Original Article

Tumor necrosis factor-like weak inducer of apoptosis expression in healthy oral mucosa, oral dysplasia and oral squamous cell carcinoma

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Abstract

Objective: Tumor necrosis factor-like weak inducer of apoptosis (TWEAK) has been implicated in the pathogenesis of cancer, as it participates in the progression of internal malignancies. However, its role in the biology of squamous cell carcinoma (SCC) is uncertain. Studies regarding TWEAK in SCC have shown inconsistent results. We aimed to study the expression of TWEAK in healthy oral mucosa, oral dysplastic lesions and in oral SCC (OSCC).

Methods: Immunohistochemistry for TWEAK was performed on one hundred oral mucosal tissues, healthy control (HC) ($n = 20$), oral dysplasia (OD) ($n = 20$) and OSCC ($n = 60$). Staining intensity, extent of staining (ES) and immunoreactive Score (IRS) were assessed for each sample. Kruskal–Wallis ANOVA, Mann–Whitney U, Chi-square and Spearman's rank correlation coefficient were applied.

Results: TWEAK was expressed in 55% of HC, 90% of OD and in all cases of OSCC, with variable intensities. A significant difference in the ES and IRS of TWEAK was noted among the three groups. ES and IRS were highest in OSCC group. ES of TWEAK was significantly higher at invasive tumor front (ITF) than in the whole tumor, with a significant positive correlation. TWEAK expression showed a significant association with invasive front grading, pattern of invasion and surgical margins of OSCC.

Conclusions: TWEAK may contribute to the progression of OSCC. It might also sustain altered differentiation, invasion and migration of tumor cells at ITF.

Keywords: Clinicopathologic parameters, oral squamous cell carcinoma, tumor necrosis factor-like weak inducer of apoptosis

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Received: 08.05.2019, **Accepted:** 19.07.2019

INTRODUCTION

Biomarkers are progressively employed in managing cancer patients.[1] Current investigations have made evident that the tumor necrosis factor (TNF)-like weak inducer of apoptosis-fibroblast growth factor inducible 14 (TWEAK-Fn14) has a task in the progression of malignant tumors.[2] TWEAK is a multifunctional

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.JOMFP_151_19

How to cite this article: Acharya S, Prabhu P, Patil V, Acharya AB, Desai A, Nikhil K. Tumor necrosis factor-like weak inducer of apoptosis expression in healthy oral mucosa, oral dysplasia and oral squamous cell carcinoma. J Oral Maxillofac Pathol 2019;23:369-77.

cytokine that, through its specific receptor Fn14, directs several cellular activities, including proliferation, adhesion and migration, differentiation, survival, apoptosis, angiogenesis and inflammation.[3] Overexpression of TWEAK has been detected in cancer of esophageal, liver, pancreatic, colorectal, ovarian, bladder and prostate, whereas prominent Fn14 upregulation has been noted in brain glioma, cancer of lung, breast, colorectal and prostate and melanoma.[2,4] Recently, TWEAK has been researched as a marker of the outcome after treatment in head-and-neck squamous cell carcinoma (HNSCC). Patients with HNSCC without local, regional or distant tumor recurrence presented higher TWEAK levels than patients with a poor prognosis. Furthermore, it has been observed that the expression levels of TWEAK and Fn14 are closely associated with the grading and prognosis of HNSCC.[1,5] Hence, TWEAK-Fn14 signals participate in the tumorigenicity of various malignancies.[6]

Even the gene expression pattern showed a gradual and significant increase in the expression pattern of TWEAK and Fn14 genes from control to cancerous tissue. Gene expression of TWEAK increased in HNSCC.[5] On the other hand, immunohistochemical (IHC) analysis has supported that both TWEAK and Fn14 are upregulated in cutaneous SCC although TWEAK expression is sometimes weak in these tissue samples.[7,8] For HNSCC, both TWEAK and Fn14 are expressed more in the tumor tissue than that in the adjacent and distal tissue.[5] Hence, it is ambiguous whether TWEAK-Fn14 activation provides a contributive or protective role in the development of SCC.[6]

In a few reports, expression of TWEAK in SCC ranged from strong to completely absent. In biopsies of SCC, expression of TWEAK greatly varied depending on the level of tumor differentiation.[7] IHC expression of TWEAK significantly decreased in oral SCC (OSCC) compared to healthy mucosa.[9] It was also reported that TWEAK expression is decreased, whereas Fn14 expression is increased significantly in cervical carcinoma and intraepithelial neoplasm specimens compared with normal control.[10] Thus, the literature lacks clarity regarding the expression of TWEAK in the mucosal tissue samples both in health and disease. Reviews imply that TWEAK levels are different in mucosal pathologies as compared to healthy mucosa. In addition, the aforementioned studies revealed inconsistent results pertaining to the role of TWEAK-Fn14 signaling in SCC. Furthermore, the expression of TWEAK in tumor tissue and their potential function in OSCC has been barley investigated. Hence, the study aimed to analyze the expression and distribution of TWEAK in the oral mucosal tissues of healthy controls (HCs), oral dyplasias (ODs) and in OSCCs.

METHODS

An IHC analysis was undertaken to evaluate and compare the expression of TWEAK in hundred oral mucosal tissue samples obtained from HC (n = 20), patients with OD (n = 20) and with OSCC (n = 60). Department of Oral Surgery and Craniofacial Unit (CFU) of the institution provided the mucosal samples from patients who underwent treatment. The ethical clearance for the proposal was obtained from the Institutional Ethical Committee (IRB.No. 2016/S/OP/51). Histopathologically confirmed cases of OD and OSCC were included in the study. For the control group, HC samples were obtained from systemically healthy participants without carcinoma and dysplasia who were undergoing minor oral surgical procedures or extraction for orthodontic treatment or impacted teeth. Following OSCC cases were excluded: participants with systemic diseases and allergic conditions, participants treated elsewhere before reporting to our institution, participants with preoperative chemotherapy or radiotherapy before surgery and participants with local resection without neck dissection.

Clinical data were obtained by examining the participants in the Department of Oral Medicine, and histopathological details were analyzed in the Department of Oral Pathology by reviewing the hematoxylin and eosin-stained sections obtained from participants' tissue samples. Treatment and recurrence details of OSCC cases were obtained from the database of the department and CFU of the institution.

Tissue sections were dewaxed with xylene, hydrated using graded alcohol and treated with hydrogen peroxide in methanol for 10 min to eliminate endogenous peroxidase activity. Antibody TWEAK/TNFSF12 (CAT#NBP1-76695, Novus Biologicals, CO, USA) was used after antigen retrieval using microwave. The secondary antibody was obtained from Thermo Scientific-Quanto Detection system. Standardization was performed as per the manufacturer's instructions. Staining was performed according to the manufacturer's protocol using automatic stainer. Sections of skin with known TWEAK expression were used as a positive control. Negative control was made by omission of each primary antibody. A brown precipitate seen in the cytoplasm confirmed the presence of TWEAK. The IHC stained slides were examined imaged and analyzed using a Leica microscope. The number of samples stained, localization, staining intensity (SI) and extent of staining (ES) were assessed. All the hundred TWEAK stained sections were analyzed by two observers who followed uniform criterion and were blinded to the final outcome. Before the commencement of the

principal investigation, a pilot analysis was carried out to assess intra- and interobserver consistency with few TWEAK-stained sections.

The percentage of TWEAK immunopositive cells was obtained from 20 random fields per section using a 20× objective lens. Results were classified as follows for the percentage of positive tumor cells (PC): Score 0 = no immunoreactivity; Score 1+ = <10% PC; Score 2+ = 10%–50% PC; Score 3+ = >50%–80% PC and Score 4+ = >80%–100% PC. The SI was evaluated by two independent observers, in a random order, at ×200. Overall epithelial expression of TWEAK was scored from 0 (no staining), 1+ (mild staining), 2+ (moderate staining) and 3+ (strong staining). Results for PC and SI were multiplied, resulting in an immunoreactive score (IRS) 0–12. IRS 9–12 was defined as $TWEAK_{High}$ and IRS 0–8 was defined as $TWEAK_{Low}$.

The data were analyzed using SPSS 19.0 (IBM®SPSS® Inc., Armonk, NY, USA). The data were tabulated as mean ± standard deviation, median, range and percentages. The normality of continuous variable distribution was assessed using the Kolmogorov–Smirnov test. Kruskal–Wallis ANOVA, Mann–Whitney U test, Chi-square test and Spearman's rank correlation coefficient tests were applied. $P < 0.05$ were regarded as statistically significant.

RESULTS

Immunoreactivity to TWEAK appeared as diffuse brown cytoplasmic staining. TWEAK was expressed in 55% of HC, 90% of OD, and in all cases of OSCC, with variable intensities. There was no statistically significant difference in the SI among HC, OD and OSCC groups [Figure 1]. ES showed a significant difference among HC, OD and OSCC groups. ES was highest in the OSCC group, followed by OD and HC. Pair-wise comparison showed a significant difference between HC and OD, OD and OSCC and HC and OSCC groups [Table 1]. IRS score showed a significant difference among HC, OD and OSCC groups. Pair-wise comparison showed a significant difference between HC and OD groups and HC and OSCC [Table 2].

Among twenty oral dysplastic lesions, ten cases were with moderate dysplasia and severe dysplasia in ten. The ES and IRS of TWEAK were higher in severe dysplasia than moderate. However, the difference was not statically significant (45.50 ± 23.85 vs. 38.76 ± 27.20) ($P > 0.05$).

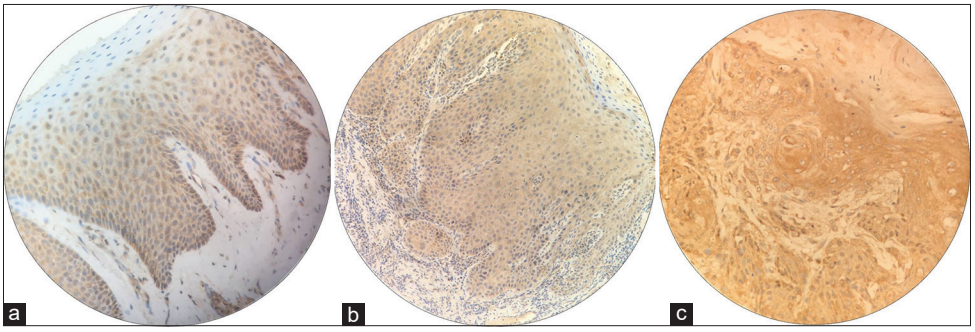

Figure 1: Photomicrograph depicting the staining intensity of tumor necrosis factor-like weak inducer of apoptosis in mucosal samples. (a) Healthy mucosa, (b) dysplasia, (c) oral squamous cell carcinoma. (Objective magnification 10×, 20×, 3,3' Diaminobenzidine chromogen, tumor necrosis factor-like weak inducer of apoptosis monoclonal antibody)

Table 1: Extent of TWEAK expression in the study groups

a. Comparison of extent TWEAK expression among HC, OD and OSCC groups by Kruskal-Wallis ANOVA				
Study groups	**TWEAK Expression Mean±SD**	**Kruskal-Wallis ANOVA value**	***P***	**Significance**
HC	14.38±5.7	40.618	0.001	S
OD	42.13±26.47			
OSCC	73.61±19.84			
b. Pair-wise comparison of TWEAK expression among HC, OD and OSCC groups with Mann-Whitney test				
Parameter	**Study groups**	**Study groups**	***U* statistic**	***P***
TWEAK expression (ES) Mean±SD	HC	OD	41.0	0.004
	OD	OSCC	209.0	0.001
	HC	OSCC	0.000	0.001

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis, ES: Extent of staining, HC: Healthy control, OD: Oral dysplasia, OSCC: Oral squamous cell carcinoma, SD: Standard deviation, S: Significant

Table 2: IRS of TWEAK in the study groups

a. Comparison of TWEAK IRS among HC, OD and OSCC groups by Kruskal-Wallis ANOVA				
Study groups	**TWEAK IRS Mean±SD**	**Kruskal-Wallis ANOVA value**	***P***	**Significance**
HC	4.09±2.16	14.328	0.001	S
OD	5±3.34			
OSCC	7.88±3.58			
b. Pair-wise comparison of TWEAK IRS among HC, OD and OSCC groups with Mann-Whitney test				
Parameter	**Study groups**	**Study groups**	***U* statistic**	***P***
TWEAK expression (IRS) Mean±SD	HC	OD	140.0	0.002
	OD	OSCC	97.0	0.583
	HC	OSCC	351.5	0.005

TWEAK: Tumor necrosis factor-like weak inducer of apoptosis, HC: Healthy control, OD: Oral dysplasia, OSCC: Oral squamous cell carcinoma, IRS: Immunoreactive scores, S: Significant, SD: Standard deviation

Table 3: The association between tumor necrosis factor-like weak inducer of apoptosis IRS and clinicopathologic parameters of oral squamous cell carcinoma patients

Parameters	Category	*n* (60)	TWEAK IRS low (0-8)	TWEAK IRS high (9-12)	χ^2	*P*
Age	≤40	15	6	9	1.090	0.296
	>40	45	25	20		
Sex	Female	8	5	3	0.434	0.510
	Male	52	26	26		
Habits	Tobacco smoking	16	10	6	1.025	0.311
	Tobacco chewing	44	21	23		
Site	Single	39	21	18	0.212	0.645
	Multiple	21	10	11		
Growth pattern	Endophytic	29	16	13	0.276	0.599
	Exophytic	31	15	16		
Size (cm)	≤4	29	15	14	0.000	0.993
	>4	31	16	15		
Clinical nodal status	pN (0)	27	12	15	1.025	0.311
	pN (+)	33	19	14		
Clinical stage	Early	14	8	6	0.219	0.640
	Advanced	46	23	23		
Broder's grade	Well	43	22	21	0.015	0.901
	Moderate-poor	17	7	10		
OSCC with OSF	Absent	44	25	19	1.753	0.185
	Present	16	6	10		
IFG	Well (1-4)	23	20	3	35.788	0.001
	Moderate (5-8)	16	11	5		
	Poor (9-12)	21	0	21		
Pathologic nodal status	pN (0)	38	18	20	0.767	0.381
	pN (+)	29	20	9		
ECS	Absent	46	24	22	0.020	0.887
	Present	14	7	7		
Surgical margins	Absent	48	28	20	4.271	0.039
	Present	12	3	9		
Skin involvement	Absent	36	19	17	0.044	0.833
	Present	24	12	12		
Bone infiltration	Absent	38	18	20	0.767	0.381
	Present	22	13	9		
PNI	Absent	50	26	24	0.013	0.908
	Present	10	5	5		
PVI	Absent	53	28	25	0.246	0.620
	Present	7	3	4		
Recurrence	Absent	48	26	22	0.601	0.438
	Present	12	5	7		
TT (cm)	≤1.5	22	13	9	0.767	0.381
	>1.5	38	18	20		
TB	Low (1-4)	30	17	13	0.601	0.438
	High (>5)	30	14	16		
POI	INFa/b	26	16	10	1.791	0.181
	INFc	34	15	19		
Stroma	Very low	25	12	13	1.270	0.598
	Low	19	9	10		
	Moderate	16	10	6		

Contd...

Table 3: Contd...

Parameters	Category	*n* (60)	IRS low (0-8)	IRS high (9-12)	χ^2	*P*
Inflammation	Weak	24	12	12	4.136	0.126
	Intermediate	17	12	5		
	Strong	19	7	12		

OSF: Oral submucous fibrosis, IFG: Invasive front grading, ECS: Extracapsular spread, PNI: Perineural Invasion, PVI: Perivascular invasion, TT: Tumor thickness, TB: Tumor budding, POI: Pattern of invasion, INFa: Infiltrative growth pattern with a distinct border, INFb: Intermediate pattern, INFc: Infiltrative growth with no distinct border, OSCC: Oral squamous cell carcinoma, IRS: Immunoreactive scores

Among 60 OSCC cases, 43 cases were well differentiated and 17 cases were moderate to poorly differentiated, according to Broder's grading. There was no significant association between IRS scores and tumor grading ($P = 0.901$) [Table 3]. ES of TWEAK was higher in moderate-to-poorly differentiated tumors than well-differentiated tumor. The difference was not statistically significant (80.10 vs. 73.86) ($P = 0.850$) [Table 4]. According to invasive front grading (IFG), 23 cases were well differentiated, 16 moderate and 21 poorly differentiated. There was a significant association between TWEAK IRS and IFG ($P = 0.001$) [Table 3]. There was a significant association between ES of TWEAK and IFG ($P = 0.001$) [Table 4].

TWEAK IRS showed also significant association with parameters like IFG [Figure 2] and surgical margins [Table 3]. ES of TWEAK revealed a significant difference in the following parameters such as IFG, pattern of invasion (POI) and surgical margins [Table 4]. Tumors with positive surgical margins, infiltrative type POI and poorly differentiated based on IFG showed significantly higher ES than their counterparts. Statistically significant difference in the ES was noted between tumors with positive and negative surgical margins, infiltrative and pushing POI and between grades of IFG.

ES of TWEAK was significantly higher at the invasive tumor front (ITF) than the whole tumor (WT), a significant positive correlation ($r = 0.83$, $P = 0.001$) [Figure 3].

DISCUSSION

The role of TNF–alpha in HNSCC has been investigated in the past. It has been a protein with potential for diagnostic utility in OSCC, an extensively expressed pro-inflammatory cytokine in the course of transformation and progression of oral cancer.[11] TWEAK is a member of the TNF ligand superfamily (TNFSF12) that was originally described as a weak inducer of apoptosis in the interferon gamma-treated tumor cell lines.[8,12,13] TWEAK which acts generally as both type II transmembrane proteins and cleaved biologically active soluble molecule which binds with high affinity to the Fn14.[8] Fn14 ligand-receptor pair likely plays an important role in variety of cellular processes and in pathogenesis of several human diseases, including cancer.[3,4,12] However, the full biological effects of TWEAK on cancer remain largely unknown because cells lacking Fn14 have also been shown to be TWEAK sensitive.[5]

TWEAK expression has been detected in many different cell lines and tissues.[10,14,15] In most cancers, TWEAK expression is increased in tumor tissues compared with normal ones,[14,15] as observed in this investigation. In this analysis, both ES and IRS of TWEAK significantly differed among the study groups and with highest expression in OSCCs. Even Alaoui *et al*. and Hu *et al*. found increased expression of TWEAK in cutaneous SCC than normal tissue samples.[6,8] This increased expression may trigger the proliferation or migration activity, but there are exceptions.[14,15]

Soluble TWEAK is produced mainly by inflammatory cells, such as macrophages, which intensively infiltrate the local tissue of SCC. TWEAK-Fn14 activation can recruit more macrophages in a feedback manner. TWEAK-Fn14 inhibition is associated with reduced macrophages infiltration in SCC xenografts, coinciding with the fact that macrophages are one of the major TWEAK-secreting cells. Hence, it is evident that intratumoral TWEAK expression increases in SCC.[6,16,17] Its expression also increases significantly in tumor tissues during cancer progression, which is associated with infiltration of inflammatory cells and activation of resident immune cells.[18,19]

Contradictory to the above findings, El-Meadawy *et al*. found that TWEAK expression was significantly downregulated in OSCC compared with that in normal mucosa.[9] Peternel *et al*. found that the TWEAK expression ranged from strong to completely absent in cutaneous SCC.[7] Zou *et al*. reported that TWEAK messenger RNA (mRNA) expression was significantly downregulated in cervical cancer compared with that in normal tissues.[10] A few researchers detected that TWEAK expression significantly decreased in carcinoma compared with normal tissue, and this may be owing to its consumption in the process of Fn14 synthesis.[3]

In certain cell lines, TWEAK also acts as a death-inducing factor. The role of TWEAK on tumor cells is inconclusive, as some studies show that TWEAK alone is able to

Table 4: Relation between the extent of staining of tumor necrosis factor-like weak inducer of apoptosis and the clinicopathologic parameters of oral squamous cell carcinoma

Parameters	Category	*n* (60)	TWEAK Expression Mean±SD	Mann-Whitney U value	*P*
Age	≤40	15	79.35±17.61	264.5	0.213
	>40	45	71.69±20.36		
Sex	Female	8	77.39±23.30	172.5	0.440
	Male	52	73.02±19.45		
Habits	Tobacco smoking	16	67.55±20.29	271.5	0.178
	Tobacco chewing	44	75.81±19.44		
Site	Single	39	73.39±18.42	387.5	0.733
	Multiple	21	74.01±22.73		
Growth pattern	Endophytic	29	69.12±19.99	335.5	0.092
	Exophytic	31	77.81±19.07		
Size (cm)	≤4	29	75.13±17.93	423.5	0.701
	>4	31	72.19±21.68		
Clinical nodal status	pN (0)	27	75.82±16.42	417.0	0.672
	pN (+)	33	71.80±22.35		
Clinical stage	Early	14	72.08±14.48	277.0	0.432
	Advanced	46	74.07±21.33		
Broders grade	Well	43	73.86±20.06	354.0	0.850
	Moderate-poor	17	80.10±20.133		
OSCC with OSF	Absent	44	72.37±19.39	221.5	0.219
	Present	16	78.56±21.71		
Pathologic nodal status	pN (0)	38	74.92±18.503	382.5	0.586
	pN (+)	22	71.351±22.25		
ECS	Absent	46	72.85±20.09	292.5	0.606
	Present	14	76.10±19.54		
Surgical margins	Absent	48	69.93±20.35	209.0	0.017
	Present	12	83.71±14.63		
Skin involvement	Absent	36	73.20±19.21	412.0	0.763
	Present	24	74.22±21.16		
Bone infiltration	Absent	38	72.27±17.69	346.0	0.269
	Present	22	75.92±23.37		
PNI	Absent	50	72.60±20.22	214.5	0.481
	Present	10	78.62±17.96		
PVI	Absent	53	72.66±20.06	148.5	0.394
	Present	7	80.75±17.77		
Recurrence	Absent	48	72.74±20.069	245.5	0.432
	Present	12	77.08±19.379		
TT (cm)	≤1.5	22	71.91±17.22	363.000	0.399
	>1.5	38	74.59±21.38		
TB	Low (1-4)	30	69.48±19.75	331.0	0.079
	High (>5)	30	77.74±19.39		
POI	INFa/b	26	68.17±19.98	308.0	0.046
	INFc	34	77.76±18.98		
Parameters	**Category**	***n***	**Mean±SD**	**Kruskal-Wallis ANOVA value**	***P***
Inflammation	Weak	24	78.51±16.76	3.721	0.156
	Intermediate	17	65.14±21.58		
	Strong	19	74.99±20.40		
Stroma	Very low	25	77.27±20.87	2.047	0.359
	Low	19	69.71±18.53		
	Moderate	16	72.51±19.93		
IFG	Well (1-4)	23	54.07±14.04	38.941	0.001
	Moderate (5-8)	16	80.00±11.08		
	Poor (9-12)	21	90.13±9.742		
Parameters	**Category**	***n***	**Mean±SD**	**Mann-Whitney U value**	***P***
IFG	Well	23	54.07±14.04	27.000	0.001
	Moderate	16	80.00±11.08		
	Moderate	16	80.00±11.08	91.000	0.018
	Poor	21	90.13±9.742		
	Well	23	54.07±14.04	4.000	0.001
	Poor	21	90.13±9.742		

OSF: Oral submucous fibrosis, IFG: Invasive front grading, ECS: Extracapsular spread, PNI: Perineural invasion, PVI: Perivascular invasion, TT: Tumor thickness, TB: Tumor budding, POI: Pattern of invasion, INFa: Infiltrative growth pattern with a distinct border, INFb: Intermediate pattern, INFc: Infiltrative growth with no distinct border, OSCC: Oral squamous cell carcinoma, SD: Standard deviation

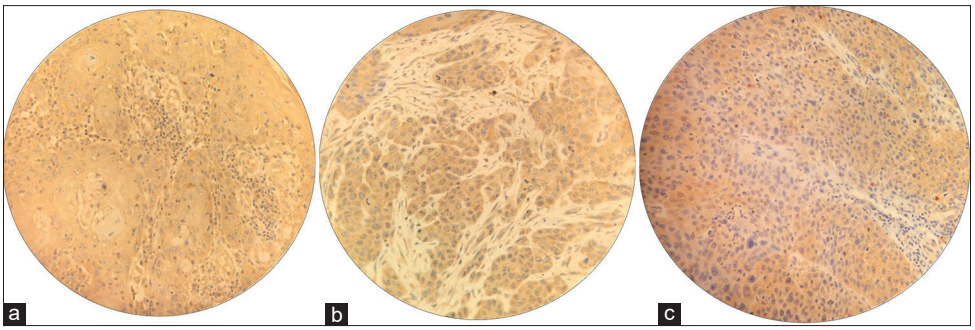

Figure 2: Photomicrograph depicting the extent of tumor necrosis factor-like weak inducer of apoptosis staining among various grades of oral squamous cell carcinoma. (a) Well differentiated, (b) moderately differentiated, (c) poorly differentiated. (objective magnification 20×, 3,3' Diaminobenzidine chromogen, tumor necrosis factor-like weak inducer of apoptosis monoclonal antibody)

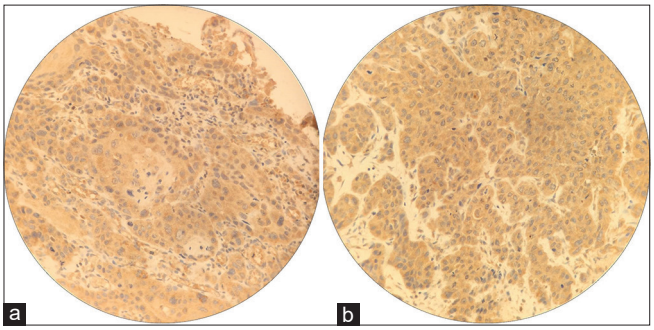

Figure 3: Photomicrograph depicting the extent of tumor necrosis factor-like weak inducer of apoptosis staining in oral squamous cell carcinoma sample. (a) Superficial tumor, (b) invasive tumor front. (objective magnification 20×, 3,3' Diaminobenzidine chromogen, tumor necrosis factor-like weak inducer of apoptosis monoclonal antibody)

promote cell proliferation, while others show that TWEAK promotes cell death.[1] In most studies, TWEAK was just one component of a death-inducing cytokine cocktail.[3] TWEAK has been known to be an inducer of apoptosis of keratinocytes by engaging the Fn14 receptor. TWEAK-induced cell death is not well understood but appears to involve multiple context-dependent mechanisms.[4]

Fn14–TNF receptor (TNFR)-associated factor 2 (TRAF2)–TNFR axis regulates the apoptosis and proliferation of tumor cells. TWEAK-Fn14 interaction activates the TRAF2 (anti-apoptotic protein) signaling pathway. Fn14–TRAF2–TNFR1 may play a role in the apoptosis of cells, and Fn14–TRAF2–TNFR2 may be responsible for cell proliferation. TWEAK has been reported to promote the proliferation of normal endothelial cells and keratinocytes infected by human papillomavirus (HPV).[2] HPV16-induced keratinocyte immortalization has been shown to be closely related to epidermis originated malignancies, and TWEAK-Fn14 activation accompanies HPV16 infection inducing the proliferation of keratinocytes.[2,20] There is a causal relationship between HPV and OSCC.[21] Normal keratinocytes mostly express TNFR1 and reveal promoted apoptosis and unmoved proliferation upon TWEAK stimulation. However, HPV infection switches keratinocytes from an apoptotic to proliferative fate under TWEAK-Fn14 interaction through upregulating TNFR2 expression.[6,20] HPV16 E6/E7-harboring keratinocytes express high levels of Fn14, which interacts with TWEAK causing proliferation of keratinocytes.

In this analysis, TWEAK showed significant association with parameters such as IFG, POI and surgical margins. Expression of TWEAK was significantly higher in poorly differentiated tumors (based on IFG), tumors with infiltrative growth pattern and tumors with positive surgical margins than their counterparts, respectively. The differentiation status mirrors the clinical behavior of SCC and scores a biological inequality between low-risk and high-risk SCC.[6] Fn14 is expressed more in undifferentiated or less-differentiated cells, including HPV-infected keratinocytes.[20] Fn14 expression prefers to be stronger in the poorly differentiated subtypes. It is possible that TWEAK-Fn14 signaling contributes more to the tumors with poor differentiation stage. However, Hu *et al*. found no significant difference in Fn14 or TWEAK expression between the well- and poorly-differentiated SCC samples.[6] Contradictory to the above-mentioned results, El-Meadawy *et al*. found the expression of TWEAK in OSCC to be inversely proportional to the histologic grades.[9] Zou *et al*. demonstrated a close correlation between reduced expression of TWEAK and increased histological grades and interstitial invasive depth in cervical carcinoma. In the view of downregulated TWEAK being closely related to grade and depth, but not with size and lymph node metastasis, the authors of one investigation hypothesized that TWEAK acts on local cancer tissue permeation than a distant metastasis.[10] Peternel *et al*. found that although TWEAK was regularly expressed in moderately- and well-differentiated SCC, it was completely absent in

poorly-differentiated tumors and stated that TWEAK might serve as a novel differentiation marker whose expression in atypical and malignant epidermal neoplasms with squamous differentiation inversely correlates with the degree of atypia. As TWEAK is shown to be downregulated in proliferating keratinocytes and also found to be reduced in pathologies with altered keratinocyte differentiation. One group of researchers concluded that downregulation of TWEAK expression might be an early indicator of disturbed differentiation or pathologic proliferation of keratinocytes in cutaneous neoplasias.[7]

Invasion and migration are the most vital qualities of malignant tumors and are intimately related to prognosis of the tumor. Several studies have stated that TWEAK-Fn14 pathway plays a significant part in the invasion and migration of tumors. TWEAK-Fn14 signaling fuels the invasion and migration of tumor cells through TNFR-associated factor 2. TWEAK augments the mRNA level of matrix metalloproteinase (MMP)-9, which supports the invasiveness of tumor cells.[2] In the present study, we found the expression of TWEAK to be higher at the invasive front than the WT and extent of TWEAK expression was higher in moderate- and poorly-differentiated OSCC than well-differentiated tumors based on IFG. Thus, it is opined that TWEAK may promote the cellular expressions of MMP-2 and MMP-9, which are suggested to contribute to the invasion of SCC cells.[6,22] MMPs can assist the movement of tumor cells by degrading some constituents of the extracellular matrix.[22] It is also cited that TWEAK lessens the expression of E-cadherin in cultured SCC cells. E-cadherin is an elementary transmembrane glycoprotein and functions in the calcium-dependent homotypic cell-cell adhesions. The downregulation of E-cadherin can increase SCC metastasis through reducing intercellular constraints.[6] Downregulation of E-cadherin is a crucial step in epithelial–mesenchymal transition which occurs at the ITF.[2] Thus, TWEAK may bring phenotypic transformation of epithelial cells. Therefore, TWEAK-Fn14 activation helps the invasion and migration of SCC cells via multiple downstream pathways.[6]

CONCLUSIONS

TWEAK was considerably expressed in the epithelium of oral mucosal samples. TWEAK expression significantly increased in mucosal pathologies compared with that in the normal oral mucosa. Increased expression in mucosal pathologies might suggest that they aid in altered proliferation and differentiation, which accompanies inflammatory or neoplastic process. Results suggest that TWEAK-Fn14 signals contribute to the progression of OSCC, most likely via Fn14–TRAF2–TNFR2 axis. It might also sustain altered differentiation, invasion and migration of tumor cells at the ITF, as TWEAK expression was substantial. TWEAK expression also showed significant association with few histopathologic prognosticators.

TWEAK can be considered as a potential target for anticancer therapy, as it is expressed in the tumor microenvironment and currently known to play a part in the tumor biology. This protein can be a new molecular target for cancer drug development. Rationale exists for inhibition of the TWEAK-Fn14 pathway as an approach to cancer treatment; blocking antibodies to TWEAK have been described in the literature. Additional studies are required to determine TWEAK as a novel molecular target for anticancer therapy in humans.

Further explorations are required to establish the function and mode of action of TWEAK in OSCC development. In this study, IHC expression of TWEAK was assessed in a small group of oral mucosal samples with and without pathologies, which may be a limitation. Simultaneous assessment of TWEAK and Fn14 expression in a larger cluster would better the understanding about their role in OSCC.

Acknowledgments

The authors thank the Department of Oral Pathology, Department of Oral and Maxillofacial Surgery and Dr. Srinath Thakur, Principal, SDM College of Dental Sciences and Hospital, Dharwad, India. This study was funded by Rajiv Gandhi University of Health Sciences (RGUHS) Advanced Research Wing, Bengaluru, India. Our sincere thanks to Dr. G.S Venkatesh, Director of RGUHS Advanced Research Wing, Bengaluru, India. The authors express gratitude to Dr. Sanjay Navani, LabSurgpath, Mumbai, India, for his expertise in IHC, Mrs. Hemalatha Gudgudi, Dr. Jyothi Kale and Dr. Dewin Arnold for procuring the tissue samples and data.

Financial support and sponsorship

This work was supported by the Rajiv Gandhi University of Health Sciences (RGUHS) Advanced Research Wing (Grant Number (project Code): D010, year: 2016-2017) Bengaluru, India.

Conflicts of interest

There are no conflicts of interest.

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