Received: 4 January 2019 | Accepted: 20 August 2019

DOI: 10.1111/jicd.12469



WILEY

ORIGINAL ARTICLE

Oral Pathology

# Immunohistochemical expression of tumor necrosis factor-like weak inducer of apoptosis and fibroblast growth factor-inducible immediate early response protein 14 in oral squamous cell carcinoma and its implications

Swetha Acharya[1] | Prashant Prabhu[1] | Vidya S. Patil[2] | Anirudh B. Acharya[3] | Krithi Nikhil[4]

[1]Department of Oral Pathology and Microbiology, SDM College of Dental Sciences and Hospital, Dharwad, India

[2]Department of Biochemistry, SDM College of Medical Sciences and Hospital, Dharwad, India

[3]Department of Periodontics, SDM College of Dental Sciences and Hospital, Dharwad, India

[4]Biostatistician, Department of Public Health Dentistry, SDM College of Dental Sciences & Hospital, Dharwad, India

**Correspondence**
Dr Swetha Acharya, Department of Oral Pathology and Microbiology, S.D.M College of Dental Sciences and Hospital, A Constituent Unit of Shri Dharmasthala Manjunatheswara University, Dharwad, India.
Email: sbacharya@gmail.com

**Funding information**
Rajiv Gandhi University of Health Sciences, Grant/Award Number: D010

## Abstract

**Aim:** To study the expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and fibroblast growth factor-inducible immediate early response protein 14 (Fn14) in oral squamous cell carcinoma (OSCC), to elucidate the possible role of TWEAK-Fn14 in OSCC development.

**Methods:** Immunohistochemistry for TWEAK-Fn14 was performed on 61 oral mucosal samples: healthy oral mucosa (HOM; N = 15); oral dysplastic lesions (ODL; N = 15); and OSCC (N = 31). Extent of staining (ES) and immunoreactive score (IRS) were assessed. The data was statistically analyzed.

**Results:** All OSCC expressed TWEAK, and the Fn14 expression was noted in 90% of OSCC. A significant difference in the TWEAK and Fn14 expression was noted among the groups. ES and IRS of TWEAK-Fn14 significantly increased in OSCC compared with ODL and HOM. ES of TWEAK was significantly higher than Fn14 in all 3 groups. ES of TWEAK-Fn14 was significantly higher at the invasive tumor front (ITF) than in the whole tumor. TWEAK-Fn14 showed a significant association with clinicopathological parameters of prognostic significance.

**Conclusion:** Findings suggest that TWEAK and Fn14 may participate in the growth and progression of OSCC. Increased expression of TWEAK-Fn14 at the ITF may facilitate increased proliferation, altered differentiation and invasion.

KEYWORDS

clinicopathological parameters, fibroblast growth factor-inducible immediate early response protein 14, immunohistochemistry, oral squamous cell carcinoma, tumor necrosis factor-like weak inducer of apoptosis

## 1 | INTRODUCTION

Cancer-related cytokines have surfaced as 1 of the hallmarks of cancer.[1] Amongst them, tumor necrosis factor, interleukin (IL)-1, IL-6 and IL-8 act as vital intermediaries of inflammation-mediated tumorigenesis.[2] protein whose potential diagnostic value in oral squamous cell carcinoma (OSCC) has been publicized is tumor necrosis factor-α (TNF-α), a pro-inflammatory cytokine extensively articulated all through transformation and progression of oral cancer.[3]

The TNF superfamily consists of numerous cytokine ligands and receptors that control several biological processes like cell death and survival, proliferation and differentiation.[4,5] Tumor

  

necrosis factor-like weak inducer of apoptosis (TWEAK, also familiar as TNFSF12, APO3L, CD255), recognized in 1997 as a part of the TNF superfamily of ligands, is a multifunctional cytokine; and due to its pro-inflammatory properties it has been suggested that it plays a vital task in various physiological and pathological conditions.[6-8] TWEAK, through its receptor fibroblast growth factor inducible 14-kDa protein (fibroblast growth factor-inducible immediate early response protein 14 [Fn14] also recognized as TNFRSF12A, TWEAKR, CD266), which was first illustrated in 1999 as a growth factor-inducible protein, commands several cellular activities.[6] TWEAK signaling through its receptor Fn14, contains a TNF receptor-associated factor (TRAF) binding sequence, resulting in the activation of the canonical nuclear factor (NF)-κB, non-canonical NF-κB and mitogen-activated protein kinase pathways. This differential activation of NF-κB pathways by TWEAK is based on the capacity of Fn14 signaling.[9] Therefore, TWEAK-Fn14 has the potential to influence multiple cellular responses depending on the cell type and circumstance.[8]

TWEAK is usually expressed in tissues and synthesized as a type II transmembrane glycoprotein that, after a proteolytic cleavage, circulates in plasma as a soluble active form (sTWEAK).[10] TWEAK acts through its cognate receptor, a type I transmembrane protein, Fn14, which has been reported by Wiley et al. in [11]. It is 102 a.a. in length following signal peptide cleavage, making it the smallest in the TNF receptor superfamily. The Fn14 cytoplasmic tail, important for signal transduction, is 28 a.a. in length. TWEAK is the only TNF superfamily associate that binds to Fn14.[12] TWEAK plays a physiological role in tissue repair after acute injury. In contrast, TWEAK plays a pathological role in chronic inflammatory disease; when constantly triggered, it promotes chronic inflammation, pathological hyperplasia, angiogenesis and hinders differentiation of progenitor cells.[8] Fn14 is almost not present in healthy tissues. After injury, Fn14 is upregulated, assisting the communication with its ligand TWEAK and its trimerization. In chronic injury, the TWEAK-Fn14 interaction partakes in pathological tissue remodeling, promoting proliferation, migration, differentiation, apoptosis, inflammation, angiogenesis and matrix degradation.[13]

TWEAK is a trimeric cytokine; it is likely that TWEAK binding promotes Fn14 trimerization, TRAF association, signal pathway activation, changes in gene expression and thus cellular response.[7] Therefore, it is involved in the pathogenesis of various diseases. The Fn14 ligand receptor pair likely plays an important role in a variety of cellular processes and in the pathogenesis of several human diseases, including cancer.[14] Despite the variety of pathologies that TWEAK has been allied with, there is currently not enough information regarding its expression in OSCC. Studies have indicated that the expression of TWEAK-Fn14 is upregulated in many solid tumors compared with healthy tissues.[15] Currently, limited data exists regarding TWEAK-Fn14 in OSCC. So, this study was undertaken to evaluate the relation of TWEAK-Fn14 in oral carcinogenesis by examining TWEAK and Fn14 expressions in different oral tissue samples. An immunohistochemical (IHC) evaluation of TWEAK-Fn14 expressions in healthy oral mucosa (HOM), oral dysplasia lesions (ODL) and OSCC tissue samples was carried out. This investigation also analyzed the association of TWEAK-Fn14 expression with clinicopathological features of OSCC.

## 2 | MATERIALS AND METHODS

A case-control study was undertaken to assess the expression of TWEAK and Fn14 in OSCC and to compare it with oral dysplasias and healthy controls. Immunostaining for TWEAK and Fn14 was performed on 61 oral mucosal samples: HOM (N = 15), ODL (N = 15) and OSCC (N = 31). All specimens were obtained from subjects who underwent treatment in the Department of Oral Surgery or in the Cranio-facial Unit (CFU) of the institution. Institutional ethical clearance for the proposal was obtained from the institutional ethical committee (IRB no. 2016/S/OP/51). Confirmed cases of ODL and OSCC were included in the study. For the control group, samples were obtained from systemically healthy participants who were undergoing minor oral surgical procedures. Cases with systemic diseases and OSCC cases who had received preoperative chemotherapy or radiotherapy before surgery were excluded.

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

61 oral mucosal samples were subjected to IHC with primary antibodies: TWEAK antibody (catalogue no. NBP1-76695) and Fn14/TWEAK-R antibody (catalogue no. NBP2-34499), procured from Novus Biologicals (Centennial, CO, USA). Secondary antibodies were obtained from the Quanto Detection system (Thermo Fisher Scientific, Waltham, MA, USA). Standardization was performed as per the manufacturer's instructions: TWEAK and Fn14 are prestige antibodies which are available in the concentrated form (1 unit = 0.1 mg). Staining was performed according to the manufacturer's protocol. The IHC-stained slides were examined, imaged and analyzed using a Leica microscope (Wetzlar, Germany). The number of samples stained, localization, extent of staining (ES) and staining intensity (SI) were assessed.

The percentage of TWEAK/Fn14 immunopositive cells was obtained from 20 random fields per case/section using a 20x objective lens. Results were classified as follows for the percentage of positive tumor cells (PC): score 0, no immunoreactivity; score 1+, 1 to less than 10% PC; score 2+, 10%-50% PC; score 3+, more than 50% to 80% PC; score 4+, more than 80% to 100% PC. The SI was evaluated by 2 independent observers, in a random order, at ×200 magnification. Overall epithelial expression of TWEAK and Fn14 was scored from 0 (no staining), 1+ (mild staining), 2+ (moderate staining) to 3+ (strong staining). Results for PC and SI were multiplied, resulting in an immunoreactivity score (IRS) ranging 0-12. IRS 9-12 was defined as TWEAK/$Fn14_{High}$ and IRS 0-8 was defined as TWEAK/$Fn14_{Low.}$

**TABLE 1** Comparison of quantitative expression (extent of staining) of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) among the groups

| | TWEAK | | | |
|---|---|---|---|---|
| **Groups** | **Mean ± SD** | | **ANOVA** | ***P*** |
| HOM | 13.50 ± 5.61 | | 22.090 | .001 |
| ODL | 40.36 ± 25.38 | | | |
| OSCC | 72.65 ± 22.82 | | | |
| Pairwise comparison of quantitative expression of TWEAK between the groups | | | | |
| Parameter | Groups | Groups | *U* | *P* |
| TWEAK | HOM | ODL | 9.000 | .016 |
| | ODL | OSCC | 59.0 | .001 |
| | HOM | OSCC | 0.000 | .001 |

HOM, healthy oral mucosa; ODL, oral dysplastic lesions; OSCC, oral squamous cell carcinoma.

The data were analyzed using SPSS version 19.0 software (IBM, Armonk, NY, USA). The data were tabulated as mean ± SD, median, range and percentages. Kruskal-Wallis ANOVA, Mann-Whitney *U*-test, $\chi^2$-test and Spearman's rank correlation coefficient tests were applied. $P < .05$ was regarded as statistically significant.

## 3 | RESULTS

Positive IHC reactivity to TWEAK and Fn14 showed as diffuse brown cytoplasmic staining. TWEAK-Fn14 expression was mostly localized in the cytoplasm and very little showed membrane locations. In biopsies of HOM, mild-moderate cytoplasmic staining for TWEAK and Fn14 was observed in all basal and suprabasal keratinocytes. Mild-strong cytoplasmic staining of TWEAK-Fn14 was noted in the keratinocytes of the ODL. OSCC showed 100% immunopositivity for TWEAK. Amongst these, 48% showed strong reaction, while the remaining 52% cases revealed mild to moderate reaction. Positive cytoplasmic expression of TWEAK was detected in some inflammatory cells around the tumor. Fn14 expression was observed in 90% of cases. Among these, 57% showed strong reaction, while the remaining 42.8% cases revealed mild to moderate reaction.

The ES of TWEAK showed a significant difference among study groups. A trend of gradually increasing mean of TWEAK from HOM to OSCC tissues was observed. ES was highest in the OSCC group followed by ODL and HOM, and with significant differences as noted by the pairwise comparisons between groups. TWEAK expression was significantly increased in ODL and OSCC compared with HOM (Table 1, Figure 1). There was no statistically significant difference in the SI among study groups. A significant difference in the IRS among the study groups, and a significant comparative difference between ODL and OSCC ($P = .016$), and HOM and OSCC ($P = .019$), was seen.

The ES of Fn14 showed a significant difference among study groups. ES was highest in the OSCC group followed by ODL and HOM. Pairwise comparisons revealed a significant difference between ODL and OSCC, and HOM and OSCC groups (Table 2, Figure 2). A significant difference in the SI was observed among study groups and significant differences between ODL and OSCC ($P = .009$), and HOM and OSCC ($P = .016$) by pairwise comparison. The difference in the IRS among the study groups was significant, and also between ODL and OSCC groups ($P = .001$), and HOM and OSCC ($P = .005$) by pairwise comparison.

Statistically significant differences between the extent of TWEAK and Fn14 staining in all 3 groups were noted ($P < .05$). The ES was higher with TWEAK than with Fn14 in all 3 groups. The extent of TWEAK and FN14 staining showed a significant positive correlation in OSCC ($P = .001$, $r = .79$).

The extent of TWEAK staining was significantly higher at the invasive tumor front (ITF) than in the whole tumor (WT) with significant positive correlation ($P = .001$, $r = .82$).The extent of FN14 staining was significantly higher at the ITF than in the WT with a significant positive correlation ($P = .001$, $r = .93$).

TWEAK IRS showed significant association with OSCC associated with oral submucous fibrosis (OSF), invasive front grading (IFG),

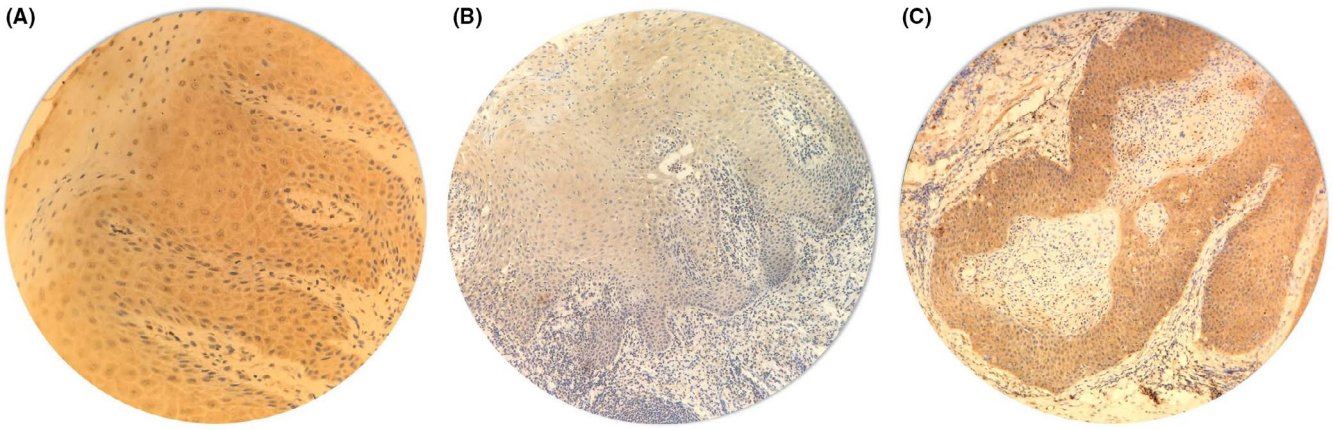


**FIGURE 1** Photomicrograph depicting the expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in (A) healthy oral mucosa, (B) oral dysplasia and (C) oral squamous cell carcinoma (Objective magnification 10x, 20x; 3,3′-diaminobenzidine-tetrachloride chromogen, TWEAK monoclonal antibody)

**TABLE 2** Comparison of quantitative expression (extent of staining) of fibroblast growth factor-inducible immediate early response protein 14 (Fn14) among the groups

| | Fn14 | | | |
|---|---|---|---|---|
| **Groups** | **Mean ± SD** | | **ANOVA** | ***P*** |
| HOM | 5.48 ± 7.31 | | 19.009 | .001 |
| ODL | 18.31 ± 18.69 | | | |
| OSCC | 54.83 ± 29.01 | | | |
| Pairwise comparison of quantitative expression of Fn14 between the groups | | | | |
| Parameter | Group | Group | *U* | *P* |
| Fn14 | HOM | ODL | 17 | .103 |
| | ODL | OSCC | 53.0 | .001 |
| | HOM | OSCC | 13.5 | .001 |

HOM, healthy oral mucosa; ODL, oral dysplastic lesions; OSCC, oral squamous cell carcinoma.

surgical margins and pattern of invasion (POI) (Table 3). A statistically significant difference in the extent of TWEAK staining was noted between tumors with positive and negative surgical margins ($P = .038$), infiltrative and pushing POI ($P = .028$), and between grades of IFG ($P = .001$). Tumors with positive surgical margins, infiltrative type PI and poor differentiation based on IFG showed a significantly higher extent of TWEAK staining than their counterparts (Figure 3).

Fn14 IRS showed significant association with age, OSCC associated with OSF, IFG, surgical margins, TB and POI ($P < .05$). The extent of Fn14 staining revealed a significant difference in parameters like surgical margins, bone involvement (BI), tumor thickness (TT), extraoral swelling, TB, POI and IFG ($P < .05$) (Figure 4). Tumors with positive surgical margins, with bone involvement, TT of more than 1.5 cm, large tumors with extraoral swelling, tumors with high-intensity TB, infiltrative type POI and poor differentiation based on IFG showed a significantly higher extent of Fn14 staining than their counterparts (Table 4).

## 4 | DISCUSSION

TWEAK-Fn14 is comparatively low in normal tissues but undergoes a dramatic upregulation in settings of injury and disease.[8] TWEAK-Fn14 is expressed in tumor tissue and TWEAK stimulates some cellular processes coupled with tumor progression.[7] The expression of TWEAK in OSCC is entirely cytoplasmic. The absence of nuclear TWEAK indicates probable inactivation of its protective function in tumor progression.[16] TWEAK expression is significantly increased locally in target tissues in cancer, all of which are related to infiltration of inflammatory cells or activation of resident innate immune cells.[6,8] In OSCC, a tumor that is characterized by an intense inflammatory component, TWEAK and Fn14 are highly expressed, as observed in this study.[17] Fn14 has been reported to be overexpressed in most solid tumors relative to non-tumors,[12,18] even though it has been hardly addressed in OSCC. In this study, the expression of Fn14 was significantly upregulated in tumors compared with that of ODL and HOM. In all probability, there is a constant or continued TWEAK-Fn14 activation which may be accountable for increased expression in tumors. Gene expression analysis of TWEAK-Fn14 in OSCC tissues found a gradual significant increase in the expression pattern of these molecules from control to cancerous tissues.[18] In most analyses, TWEAK expression is increased in tumor tissues compared with normal, which may trigger the proliferation or migration activity.[12] Hence, TWEAK may contribute to progression of tumors.[16] TWEAK-Fn14 upregulates vascular endothelial growth factor expression to promote ovarian cancer metastasis and regulates the invasive capacity of breast cancer cells.[18]

Meadawy et al.[16] found that TWEAK expression was significantly downregulated in OSCC compared with that in normal mucosa. Zou et al.[12] reported that TWEAK mRNA expression was significantly downregulated in cervical cancer compared with normal. Peternel et al.[5] found that the TWEAK expression ranged from strong to completely absent in cutaneous carcinoma. A few researchers[7,12] detected that TWEAK expression significantly decreased in carcinoma compared with normal tissue, possibly because of its consumption

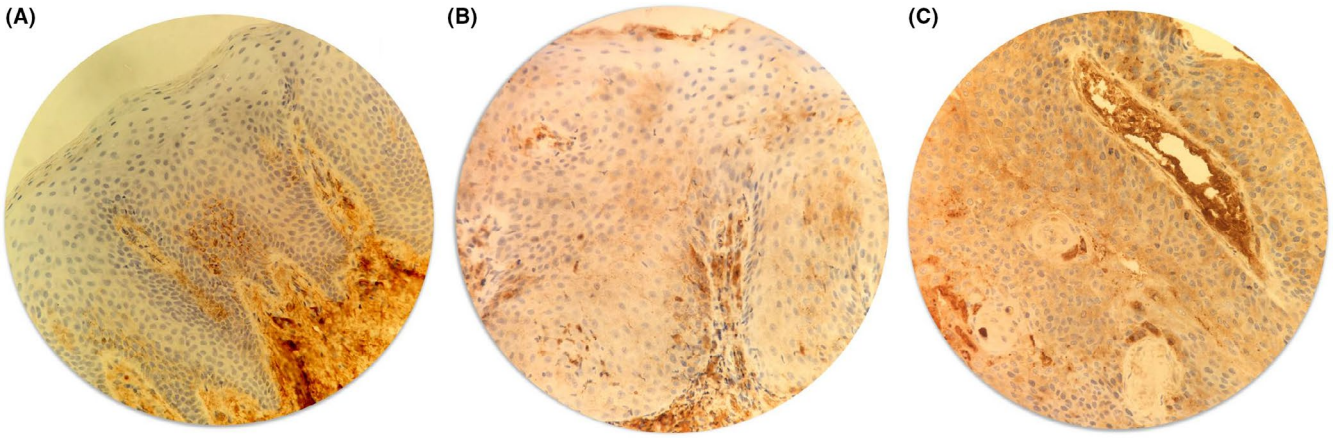


**FIGURE 2** Photomicrograph depicting the expression of fibroblast growth factor-inducible immediate early response protein 14 (Fn14) in (A) healthy oral mucosa, (B) oral dysplasia and (C) oral squamous cell carcinoma (Objective magnfication 10x, 20x; 3,3′-diaminobenzidine-tetrachloride chromogen, Fn14 monoclonal antibody)

**TABLE 3** Association between tumor necrosis factor-like weak inducer of apoptosis immunoreactive score (IRS) and the clinicopathological parameters of oral squamous cell carcinoma

| Parameters | Category | N | IRS low | IRS high | $\chi^2$ | *P* |
|---|---|---|---|---|---|---|
| Age (years) | ≤40 | 8 | 3 | 5 | 0.860 | .3543 |
| | >40 | 23 | 13 | 10 | | |
| Sex | Female | 5 | 3 | 2 | 0.168 | .682 |
| | Male | 26 | 13 | 13 | | |
| Habits | Tobacco smoking | 9 | 6 | 3 | 1.151 | .0283 |
| | Gutkha chewing | 22 | 10 | 12 | | |
| Growth pattern | Endophytic | 17 | 10 | 7 | 0.784 | .376 |
| | Exophytic | 14 | 6 | 8 | | |
| Size | ≤4 cm | 11 | 6 | 5 | 0.059 | .809 |
| | >4 cm | 20 | 10 | 10 | | |
| Stage | Early | 5 | 3 | 2 | 0.168 | .682 |
| | Advanced | 26 | 13 | 13 | | |
| Association with OSF | Absent | 21 | 14 | 7 | 5.907 | .015 |
| | Present | 10 | 2 | 8 | | |
| Broder's grade | Well | 21 | 12 | 9 | 0.79 | .320 |
| | Moderate-poor | 10 | 4 | 6 | | |
| IFG | Well | 17 | 14 | 3 | 15.387 | .001 |
| | Moderate | 7 | 2 | 5 | | |
| | Poor | 7 | 0 | 7 | | |
| LNM | pN (0) | 16 | 7 | 9 | 0.819 | .366 |
| | pN (+) | 15 | 9 | 6 | | |
| ECS | Absent | 20 | 10 | 10 | 0.059 | .809 |
| | Present | 11 | 6 | 5 | | |
| Surgical margins | Absent | 25 | 15 | 10 | 3.638 | .05 |
| | Present | 6 | 1 | 5 | | |
| PNI | Absent | 26 | 14 | 12 | 0.322 | .570 |
| | Present | 5 | 2 | 3 | | |
| Recurrence | Absent | 24 | 13 | 11 | 0.278 | .598 |
| | Present | 7 | 3 | 4 | | |
| TT | ≤1.5 cm | 9 | 7 | 2 | 3.476 | .062 |
| | >1.5 cm | 22 | 9 | 13 | | |
| TB | Low | 15 | 10 | 5 | 2.637 | .104 |
| | High | 16 | 6 | 10 | | |
| POI | 1-2 | 13 | 10 | 3 | 5.743 | .017 |
| | 3-4 | 18 | 6 | 12 | | |
| Stroma | Very low | 11 | 4 | 7 | 1.621 | .445 |
| | Low | 8 | 5 | 3 | | |
| | Moderate | 12 | 7 | 5 | | |
| Inflammation | Weak | 11 | 5 | 6 | 4.327 | .115 |
| | Intermediate | 7 | 6 | 1 | | |
| | Strong | 13 | 5 | 8 | | |

IFG, invasive front grading; LNM, lymph node metastasis; OSF, oral submucous fibrosis; PNI, perineural invasion; POI, patttern of invasion; TB, tumor budding; TT, tumor thickness.

in the process of Fn14 synthesis. The decreased levels of TWEAK in cancers may stimulate the synthesis of Fn14; there are reports of high levels of Fn14 in SCC/carcinoma in situ. It has been proposed that a high level of Fn14 is sufficient to cause biological effects without excessive TWEAK, and the increased expression of Fn14 may be in response to tumor cell-derived growth factors.[7,12]

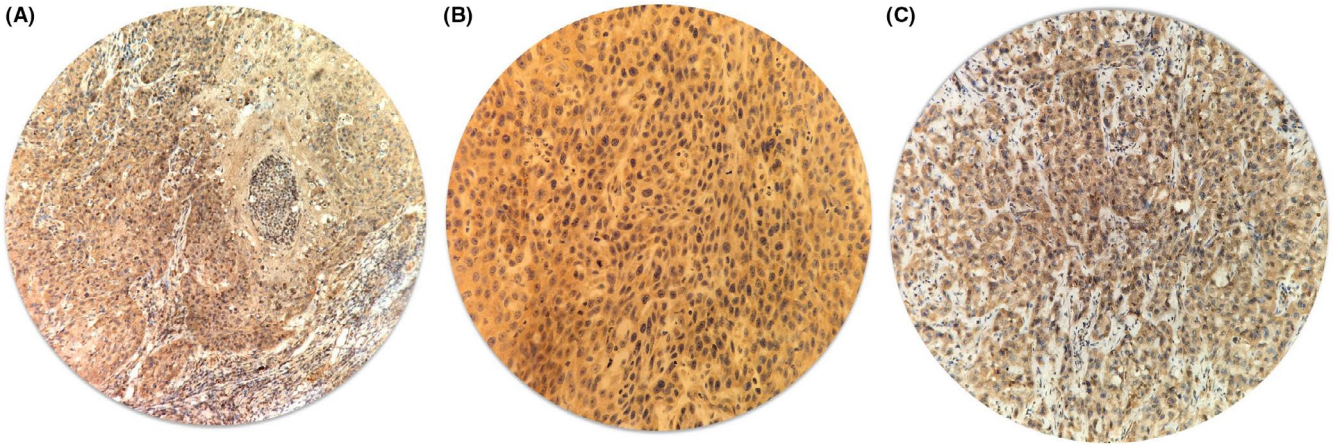


**FIGURE 3** Photomicrograph depicting the expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in (A) well-differentiated squamous cell carcinoma, (B) moderately differentiated squamous cell carcinoma and (C) poorly differentiated squamous cell carcinoma (Objective magnfication 10x, 20x; 3,3′-diaminobenzidine-tetrachloride chromogen, TWEAK monoclonal antibody)

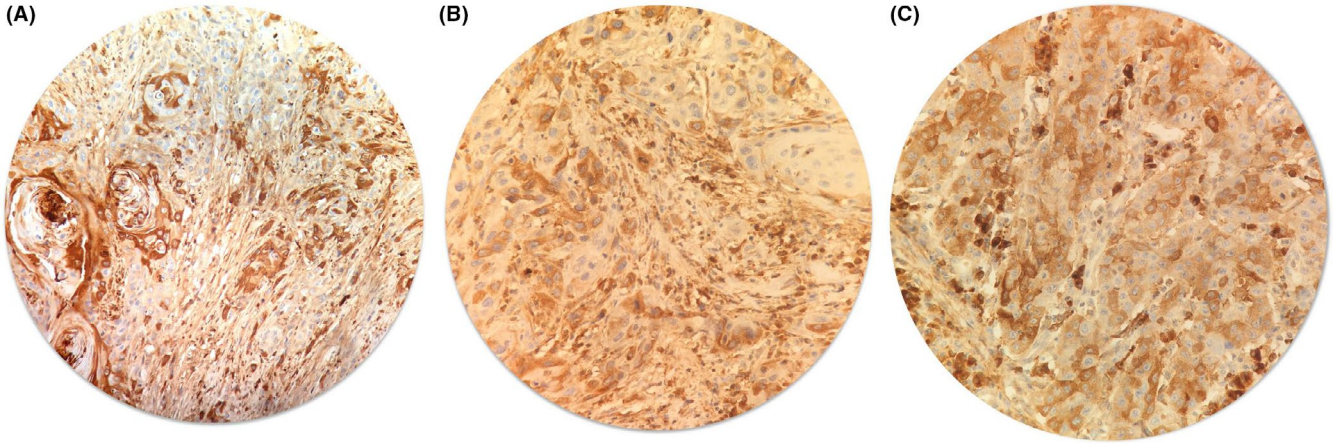


**FIGURE 4** Photomicrograph depicting the expression of fibroblast growth factor-inducible immediate early response protein 14 (Fn14) in (A) well-differentiated squamous cell carcinoma, (B) moderately differentiated squamous cell carcinoma and (C) poorly differentiated squamous cell carcinoma (Objective magnification 40x; 3,3′-diaminobenzidine-tetrachloride chromogen, Fn14 monoclonal antibody)

TWEAK-independent Fn14 signaling can occur in vitro; whether TWEAK-independent Fn14 signaling could occur in vivo is currently indefinite. TWEAK-independent Fn14 signaling can occur when intracellular Fn14 levels attain a definite threshold level. Overexpression of Fn14 on the cell surface induces spontaneous trimerization and multimerization, and this receptor clustering promotes TRAF association and intracellular cascade activation.[7] Elevated expression of Fn14 has also been found in the keratinocyte-originated cancers.[12,19,20] Elevated Fn14 expression promotes growth in gastric cancer.[20] Accordingly, Fn14 is considered as a tumor-specific cell surface biomarker and a tumor cell regulatory molecule.[12,18]

It is hypothesized that anarchy of apoptosis in cancer plays a role in the cancer development where the TWEAK-Fn14 pathway may be involved. It is also mentioned in a few studies that TWEAK acted as a tumor suppression factor which is contrary to most cancer species reported in the literature. TWEAK suppresses malignant cell growth by binding to Fn14 to promote apoptosis or necrosis. TWEAK could induce necrosis through a lysosomal cathepsin B pathway or indirect induction of apoptosis by induction of autocrine TNF-α or caspase pathways. Thus, it is contemplated that TWEAK/Fn14 may play an important role in inhibiting proliferation of cervical cancer cells and suppress the development of carcinoma.[12]

The Fn14-TRAF2-TNFR axis regulates the apoptosis and proliferation of tumor cells. TWEAK has been known to be an inducer of apoptosis of keratinocytes by engaging the Fn14 receptor if there is activation of the Fn14-TRAF2-TNFR1 axis.[15]

The literature has proposed the cell proliferative action of TWEAK as follows: it acts as a growth factor;[8] it activates the transcription factor NF-κB, which reverses the inhibition of cell proliferation; also, TWEAK promotes the proliferation of keratinocytes if infected by human papilloma virus (HPV). Activation of cytoplasmic caspase-8 propagates various apoptotic signals in keratinocytes. E6/E7 oncoproteins inhibit the caspase-8 synthesis and promote TRAF2 expression, and activation of the Fn14-TRAF2-TNR2 axis may be

**TABLE 4** The relation between the extent of Fn14 staining and the Clinicopathologic parameters of OSCC

| Parameters | Category | N | Mean ± SD | *U*-Value | *P*-value |
|---|---|---|---|---|---|
| Age | ≤40 | 8 | 63 ± 20.76 | 66.5 | .24 |
| | >40 | 23 | 52.04 ± 31.33 | | |
| Sex | Female | 5 | 54.60 ± 43.77 | 61 | .83 |
| | Male | 26 | 54.92 ± 26.58 | | |
| Habits | Tobacco smoking | 9 | 44.56 ± 30 | 81 | .43 |
| | Gutkha chewing | 22 | 59.09 ± 28.27 | | |
| Type | Endophytic | 17 | 45.71 ± 28.24 | 82 | .14 |
| | Exophytic | 14 | 66 ± 26.90 | | |
| Size | ≤4 cm | 11 | 53.27 ± 31.43 | 94 | .5 |
| | >4 cm | 20 | 55.75 ± 28.47 | | |
| Clinical nodes | pN(0) | 11 | 46.91 ± 22.34 | 82.5 | .25 |
| | pN (+) | 20 | 59.25 ± 31.84 | | |
| Clinical stage | Early | 5 | 39 ± 9.67 | 30.5 | .06 |
| | Advanced | 26 | 57.92 ± 30.63 | | |
| Association with OSF | Absent | 21 | 48.67 ± 29.12 | 67 | .1 |
| | Present | 10 | 67.90 ± 25.53 | | |
| Broders grade | Well | 21 | 54.24 ± 26.28 | 96.5 | .71 |
| | Moderate-poor | 10 | 56.2 ± 35.75 | | |
| LNM | pN(0) | 16 | 53.19 ± 26.19 | 108 | .63 |
| | pN(+) | 15 | 56.67 ± 32.68 | | |
| ECS | Absent | 20 | 51.60 ± 27.60 | 89 | .38 |
| | Present | 11 | 60.82 ± 32.01 | | |
| Surgical margins | Absent | 25 | 49.84 ± 28.79 | 33 | .036 |
| | Present | 6 | 75.83 ± 20.82 | | |
| BI | Absent | 17 | 44.53 ± 28.51 | 69 | .04 |
| | Present | 14 | 67.43 ± 25.27 | | |
| PNI | Absent | 26 | 52.35 ± 29.8 | 43.5 | .24 |
| | Present | 5 | 68 ± 22.9 | | |
| Recurrence | Absent | 24 | 53.08 ± 28.12 | 66 | .39 |
| | Present | 7 | 61 ± 33.67 | | |
| TT | ≤1.5 cm | 9 | 38.78 ± 19.14 | 47 | .02 |
| | >1.5 cm | 22 | 61.45 ± 30.17 | | |
| Extraoral swelling | Absent | 7 | 30 ± 23.60 | 30 | .01 |
| | Present | 24 | 62.13 ± 26.70 | | |
| TB | Low (1-4) | 15 | 46.27 ± 23.78 | 63.5 | .02 |
| | High (>5) | 16 | 62.94 ± 31.9 | | |
| POI | POI 1-2 | 13 | 41.77 ± 20.27 | 48 | .006 |
| | POI 3-4 | 18 | 64.33 ± 31.20 | | |
| | | | | ANOVA value | |
| Stroma | Very low | 11 | 68.73 ± 23.87 | 4.53 | .104 |
| | Low | 8 | 47.13 ± 27.73 | | |
| | Moderate | 12 | 47.33 ± 31.58 | | |
| Inflammation | Weak | 11 | 52.09 ± 31.13 | 1.063 | .588 |
| | Intermediate | 7 | 42.86 ± 20.74 | | |
| | Strong | 13 | 63.69 ± 30.21 | | |

(Continues)

**TABLE 4** (Continued)

| Parameters | Category | N | Mean ± SD | *U*-Value | *P*-value |
|---|---|---|---|---|---|
| IFG | Well | 17 | 34.59 ± 17.95 | 23.85 | .001 |
| | Moderate | 7 | 62.71 ± 9.94 | | |
| | Poor | 7 | 96.29 ± 5.05 | | |
| | | | | U value | |
| IFG | Well | 17 | 34.59 ± 17.95 | 2.000 | .001 |
| | Moderate | 7 | 62.71 ± 9.94 | | |
| | Moderate | 7 | 62.71 ± 9.94 | 0.000 | .002 |
| | Poor | 7 | 96.29 ± 5.05 | | |
| | Well | 17 | 34.59 ± 17.95 | 0.000 | .001 |
| | Poor | 7 | 96.29 ± 5.05 | | |

responsible for cell proliferation.[15,21] Cross-talk in keratinocytes between TWEAK/Fn14 signaling and HPV16 infection may collaborate in regulating cell-cycle progression.[21]

TWEAK induces phenotypic alterations of epithelial cells, accompanied by loss of E-cadherin and epithelial integrity. Downregulation of E-cadherin is considered as a critical step in epithelial-mesenchymal transition (EMT).[15] In the present study, a significantly increased expression of TWEAK-Fn14 at the invasive front compared with the whole tumor was observed, emphasizing its potential role in EMT. Also, reports mention that TWEAK induces the production of matrix metallopeptidases or upregulates them.[8,10,15] Thus, the TWEAK-Fn14 axis may fuel the invasion and migration of tumor cells in OSCC. Findings of this study suggest that TWEAK and Fn14 expression were higher in biologically aggressive tumors than their counterparts.

## 5 | CONCLUSIONS

TWEAK and Fn14 were expressed in oral tissue samples in this investigation. The expression gradually increased from HOM to ODL and OSCC, which implies that TWEAK-Fn14 may play a role in tumor growth and progression. TWEAK-Fn14 expression showed a significant association with several clinicopathological parameters of prognostic significance like IFG, POI, TB and surgical margins. Increased expression of TWEAK-Fn14 in OSCC at the ITF compared with the WT may facilitate increased proliferation, altered differentiation and invasion.

The present investigation analyzed the IHC expression of TWEAK-Fn14 in a small cohort of OSCC subjects, which may limit the statistical power of the study. The utility of TWEAK-Fn14 requires validation in a larger cohort in order to subcategorize subjects based on clinicopathological characteristics. A few reports suggest that low pretreatment sTWEAK is an independent prognostic factor for clinical outcome in OSCC and that the TWEAK pathway plays an important role in regulation of locoregional control. Because the literature cites that blood sTWEAK levels are linked to TWEAK gene expression in tumors, we recommend future prospective studies involving OSCC subjects with simultaneous gene expression and IHC evaluation of Fn14 and TWEAK in tissue specimens; pretreatment and post-treatment sTWEAK would provide clarity about their behavior as a biomarker.

## ACKNOWLEDGEMENTS

The authors thank the Department of Oral Pathology 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, 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.

## ORCID

*Swetha Acharya* https://orcid.org/0000-0001-5063-2725

*Anirudh B. Acharya* https://orcid.org/0000-0002-2062-5791

## REFERENCES

1. Prasad G, McCullough M. Chemokines and cytokines as salivary biomarkers for the early diagnosis of oral cancer. *Int J Dent*. 2013;2013:813756.
2. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell*. 2000;100:57-70.
3. Krishnan R, Thayalan DK, Padmanaban R, Randas R, Annasamy RK, Anandan N. Association of serum and salivary tumor necrosis factor-α with histological grading in oral cancer and its role in differentiating premalignant and malignant oral disease. *Asian Pac J Can Prev*. 2014;15:7141-7148.
4. Pettersen I, Baryawno N, Abel F, et al. Expression of TWEAK/Fn14 in neuroblastoma: implications in tumorigenesis. *Int J Oncol*. 2013;42(4):1239-1248.
5. Peternel S, Manestar-Blazic′ T, Brajac I, Prpic′-Massari L, Kastelan M. Expression of TWEAK in normal human skin, dermatitis and epidermal neoplasms: association with proliferation and differentiation of keratinocytes. *J Cutan Pathol*. 2011;38:780-789.
6. Gu L., Dai L., Cao C., et al. Functional expression of TWEAK and the receptor Fn14 in human malignant ovarian tumors: possible implication for ovarian tumor intervention. *PLoS One*. 2013;8(3):e57436.

7. Winkles JA. The TWEAK-Fn14 cytokine-receptor axis: discovery, biology and therapeutic targeting. *Nat Rev Drug Discov*. 2008;7:411-425.
8. Burkly LC, Michaelson JS, Hahm K, Jakubowski A, Zheng TS. TWEAKing tissue remodeling by a multifunctional cytokine: role of TWEAK/Fn14 pathway in health and disease. *Cytokine*. 2007;40:1-16.
9. Enwere EK, Lacasse EC, Adam NJ, Korneluk RG. Role of the TWEAK-Fn14-cIAP1-NF-κB signaling axis in the regulation of myogenesis and muscle homeostasis. *Front Immunol*. 2014;5(34):1-13.
10. Winkles JA, Tran NL, Berens ME. TWEAK and Fn14: new molecular targets for cancer therapy? *Cancer Lett*. 2006;235:11-17.
11. Wiley SR, Cassiano L, Lofton T, et al. A novel TNF receptor family member binds TWEAK and is implicated in angiogenesis. *Immunity*. 2001;15:837–846.
12. Zou H, Wang D, Gan X, et al. Low TWEAK expression is correlated to the progression of squamous cervical carcinoma. *Gynecol Oncol*. 2011;123(1):123-128.
13. Blanco-Colio Luis M. TWEAK/Fn14 axis: a promising target for the treatment of cardiovascular diseases. *Front Immunol*. 2014;5(3):2-13.
14. Winkles JA, Tran NL, Brown SA, Stains N, Cunliffe HE, Berens ME. Role of TWEAK and Fn14 in tumor biology. *Front Biosci*. 2007;12:2761-2771.
15. Hu G, Zeng W, Xia Y. TWEAK/Fn14 signaling in tumors. *Tumor Biol*. 2017;39:1-9.
16. El-Meadawy SH, Salama NM, EL-Nagdy SY, Zyada MM. Immunohistochemical study of TNF like weak inducer of apoptosis (TWEAK) in oral squamous cell carcinoma tissues & its relation to 8-Hydroxy-2-Deoxyguanosine (8-OHDG) expression in saliva. *Egypt Dent J*. 2013;59:481-494.
17. Sabour Alaoui S, Dessirier V, de Araujo E, et al. TWEAK affects keratinocyte G2/M growth arrest and induces apoptosis through the translocation of the AIF protein to the nucleus. *PLoS One*. 2012;7(3):e33609.
18. Aviles-Jurado FX, Terra X, Gomez D, et al. Low blood levels of sTWEAK are related to locoregional failure in head and neck cancer. *Eur Arch Otorhinolaryngol*. 2015;272:1733-1741.
19. Yoriki R, Akashi S, Sho M, et al. Therapeutic potential of the TWEAK/Fn14 pathway in intractable gastrointestinal cancer. *Exp Ther Med*. 2011;2:103-108.
20. Kwon O, Park S, Kang T, et al. Elevated fibroblast growth factor-inducible 14 expression promotes gastric cancer growth via nuclear factor-kB and is associated with poor patient outcome. *Cancer Lett*. 2012;314:73-81.
21. Cheng H, Zhan N, Ding D, et al. HPV type 16 infection switches keratinocytes from apoptotic to proliferative fate under TWEAK/Fn14 interaction. *J Invest Dermatol*. 2015;135:2427-2443.

**How to cite this article:** Acharya S, Prabhu P, Patil VS, Acharya AB, Nikhil K. Immunohistochemical expression of tumor necrosis factor-like weak inducer of apoptosis and fibroblast growth factor-inducible immediate early response protein 14 in oral squamous cell carcinoma and its implications. *J Invest Clin Dent*. 2019;00:e12469. https://doi.org/10.1111/jicd.12469