-κB can be negatively regulated by the metastasis inhibitor RKIP to inhibit tumor growth. The NF-κB pathway regulates EMT either directly or indirectly through the transcription of the gene products involved in EMT. In conclusion, TGM2 and RKIP play antagonistic roles in EMT regulation through NF-κB. Survival analysis in this study showed that the combined expression of TGM2 and CMTM4 could indicate a poor disease prognosis. Because protein regulation is affected by many factors, there was no significant correlation between these factors in the multivariate analysis.

CMTM4 is located at 16q22.1, and is expressed as a tumor suppressor gene in hepatocellular carcinoma, lung squamous cell carcinoma, esophageal cancer, ovarian cancer, and type I renal clear cell carcinoma.[6–8,12–14,29] The expression of CMTM4 is significantly upregulated in human HNSCC and HNSCC cell lines. Previous studies have shown that CMTM4 is involved in the induction of CSC phenotype and PD-L1 expression by regulating EMT in HNSCC.[30] The expression level can be used as a predictive marker for the response of patients to anti-PD-L1 treatment. Another study demonstrated that depletion of CMTM4 has the potential to improve the clinical efficacy of anti-PD-L1 immunotherapy in patients with HCC.[31] CMTM4 plays a role in regulating EMT by inhibiting the migration and invasion of tumor cells.[32]

In the present study, CMTM4 expression was upregulated in oral cancer tissues and downregulated or absent in the adjacent cancer tissues. At the same time, the expression intensity increased with a decrease in the degree of cancer differentiation and was negatively correlated with the survival of patients,

Table 4

Multivariate analysis of individual protein expression.

	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
RKIP	0.77 [0.32, 1.88]	.568				
TGM2			3.55 [1.03, 12.26]	.046		
CMTM4					2.23 [0.71, 7.01]	.168
Age	1.98 [0.88, 4.44]	.097	2.03 [0.96, 4.29]	.065	1.60 [0.74, 3.48]	.235
Pathologic grade	2.06 [0.94, 4.53]	.072	2.04 [0.91, 4.58]	.085	1.64 [0.71, 3.81]	.245
Lymph nodes metastasis	1.70 [0.43, 6.72]	.450	1.82 [0.47, 6.99]	.383	1.62 [0.41, 6.34]	.491
Clinical stage	1.66 [0.41, 6.74]	.479	1.12 [0.26, 4.78]	.874	1.59 [0.38, 6.59]	.525
Tumor location						
Tongue	Ref (1.00)		Ref (1.00)		Ref (1.00)	
Gingiva	0.91 [0.36, 2.32]	.842	1.12 [0.44, 2.86]	.819	0.91 [0.36, 2.30]	.839
Sublingual	1.70 [0.60, 4.86]	.322	1.72 [0.58, 5.10]	.332	1.57 [0.55, 4.49]	.395
Other	0.64 [0.24, 1.77]	.394	0.75 [0.27, 2.08]	.587	0.63 [0.23, 1.70]	.363
Tumor size	1.73 [0.76, 3.93]	.191	1.85 [0.76, 4.51]	.173	1.56 [0.67, 3.64]	.307

95% CI = 95% confidence interval, CMTM4 = protein CMTM4, HR = hazard ratio, RKIP = Raf kinase inhibitor Protein, TGM2 = transglutaminase2.

Table 5

Multivariate analysis of protein co-expression.

	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
RKIP and TGM2	1.68 [0.72, 3.91]	.226				
RKIP and CMTM4			0.83 [0.35, 1.95]	.666		
TGM2 and CMTM4					3.32 [1.24, 8.91]	.017
Age	1.70 [0.80, 3.63]	.169	1.97 [0.86, 4.49]	.109	1.74 [0.80, 3.78]	.160
Pathologic grade	1.95 [0.88, 4.33]	.098	2.15 [0.95, 4.86]	.065	1.37 [0.57, 3.30]	.488
Lymph nodes metastasis	1.50 [0.39, 5.77]	.558	1.64 [0.42, 6.42]	.474	1.71 [0.44, 6.68]	.441
Clinical stage	1.44 [0.35, 5.97]	.616	1.69 [0.42, 6.80]	.461	1.24 [0.29, 5.30]	.769
Tumor location						
Tongue	Ref (1.00)		Ref (1.00)		Ref (1.00)	
Gingiva	1.18 [0.44, 3.14]	.744	0.93 [0.37, 2.36]	.875	0.97 [0.38, 2.46]	.950
Sublingual	1.71 [0.59, 4.99]	.324	1.75 [0.61, 5.09]	.300	1.80 [0.61, 5.29]	.288
Other	0.73 [0.27, 1.97]	.530	0.68 [0.25, 1.83]	.448	0.67 [0.24, 1.88]	.448
Tumor size	1.76 [0.76, 4.11]	.189	1.75 [0.77, 3.97]	.183	1.62 [0.66, 4.01]	.293

95% CI = 95% confidence interval, CMTM4 = protein CMTM4, HR = hazard ratio, RKIP = Raf kinase inhibitor protein, TGM2 = transglutaminase 2.

suggesting that high expression of CMTM4 may predict a high degree of malignancy and poor prognosis of OSCC patients.

The study has some limitations that need further addressing. First, the patients have regionalism, and the sample size was relatively small, which may have introduced some bias in the outcomes. Second, this study only verified the expression intensity of RKIP, TGM2, and CMTM4 in OSCC and its effect on the prognosis of OSCC at the tissue level. Follow-up studies need to expand the patient sample size, to investigate the predictive effect of RKIP, TGM2, and CMTM4 on metastasis and prognosis of OSCC patients, and further explore the molecular mechanism of RKIP, TGM2, and CMTM4 in the occurrence and development of OSCC.

5. Conclusions

Based on immunohistochemical studies and data analysis, the expression of TGM2 was up-regulated in OSCC tissues and could be used as an independent prognostic factor for patients with OSCC. The combined expression of TGM2 and CMTM4 can be used as an indicator to evaluate the risk of metastasis and prognosis of OSCC. Thus, and can be used as potential markers for the diagnosis of oral cancer.

Author contributions

Conceptualization: Tao Xu.
Investigation: Tianyu Luo.
Methodology: Hongfei Ci.
Resources: Yurong Ou.
Software: Junhui Sun.
Supervision: Tao Xu.
Validation: Yurong Ou.
Visualization: Junhui Sun.
Writing – original draft: Tianyu Luo.
Writing – review & editing: Hongfei Ci.

References

[1] Lingen MW, Kalmar JR, Karrison T, et al. Critical evaluation of diagnostic aids for the detection of oral cancer. Oral Oncol. 2008;44:10–22.
[2] Joseph JP, Harishankar MK, Pillai AA, et al. Hypoxia induced EMT: a review on the mechanism of tumor progression and metastasis in OSCC. Oral Oncol. 2018;80:23–32.
[3] Yeung K, Janosch P, McFerran B, et al. Mechanism of suppression of the Raf/MEK/extracellular signal-regulated kinase pathway by the raf kinase inhibitor protein. Mol Cell Biol. 2000;20:3079–85.
[4] Lin K, Baritaki S, Militello L, et al. The role of B-RAF mutations in melanoma and the induction of EMT via dysregulation of the NF-κB/Snail/RKIP/PTEN circuit. Genes Cancer. 2010;1:409–20.
[5] Cessna H, Baritaki S, Zaravinos A, et al. The role of RKIP in the regulation of EMT in the tumor microenvironment. Cancers (Basel). 2022;14:4596.
[6] Tabolacci C, De Martino A, Mischiati C, et al. The role of tissue transglutaminase in cancer cell initiation, survival and progression. Med Sci (Basel). 2019;7:19.
[7] Mehta K, Kumar A, Kim HI. Transglutaminase 2: a multi-tasking protein in the complex circuitry of inflammation and cancer. Biochem Pharmacol. 2010;80:1921–9.

[8] Han W, Ding P, Xu M, et al. Identification of eight genes encoding chemokine-like factor superfamily members 1–8 (CKLFSF1-8) by in silico cloning and experimental validation. Genomics. 2003;81:609–17.
[9] Chen L, Yang QC, Li YC, et al. Targeting CMTM6 suppresses stem cell-like properties and enhances antitumor immunity in head and neck squamous cell carcinoma. Cancer Immunol Res. 2020;8:179–91.
[10] Yuan W, Li T, Mo X, et al. Knockdown of CMTM3 promotes metastasis of gastric cancer via the STAT3/Twist1/EMT signaling pathway. Oncotarget. 2016;7:29507–19.
[11] Wu J, Li L, Wu S, et al. CMTM family proteins 1-8: roles in cancer biological processes and potential clinical value. Cancer Biol Med. 2020;17:528–42.
[12] Li T, Cheng Y, Wang P, et al. CMTM4 is frequently downregulated and functions as a tumour suppressor in clear cell renal cell carcinoma. J Exp Clin Cancer Res. 2015;34:122.
[13] Wang Y, Li J, Cui Y, et al. CMTM3, located at the critical tumor suppressor locus 16q22.1, is silenced by CpG methylation in carcinomas and inhibits tumor cell growth through inducing apoptosis. Cancer Res. 2009;69:5194–201.
[14] Zhang M, Wang J, Yue H, et al. Identification of prognostic biomarkers in the CMTM family genes of human ovarian cancer through bioinformatics analysis and experimental verification. Front Genet. 2022;13:918319.
[15] Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
[16] Siegel RL, Miller KD, Wagle NS, et al. Cancer statistics, 2023. CA Cancer J Clin. 2023;73:17–48.
[17] Yokoyama J, Hasegawa Y, Sugasawa M, et al. Long term-follow-up multicenter feasibility study of ICG fluorescence-navigated sentinel node biopsy in oral cancer. Mol Clin Oncol. 2020;13:41.
[18] Bugshan A, Farooq I. Oral squamous cell carcinoma: metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. F1000Res. 2020;9:229.
[19] Chu HW, Chang KP, Hsu CW, et al. Identification of salivary biomarkers for oral cancer detection with untargeted and targeted quantitative proteomics approaches. Mol Cell Proteomics. 2019;18:1796–806.
[20] Wang Y, Zhang X, Wang S, et al. Identification of metabolism-associated biomarkers for early and precise diagnosis of oral squamous cell carcinoma. Biomolecules. 2022;12:400.
[21] Pillai J, Chincholkar T, Dixit R, et al. A systematic review of proteomic biomarkers in oral squamous cell cancer. World J Surg Oncol. 2021;19:315.
[22] Ji Y, Jones C, Baek Y, et al. Near-infrared fluorescence imaging in immunotherapy. Adv Drug Deliv Rev. 2020;167:121–34.
[23] Madhura MG, Rao RS, Patil S, et al. Advanced diagnostic aids for oral cancer. Dis Mon. 2020;66:101034.
[24] Zhang L, Li Q, Yang J, et al. Cytosolic TGM2 promotes malignant progression in gastric cancer by suppressing the TRIM21-mediated ubiquitination/degradation of STAT1 in a GTP binding-dependent modality. Cancer Commun (Lond). 2023;43:123–49.
[25] Kang S, Oh SC, Min BW, et al. Transglutaminase 2 regulates self-renewal and stem cell marker of human colorectal cancer stem cells. Anticancer Res. 2018;38:787–94.
[26] Leicht DT, Kausar T, Wang Z, et al. TGM2: a cell surface marker in esophageal adenocarcinomas. J Thorac Oncol. 2014;9:872–81.
[27] Sun W, Qin Y, Wang Z, et al. The NEAT1_2/miR-491 axis modulates papillary thyroid cancer invasion and metastasis through TGM2/NFκb/FN1 signaling. Front Oncol. 2021;11:610547.
[28] Lu MM, Guo XJ, Lyu CQ, et al. Clinical significance of raf-1 kinase inhibitor protein in oral squamous cell carcinoma and its role in cell metastasis. Eur Rev Med Pharmacol Sci. 2020;24:12194–9.
[29] Pei Y, Zhang Z, Tan S. Current opinions on the relationship between CMTM family and hepatocellular carcinoma. J Hepatocell Carcinoma. 2023;10:1411–22.
[30] Li H, Liu YT, Chen L, et al. CMTM4 regulates epithelial-mesenchymal transition and PD-L1 expression in head and neck squamous cell carcinoma. Mol Carcinog. 2021;60:556–66.
[31] Chui NN, Cheu JW, Yuen VW, et al. Inhibition of CMTM4 sensitizes cholangiocarcinoma and hepatocellular carcinoma to T cell-mediated antitumor immunity through PD-L1. Hepatol Commun. 2022;6:178–93.
[32] Greenburg G, Hay ED. Epithelia suspended in collagen gels can lose polarity and express characteristics of migrating mesenchymal cells. J Cell Biol. 1982;95:333–9.