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Expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and its receptor, fibroblast growth factor-inducible 14 protein (Fn14), in healthy tissues and in tissues affected by periodontitis

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Background and Objective: Host-derived enzymes, cytokines and other proinflammatory mediators play an integral role in periodontal destruction. The levels of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and its receptor, fibroblast growth factor-inducible 14 protein (Fn14), are elevated in tissues from a number of chronic inflammatory diseases. The aim of the present study was to investigate the expression of TWEAK and Fn14 at the protein and mRNA levels in gingival biopsies from periodontitis patients and from clinically healthy patients.

Materials and Methods: Gingival biopsies were obtained from healthy sites ($n = 7$) and from sites affected by periodontitis ($n = 27$). The expression of TWEAK and Fn14 was investigated by immunohistochemistry in formalin-fixed, paraffin-embedded tissues. The levels of mRNA for TWEAK and Fn14 were also investigated by RT-PCR.

Results: The expression of TWEAK and Fn14 proteins was significantly higher in periodontitis tissue than in healthy tissue. In periodontitis tissues, TWEAK and Fn14 proteins were mainly expressed by mononuclear leukocytes (morphologically resembling lymphocytes and plasma cells), by cells lining blood vessels, by spindle-shaped cells resembling fibroblasts and by multinucleated cells. The Fn14 mRNA level in periodontitis tissue was significantly higher than that in healthy tissue. A moderate correlation between TWEAK/Fn14 expression and inflammation and bone loss, but not pocket depth, was noted.

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Conclusion: This study demonstrates higher expression of TWEAK protein and of Fn14 mRNA and protein in periodontitis tissues than in clinically healthy controls. Our data support the concept that TWEAK/Fn14 signaling is an additional player in the pathogenesis of periodontitis and adds to the increasing number of cytokine networks involved in periodontal inflammation.

Periodontitis is a chronic inflammatory disease wherein microbial factors induce complex inflammatory and immune responses in a susceptible host (1,2). In periodontitis, host-derived enzymes, cytokines and other proinflammatory mediators play an integral role in the destruction of tooth-supporting structures (3,4). Thus, bacteria are considered to be necessary factors for periodontal disease initiation but they are not sufficient to cause disease progression. Cytokines, the major regulators of the immuno-inflammatory response seen in periodontitis, affect tissue destruction by acting either directly on cells or through inducing the production of other cytokines.

Tumor necrosis factor (TNF)-like weak inducer of apoptosis (TWEAK) belongs to the TNF superfamily (5). It is expressed by a variety of inflammatory cells, including monocytes, macrophages, activated T-cells and plasma cells (5–8). In addition, it is expressed by fibroblasts (9). The receptor for TWEAK, fibroblast growth factor-inducible 14 protein (Fn14) (10), is expressed by many cell types in response to injury, including epithelial cells, mesenchymal cells and endothelial cells (11–15). Moreover, many tissue progenitor cells of mesenchymal lineage, embryonic stem cells and osteoblasts also express Fn14 (11,15–17).

TWEAK/Fn14 signaling results in multiple biologic effects, including the induction of inflammatory cytokines and matrix metalloproteinases, modulation of the immune response, angiogenesis and the stimulation of apoptosis (5,13,15,18–24). Other studies have shown that TWEAK has a role to play in tissue repair and regeneration following acute tissue injury (11). TWEAK also has an inhibitory effect on the differentiation of osteoblasts (17). We recently reported that TWEAK, alone and in the presence of TNF-α, potently up-regulated the expression of the Wnt/bone morphogenetic protein inhibitor, sclerostin, in human primary osteoblasts (17), which probably accounts for at least some of the inhibitory effects of TWEAK on these cells. A single study found that TWEAK directly promotes the osteoclastic differentiation of cells from the monocyte/macrophage lineage, although that study did not find evidence of Fn14 expression by the responding cells (25).

Some studies have demonstrated the expression of TWEAK and its receptor Fn14 in tissues and cells from patients with different chronic inflammatory diseases such as rheumatoid arthritis and periodontitis (15,24,26). These studies indicate that TWEAK might play a role in the pathogenesis of these chronic inflammatory diseases. It has been proposed that TWEAK/Fn14 signaling might be a universal mechanism which plays a role in various inflammatory and autoimmune disorders mediating pathologic tissue remodeling (27). Accordingly, TWEAK might serve as an additional therapeutic target in the treatment of chronic inflammatory diseases (28–30). Therefore, the aim of the present study was to investigate the expression of TWEAK and Fn14 in more detail in healthy and inflamed periodontal tissues from a larger number of patients than has been previously reported. In addition, this present study investigated the correlation between TWEAK/Fn14 expression and some important parameters of periodontitis, such as inflammation, bone loss and pocket depth.

Material and methods

Patient demographics

The study included gingival samples taken from 27 patients (18 women and 9 men, 30–77 years of age, mean age 55.2 years) with generalized chronic and aggressive (moderate to severe) periodontitis. The study population included 23 patients with generalized chronic periodontitis and four patients with generalized aggressive periodontitis. The patients were classified according to the American Academy of Periodontology 1999 classification (31). The gingival biopsy sites of the patients from the chronic and aggressive periodontitis group had clinical probing pocket depths and clinical attachment loss of > 5 mm, with radiographic evidence of bone loss ranging from 50 to 90% of the root length. Bone loss and pocket depth at the biopsy sites were recorded and grouped as shown in Table 1. The test samples included mostly interdental tissue and granulation tissue removed next to periodontal bony defects. The healthy tissue samples consisted of gingival tissue removed from seven patients (four men and three women, 23–70 years of age at the time of surgery; mean age 45.5 years) undergoing crown-lengthening surgery not affected by periodontitis. Informed consent was obtained from the patients attending the Periodontics postgraduate clinic. This study was approved by the University of Adelaide Human Ethics committee, in accordance with the guidelines of the National Health and Medical Research Council of Australia.

Antibodies

The following monoclonal antibodies were used for this study: mouse anti-human TWEAK (P2D10, subclass IgG2a, gifted by Timothy S. Zheng; Biogen Idec Inc., Cambridge, MA, USA) (32) and purified mouse anti-human Fn14 (subclass IgG1, clone ITEM 1; catalogue number 314002; BioLegend, Karrinyup, WA, Australia) (24). For control sections, staining was performed using irrelevant isotype-matched mouse monoclonal antibodies (1D4.5 for IgG2a

Table 1. Grading of inflammation, pocket depth and bone loss at a biopsy site

Inflammation score at a biopsy site (histology score)
Score 0 (minimal): 0–10% chronic inflammatory cells
Score 1 (mild): 11–25% chronic inflammatory cells
Score 2 (mild to moderate): 26–50% chronic inflammatory cells
Score 3 (moderate to severe): 51–75% chronic inflammatory cells
Score 4 (severe): 76 to 100% chronic inflammatory cells
Bone loss at the biopsy site (radiographic score)
Score 1: no discernable radiographic evidence of bone loss
Score 2: (mild) proximal bone loss reaching at most one-third of normal bone height
Score 3: (moderate) proximal bone loss between one-third and two-thirds of normal bone height
Score 4: (severe) proximal bone loss of more than two-thirds of normal bone height
Pocket depth at biopsy site (clinical measurement in mm)
Score 1: 0–3 mm
Score 2: 3.5–5 mm
Score 3: 5.5–7
Score 4: > 7.0 mm

and 1B5 for IgG1) under otherwise identical conditions.

Preparation of tissue for immunohistochemical detection

Following collection of the periodontal tissues from patients, the samples were immediately immersed in 10% normal buffered formalin overnight and then processed for embedding in paraffin. Sec tions (5 μm) were cut, de-waxed and rehydrated. Both TWEAK and Fn14 expression was detected using a three-step immunohistochemical detection method (33).

Routine histology staining and assessment

Routine histological assessment of hematoxylin and eosin-stained tissue samples was carried out to ensure that the tissues included were appropriate for immunohistochemical analysis (34). Following microscopic assessment, each sample was given an inflammatory score based on the percentage of chronic inflammatory cells present in the connective tissue layer of periodontal tissue (both diseased and healthy) samples (Table 1). The grading system was performed by two independent observers who were blinded to the status of the samples.

Immunohistochemical staining for TWEAK expression

For detection of TWEAK, tissue sections were pretreated with Proteinase K (100 μg/mL) at 37°C for 30 min to unmask antigen epitopes. Endogenous peroxidase activity was inhibited with phosphate-buffered saline (PBS) containing 0.3% H_2O_2 and 0.1% sodium azide. A working concentration of 14.5 μg/mL of the primary antibody, P2D10, diluted in PBS containing 1% bovine serum albumin (BSA) was determined in a preliminary experiment. The primary antibody was applied to the sections and incubated in a humid chamber overnight at room temperature. The sections were then incubated with horseradish peroxidase-conjugated goat anti-mouse IgG for 30 min at room temperature. Horseradish peroxidase-conjugated swine anti-goat IgG was then added for another 30 min at room temperature. Secondary and tertiary antibodies were diluted in PBS/1% BSA in the presence of 10% normal human serum. The sections were washed in PBS between each step. The color reaction was developed using hydrogen peroxide as the substrate and 3,9 aminoethylcarbazole (AEC) as the dye. Counterstaining was performed using Harris Hematoxylin.

Immunohistochemical staining for Fn14 expression

To detect Fn14 expression, sections underwent heat epitope retrieval in sodium citrate buffer (pH 6) for 20 min at 95°C in a water bath. Then, the sections were bench cooled for 20 min. Endogenous peroxidase activity was inhibited with 0.3% H_2O_2 in methanol. Blocking serum (normal horse serum) was added to the sections to reduce nonspecific staining. A working concentration of 20 μg/mL (diluted in PBS/1% BSA) of the primary antibody against Fn14 was determined in a preliminary experiment. The primary antibody was applied to the sections and incubated in a wet chamber overnight at room temperature. The sections were then incubated with a secondary antibody (biotinylated universal antibody; Vectastain Universal Elite ABC kit, Vector labs, Burlingame, CA, USA), at the concentration recommended by the manufacturers, for 1 h at room temperature. Then, enhancement of primary and secondary antibody was carried out using the ABC Reagent (avidin and biotinylated horseradish peroxidase macromolecular complex). The sections were washed in PBS between each step, except for when the slides were left unwashed after the addition of the blocking serum. The color reaction was developed using H_2O_2 as the substrate and diaminobenzidine tetrahydrochloride (DAB) dye. Counterstaining was performed using Harris Hematoxylin.

Immunohistochemical controls

Isotype-matched negative-control antibodies (1D4.5 for IgG2a and 1B5 for IgG1) were used at an identical concentration to that of the test primary antibodies. Positive controls were performed on rheumatoid arthritis tissue samples known to express TWEAK and Fn14.

Microscopic analysis

Following immunohistochemical staining, the sections were scored using a previously validated semiquantitative assessment (SQA) method. One sample was analysed from each patient. Because of the small size of the tissue biopsies the total area of the section was assessed for staining using a five-point scale (0–4), by two independent calibrated and blinded observers in a random order, as described previously (35,36). Scores from 0 to 4 were given

depending on the number of chronic inflammatory cells staining positive for TWEAK or Fn14 (Table 1).

Real-time PCR

Gene expression was analysed by real-time PCR using SYBR green technology, as previously described (37). Briefly, for each patient sample, five tissue sections (20 μm) were cut using a Leica Kryostat (Leica Microsystems, North Ryde, NSW, Australia), placed into 300 μL of TRIzol™ reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), and total RNA was isolated according to the manufacturer's recommendations. One microgram of total RNA was reverse transcribed using 250 ng of random hexamer (Geneworks, Adelaide, SA, Australia) and 200 units of Superscript III Reverse Transcriptase, according to the manufacturer's recommendations. Primers to Fn14 were designed using Primer3Plus (38) to span exon–intron boundaries to avoid amplification from genomic sequences. The sequences of the Fn14 primers were: Fn14 (217 bp product) 5′-TTTCTGGCTTTTTGGTCTGG-3′ (forward) and 5′-CTTGTGGTTGGAGGAGCTTG-3′ (reverse). The sequences of the TWEAK primers were: TWEAK (83 bp product) 5′-ATCGCTGTCCGCCCAGGAGC-3′ (forward) and 5′-CTGTCTGGGGATTCAGTTCCG-3′ (reverse). The endogenous reference gene human acidic ribosomal protein P0 (hARP) (39) was used to normalize cycle threshold data obtained from the genes investigated. Relative gene expression between healthy gingival and chronic periodontitis tissue samples was obtained using the comparative threshold (ΔΔCt) method (40).

Statistical analysis

Statistical analysis for the SQA results in the two groups (periodontitis and clinically healthy patients) studied was performed using SPSS version 11.5 (SPSS Inc., Chicago, IL, USA). The Mann–Whitney *U*-test for nonparametric data was used to analyze the mean ranks of semiquantitative scores for TWEAK and Fn14. Kendall's tau_b test was used to detect correlation between two parameters. For both the Mann–Whitney *U*-test and Kendall's tau_b test, a *p*-value of < 0.05 was considered as being statistically significant. Correlation between TWEAK/Fn14 expression and inflammatory score, pocket depth and bone loss, was also determined. A correlation coefficient (*r*-value) of

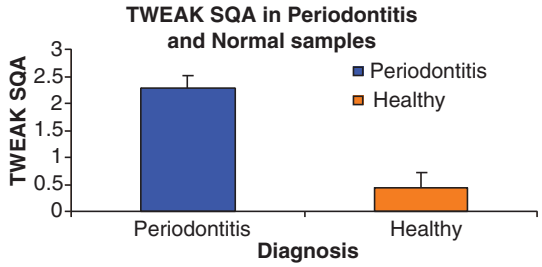

Fig. 1. Semiquantitative analysis (SQA) of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in tissue samples from patients with periodontitis and in tissue samples from healthy patients. Bars depict the mean SQA ± standard error.

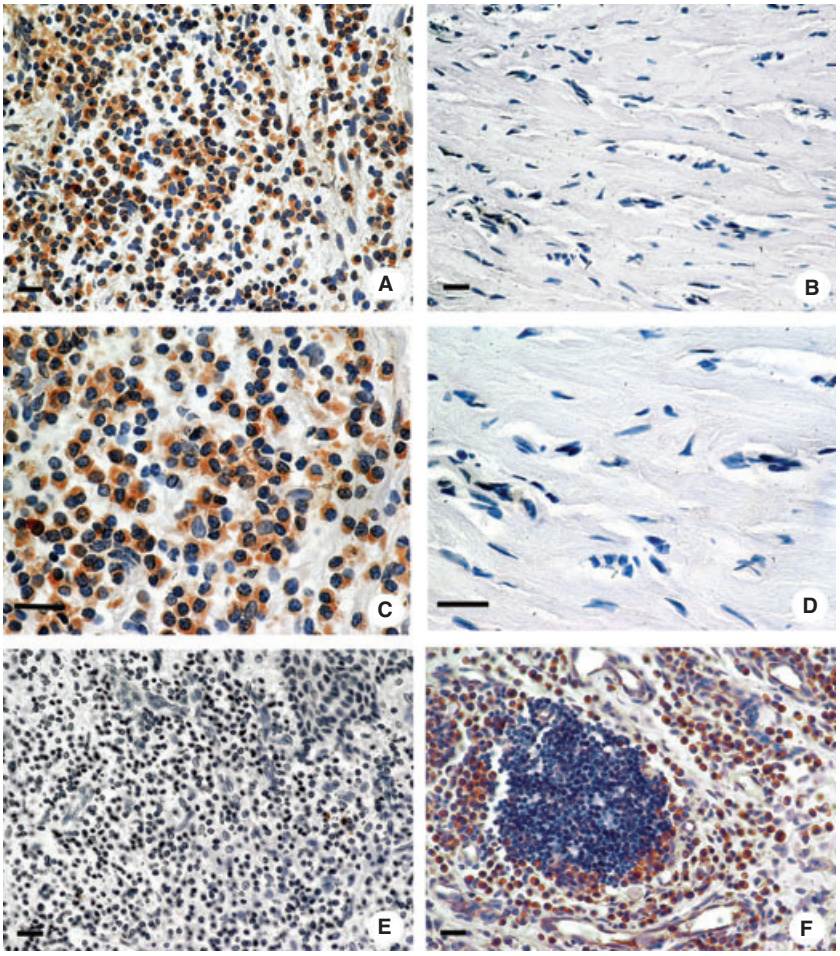

Fig. 2. Distribution of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in periodontal tissues. (A) Periodontitis tissue (200 × magnification); (B) healthy tissue (200 ×); (C) periodontitis tissue (400 ×); (D) healthy tissue (400 ×); (E) negative control (200 ×); (F) positive control – rheumatoid arthritis tissue (200 ×).

< 0.5 was considered weak, that of 0.5–0.8 was considered moderate and that of > 0.8 was considered strong. The criteria for grouping inflammatory score, pocket depth and bone loss are shown in Table 1.

Results

TWEAK expression in periodontitis tissue and healthy control tissue

The tissue sections were considered appropriate for immunohistochemical studies if they had a significant proportion of gingival epithelium along with underlying connective tissue. In this study, the clinically healthy tissue samples showed mild to moderate levels of inflammatory response. The periodontitis tissue samples showed moderate to severe levels of inflammation and included areas of granulation tissue characterized by the presence of blood vessels, loose connective tissue, spindle-shaped cells and leucocytic infiltration.

Figure 1 shows the mean TWEAK SQA in tissue samples from patients with periodontitis and from healthy tissues. Positive staining for TWEAK protein was seen in leukocytes that formed large mononuclear cell infiltrates in tissue samples from periodontitis lesions (Fig. 2A). Most of the leucocytes staining positively for TWEAK antibody had an eccentrically placed, round nucleus with a rim of cytoplasm. These mononuclear cells appeared to be morphologically similar to lymphocytes or plasma cells (Fig. 2A and 2C). In some of the sections, multinucleated cells stained positive for TWEAK protein (Fig. 3A and 3B). SQA for TWEAK in the periodontitis tissue gave a mean value of 2.27 (Table 2). Sixteen of the 26 periodontitis samples showed a high SQA of > 3. Mononuclear cells in 17 of 26 periodontitis sections showed very bright cell staining with the TWEAK antibody (Fig. 2A and 2C). This percentage ranged from 0 to 50%. In 20 of the 26 sections from periodontitis patients, cells lining the blood vessels stained positive for TWEAK protein (Fig. 3C). Positive TWEAK staining of spindle-shaped cells with morphology similar to fibroblasts was observed in 12 of 26 tissue samples (Fig. 3B).

TWEAK expression was less intense in the healthy tissues (Fig. 2B and 2D). The mean TWEAK SQA was 0.43 (Table 2), with five out of seven clinically healthy samples having a TWEAK SQA of < 1. TWEAK was also associated with cells lining blood vessels and spindle-shaped cells in five out of seven clinically healthy samples, but this staining was very weak (Fig. 2D).

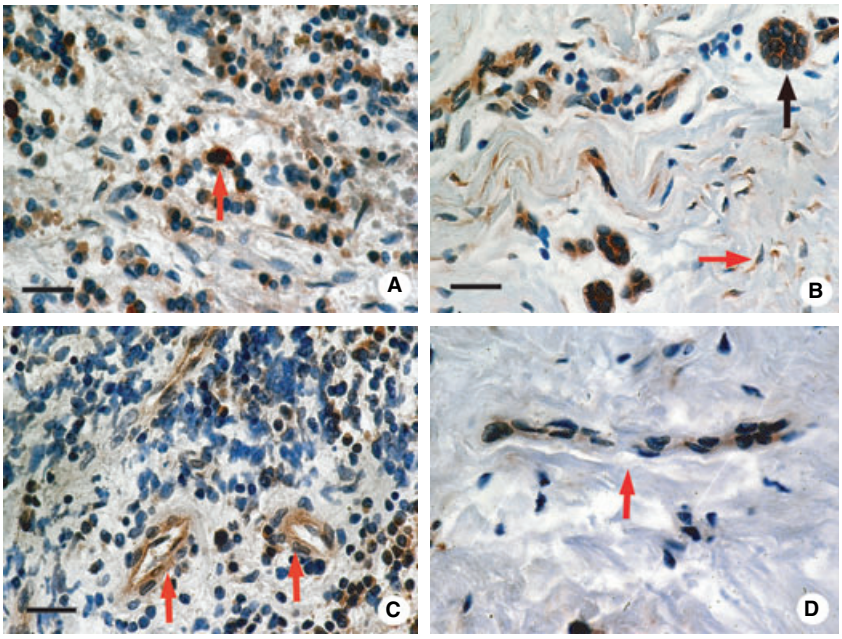

Fig. 3. Expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) by different cells in periodontal tissues. (A) Multinuclear cells (red arrow) in periodontitis tissue (400 × magnification); (B) fibroblasts (red arrow) and multinucleated cells (black arrows) in periodontitis tissue (400 ×); (C) blood vessels (arrows) in periodontitis tissue (400 ×); (D) blood vessels (arrows) in healthy tissue (400 ×).

Table 2. Assessment of immunohistochemical staining of healthy and periodontitis tissues for tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and fibroblast growth factor-inducible 14 protein (Fn14)

	TWEAK		Fn14	
	Healthy $n = 7$	Periodontitis $n = 26$	Healthy $n = 7$	Periodontitis $n = 27$
SQA 0	5 × 0 = 0	4 × 0 = 0	3 × 0 = 0	2 × 0 = 0
SQA 1	1 × 1 = 1	3 × 1 = 3	2 × 1 = 2	5 × 1 = 5
SQA 2	1 × 2 = 2	3 × 2 = 6	1 × 2 = 2	5 × 2 = 10
SQA 3	0 × 3 = 0	14 × 3 = 42	1 × 3 = 3	8 × 3 = 24
SQA 4	0 × 4 = 0	2 × 4 = 8	0 × 4 = 0	7 × 4 = 28
Total	3	59	7	67
Mean	3/7 = 0.428	59/26 = 2.269	7/7 = 1	67/272.481
Range	0–2	0–4	0–3	0–4
SD	0.7868	1.2151*	1.155	1.282**

Data represent the number of subjects in each semiquantitative analysis (SQA) category.
*$p = 0.002$ (compared with TWEAK healthy).
**$p = 0.013$ (compared with Fn14 healthy).

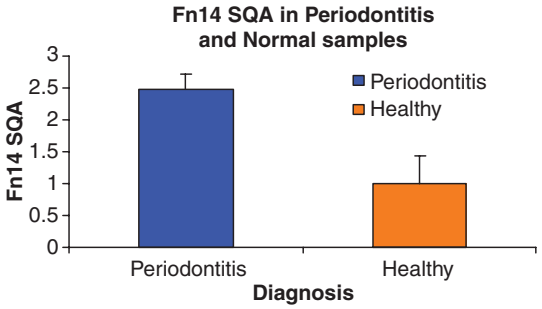

Fig. 4. Semiquantitative analysis (SQA) of fibroblast growth factor-inducible 14 protein (Fn14) in tissue samples from patients with periodontitis and in tissue samples from healthy tissues. Data are expressed as mean SQA ± standard error of the mean (SEM).

Fn14 expression in tissue from patients with periodontitis and in tissue from healthy controls

In tissue samples from periodontitis patients, high expression of Fn14 protein was observed (Fig. 4) and this was mainly associated with infiltrating leucocytes (Fig. 5A and 5C). In the periodontitis tissue samples, the mean Fn14 SQA was 2.48 (Table 2). Fifteen out of 27 patients had a high Fn14 SQA of ≥ 3. The mean Fn14 SQA of the periodontitis sample was found to be significantly greater ($p < 0.05$) than that of tissue samples collected from nonperiodontitis patients (Fig. 5B and 5D). In healthy patients the mean Fn14 SQA was 1.00 (Table 2). Five out of seven samples from clinically healthy patients had a low Fn14 SQA, of < 1.

In the inflamed tissues, cells lining blood vessels stained positive for Fn14 (Fig. 6A). This pattern of staining was noted in all but three of the periodontitis tissue samples (24/27). By contrast, blood vessels in tissues from healthy sites were positive for Fn14 protein in four out of seven tissue samples (with two of these samples showing very weak staining) (Fig. 6B).

In the tissue samples from patients from periodontitis, Fn14 protein expression was also noted in spindle-shaped cells resembling fibroblasts (Fig. 6A). By contrast, Fn14 expression was detected in spindle-shaped cells in only one of the tissue samples from healthy sites.

No staining was present in sections that were used as negative controls (Figs 2E and 5E). Rheumatoid arthritis tissue samples were used as positive controls. TWEAK and Fn14 protein were expressed strongly in sections from active rheumatoid arthritis lesions (Figs 2F and 5F).

The expression of TWEAK protein was significantly higher in periodontitis tissue than in healthy tissue (Mann–Whitney *U*-test, $p = 0.002$). Similarly, in comparison with healthy tissue, periodontitis tissue contained significantly higher levels of Fn14 protein (Mann–Whitney *U*-test, $p = 0.013$). The distribution of TWEAK and Fn14 SQA scores, according to the severity of bone loss, pocket depth and inflammation score, are shown in Table 3. The correlation between TWEAK/Fn14 expression and inflammation, pocket depth and bone loss is shown in Table 4. A weak, positive correlation was found between TWEAK and Fn14 expression (Kendall's tau_b test; $p = 0.007$, $r = 0.395$). In addition, a moderate correlation was found between the inflammatory score and TWEAK

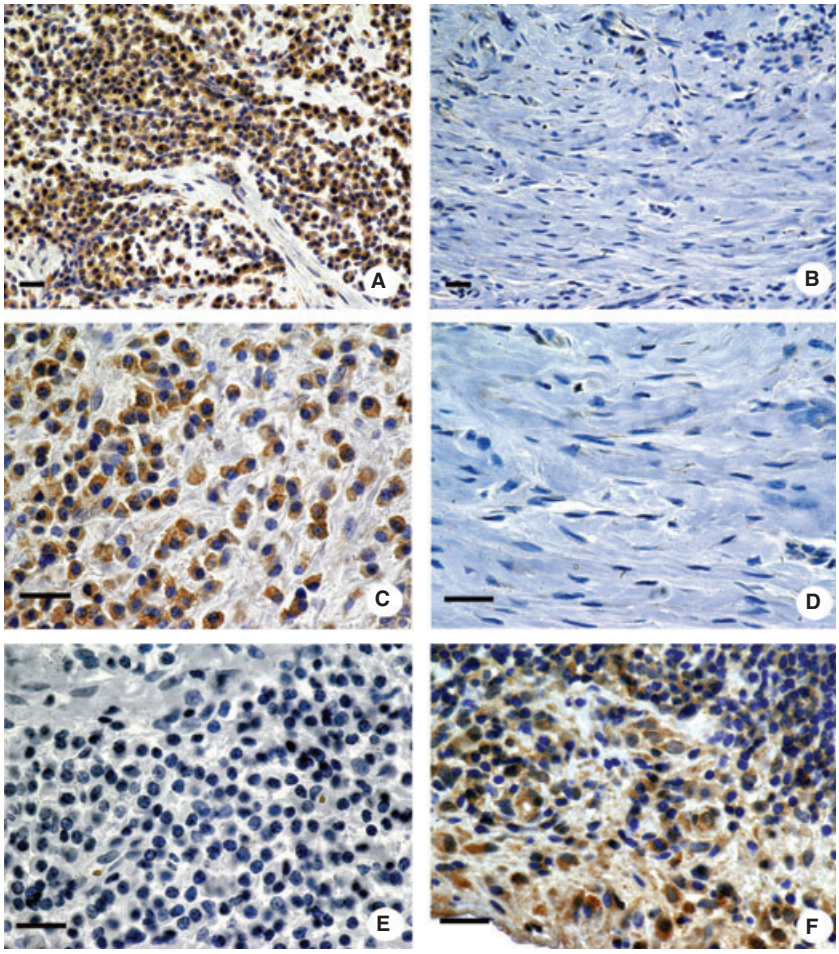

Fig. 5. Distribution of fibroblast growth factor-inducible 14 protein (Fn14) in periodontal tissues. (A) Periodontitis tissue (200 × magnification); (B) healthy periodontal tissue (200 ×); (C) periodontitis tissue (400 ×); (D) healthy tissue (400 ×); (E) negative control (400 ×); (F) positive control rheumatoid arthritis tissue (400 ×).

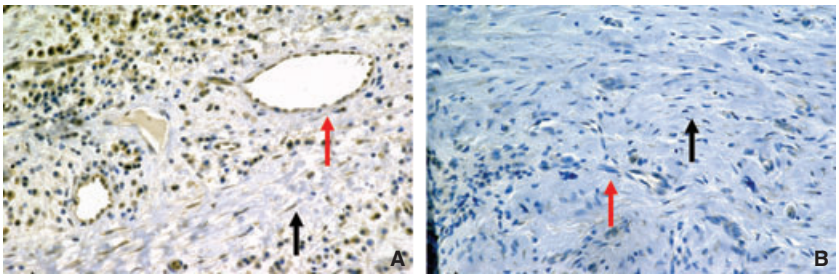

Fig. 6. Fibroblast growth factor-inducible 14 protein (Fn14) expression in cells lining blood vessels (red arrow) and fibroblasts (black arrow) in (A) periodontitis tissue (200 ×) and (B) healthy tissues (200 ×).

Table 3. Distribution of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and fibroblast growth factor-inducible 14 protein (Fn14) semiquantitative analysis (SQA) scores in normal and periodontitis tissues according to severity of bone loss, pocket depth and inflammation score

		SQA TWEAK (n = 33)					SQA Fn14 (n = 34)				
	Category	0	1	2	3	4	0	1	2	3	4
Bone loss score	0	5	1	1	–	–	3	2	1	1	–
	1	1	–	–	–	–	–	–	1	–	–
	2	–	–	2	1	–	–	1	–	3	–
	3	3	3	1	13	2	2	4	4	5	7
Pocket depth score	1	5	1	1	–	–	3	2	1	1	–
	2	1	–	–	1	–	–	–	1	–	1
	3	–	1	2	6	1	–	2	3	3	3
	4	3	2	1	7	1	2	3	1	5	3
Inflammation score	0	2	–	–	–	–	1	1	–	–	–
	1	3	1	1	1	–	3	2	–	1	–
	2	2	1	1	2	–	1	–	2	3	–
	3	2	1	2	6	1	–	4	3	2	3
	4	–	1	–	5	1	–	–	1	3	4

Table 4. Assessment of correlation between tumor necrosis factor-like weak inducer of apoptosis (TWEAK)/fibroblast growth factor-inducible 14 protein (Fn14) and inflammation, bone loss and pocket depth in biopsies from normal and periodontitis tissues

	TWEAK (n = 33)		Fn14 (n = 34)	
	r-value	p-value	r-value	p-value
Inflammation	0.494	0.001*	0.530	0.0003*
Bone loss	0.591	0.001*	0.341	0.022*
Pocket depth	0.248	0.085	0.237	0.085

*Significant correlation at $p < 0.05$.

(Kendall's tau_b test; $p = 0.001$ and $r = 0.494$) and Fn14 expression (Kendall's tau_b test; $p = 0.0001$ and $r = 0.530$). TWEAK SQA correlated better with bone loss than Fn14 SQA. Fn14 SQA has a better correlation with the inflammatory score than TWEAK SQA. No correlation was noted between TWEAK/Fn14 expression and pocket depth.

TWEAK and Fn14 mRNA

The results for real-time PCR evaluation of the expression of TWEAK and Fn14 mRNA in healthy and inflamed periodontal tissues are shown in Fig. 7. For both healthy and inflamed tissues, detectable levels of mRNA for both TWEAK and Fn14 were noted. The expression of Fn14 mRNA was significantly increased in the inflamed tissues ($p < 0.05$). TWEAK mRNA expression, while slightly increased in inflamed tissues, was not significantly different when compared to expression in healthy tissues.

Discussion

The present study demonstrated that periodontal tissues with high inflammatory scores had high levels of TWEAK and Fn14 protein expression, and this was mirrored by an increase in the mRNA levels for these two genes. Given the well-documented role of proinflammatory cytokines in the initiation and progression of periodontitis (41–44), we propose that the proinflammatory effect of TWEAK/Fn14 signalling might be an additional factor contributing to the tissue destruction in periodontitis. The correlation between TWEAK and Fn14 expression and inflammatory score in the periodontitis samples indicates that TWEAK and Fn14 are expressed by infiltrating inflammatory cells rather than by resident cell populations. This is in contrast to the findings in healthy samples wherein a low inflammatory score correlated with low TWEAK and Fn14 SQA scores. This study supports the findings of a previous study, which reported expression of Fn14 by mononuclear cells and fibroblasts in periodontitis tissue (24), and importantly by multinucleated cells and endothelial cells. These observations suggest a possible role for TWEAK/Fn14 signalling in periodontal destruction.

TWEAK is known for its multiple biologic effects, which are mediated via the Fn14 receptor and nuclear factor-kappaB signaling pathway (13,45–47). Signaling pathways other than that of nuclear factor-kappaB, such as MAPK, ERK1/2, JNK 1/2 and p38 also mediate TWEAK/Fn14 interactions (14,17,24,48). The proinflammatory role of TWEAK has been investigated in chronic immunoinflammatory and autoimmune diseases, such as rheumatoid arthritis, atherosclerosis, systemic lupus erythematosus and multiple sclerosis (22,23,26,49–51). The activities of TWEAK include

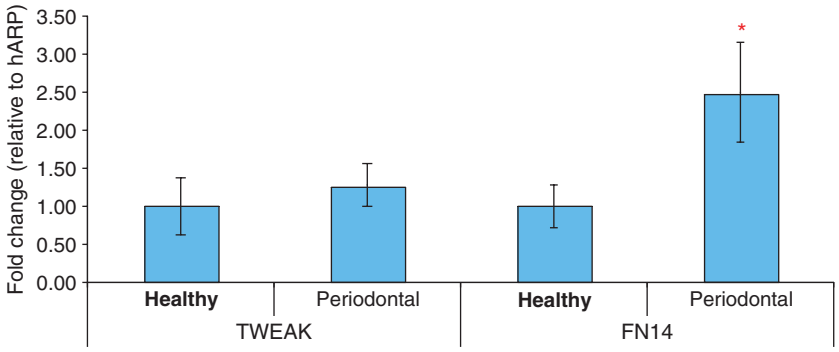

Fig. 7. Expression of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) and fibroblast growth factor-inducible 14 protein (Fn14) mRNA in healthy tissues and in inflamed periodontal tissues. *Represents $p < 0.05$. human acidic ribosomal protein P0.

induction of proinflammatory molecules, such as cytokines [interleukin (IL)-6, interferon-inducible protein-10 (IP-10)] chemokines [IL-8, monocyte chemotactic protein-1 (MCP-1)], prostaglandin E_2 and MMPs (5,13,15,22,23). IL-1beta and TNF are also known to potentiate the proinflammatory actions of the TWEAK/Fn14 pathway by up-regulating the expression of Fn14 (22,24).

TWEAK/Fn14 is known to affect endothelial cells in multiple ways. These include endothelial cell proliferation, migration, capillary formation, promoting cell survival, increasing the expression of intercellular adhesion molecule-1 and E-selectin, and inducing the secretion of proinflammatory cytokines (10,13,14,20). In this study, endothelial cells were shown to express TWEAK and Fn14 in periodontitis tissue and to express TWEAK and Fn14 to a lesser extent in healthy tissue. Any impediment in the crucial process of angiogenesis might be a pathway by which TWEAK/Fn14 signalling can contribute to the pathogenesis of periodontitis by interfering with repair and regeneration.

TWEAK also has an inhibitory effect on the differentiation of osteoblasts (17,52). This may be effected through the up-regulation of RANKL expression on osteoblasts and the induction of chemokine release from osteoblasts such as regulated on activation normal T-cell expressed and secreted (RANTES) (52). Overall, the current evidence supports the notion that TWEAK can contribute to pathologic bone loss by interfering with osteoblast differentiation. Some evidence suggests that TWEAK also affects the differentiation of osteoclasts (25), although this latter pathway is controversial because it calls into play a TWEAK receptor other than Fn14, a finding not supported by a comprehensive screen of TNF ligand and receptor family interactions in both mice and humans (53). Furthermore, research in our laboratories has found no direct effect of TWEAK on either mouse or human osteoclastogenesis (G. J. Atkins and D. R. Haynes, unpublished data). Despite this, current evidence, including the results presented here that TWEAK/Fn14 is expressed by multinucleated cells, supports our proposal that TWEAK/Fn14 may play a role in osteoclast activation and associated bone destruction, as is seen in periodontitis (55).

The observation that the expression of TWEAK/Fn14 correlated with inflammation and bone loss, but not pocket depth, is of particular interest. Current opinion indicates that treatment planning in periodontics should be less reliant on clinical features such as pocket depth, tooth mobility, occlusion and mucogingival defects and should be more focused towards an understanding of the etiology and pathogenesis of the disease (54). In this context, molecular factors that correlate strongly with pathologic features of periodontitis, such as inflammation and bone loss, may be of considerable significance.

In conclusion, there is accruing evidence demonstrating that TWEAK/Fn14 signaling contributes to chronic inflammatory conditions via its proinflammatory actions, modulation of the immune response, angiogenesis and stimulation of apoptosis. Its contribution to pathologic bone destruction is also documented. The present study has demonstrated a higher expression of TWEAK and Fn14 in inflamed periodontal tissues compared with clinically healthy periodontal tissues. This suggests that TWEAK/Fn14 signaling might be an additional player in the pathogenesis of periodontitis.

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