

**Immunohistochemistry study on expression of
Tumor Necrosis Factor Like Weak Inducer of
Apoptosis (TWEAK) and its receptor FN14 in
normal and periodontitis tissues**

**A report submitted to the University of Adelaide in partial
fulfilment of the requirements of the Degree of Doctor of
Clinical Dentistry (Periodontology)**

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Declaration

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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1.1 Abstract

Periodontitis is a chronic inflammatory disease wherein microbial factors induce complex inflammatory and immune responses in a susceptible host. In periodontitis host-derived enzymes, cytokines and other proinflammatory mediators play an integral role in the destruction of tooth supporting structures and alveolar bone. TWEAK (TNF-like weak inducer of apoptosis), one of the members of the TNF superfamily, has recently been identified as an important inflammatory mediator. Fn14 (fibroblast growth factor-inducible 14) protein/TWEAKR has been identified as the cell surface receptor for TWEAK. TWEAK/Fn14 signaling results in multiple biologic effects including induction of inflammatory cytokines, modulating immune response angiogenesis and stimulation of apoptosis. TWEAK has also been shown to promote osteoclastic differentiation of cells from the monocyte/macrophage lineage. Expression of TWEAK and its receptor Fn14 is elevated in tissues and cells cultured from a number of chronic inflammatory diseases, such as rheumatoid arthritis, atherosclerosis, inflammatory skin, kidney and airway diseases. This review considers the biology of TWEAK and its receptor Fn14 in periodontitis.

2.1 Abstract

Background: Periodontitis is a chronic inflammatory disease wherein microbial factors induce complex inflammatory and immune responses in a susceptible host. In periodontitis host derived enzymes, cytokines and other proinflammatory mediators play an integral role in the destruction of tooth supporting structures and alveolar bone. TWEAK (TNF-like weak inducer of apoptosis) is one of the newest members of the TNF superfamily to be identified. Fibroblast growth factor-inducible 14 (Fn14) protein/TWEAKR has been identified as the cell surface receptor for TWEAK. TWEAK/Fn14 signaling results in multiple biologic effects including induction of inflammatory cytokines, modulating immune response angiogenesis and stimulation of apoptosis. Recently, TWEAK has also been shown to promote osteoclastic differentiation of cells from the monocyte/macrophage lineage. Expression of TWEAK and its receptor Fn14, is elevated in tissues and cells cultured from a number of chronic inflammatory diseases such as rheumatoid arthritis, atherosclerosis, inflammatory skin, kidney and airway diseases. Accordingly, we hypothesised that the expression of TWEAK and Fn14/TWEAKR will be increased in tissue samples from periodontitis patients.

Aim: The aim of this study was to investigate the expression of TWEAK and its receptor Fn14, in gingival biopsies from periodontitis patients and clinically normal patients using immunohistochemistry techniques.

Materials and Methods: The study included 27 patients (18 females and 9 males, aged 30-77 years, mean age = 55.2 years) with generalised chronic and aggressive periodontitis. The gingival biopsy sites in the chronic and aggressive periodontitis group had clinical probing pocket depths and clinical attachment loss greater than 5 mm with radiographic evidence of bone loss ranging from 50-90% of the root length. The non-periodontitis tissue samples consisted of gingival tissue resected from seven patients (4 males and 3 females, aged 23-70 years at the time of surgery; mean age = 45.5 years) undergoing crown lengthening surgery at sites not affected by periodontitis. Using monoclonal antibodies, the expression of TWEAK and its receptor Fn14 was investigated by immunohistochemistry in formalin-fixed paraffin embedded tissues. The specimens were evaluated by a semiquantitative analysis (SQA). The Mann Whitney U-test was used to compare mean rank of SQA between two groups and Kendall's tau_b test was used to determine correlations between different parameters.

Results: Semiquantitative analyses demonstrated that the expression of TWEAK protein was significantly higher in periodontitis tissue compared to healthy tissue (Mann Whitney U-test, p value 0.002). Similarly, in comparison with healthy tissue, periodontitis-affected tissues expressed significantly higher Fn14 protein (Mann Whitney U-test, p value 0.013). A strong positive correlation was found between TWEAK and Fn14 expression (Kendall's tau_b test; p value 0.007 and r value 0.395). In periodontitis-affected tissue specimens, TWEAK and Fn14 protein was mainly expressed by mononuclear leukocytes (morphologically resembling lymphocytes and plasma cells), cells lining blood vessels, spindle shaped cells resembling fibroblasts and multinucleated cells.

Conclusion: This study demonstrates that there is a higher expression of TWEAK and Fn14 protein in periodontitis tissues as compared to clinically normal controls. This suggests that TWEAK /Fn14 signaling could be an additional player in the pathogenesis of periodontitis and adds to the increasing number of cytokine networks involved in periodontal inflammation.