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Review Article

Trefoil Factors & Oral Tissues: An Unexplored Link

Dr.H.S.Grover[1], Dr.Prateek Gupta[2], Dr.Amit Bhardwaj[3], Dr. Neha Saksena[2], Dr. Narender Yadav[2]

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Affiliation:

1. Professor and Head, Department of Periodontology, Faculty of Dental Sciences, SGT University, Budhera, Gurgaon, Haryana, India
2. Post Graduate Student, Department of Periodontology, Faculty of Dental Sciences, SGT University, Budhera, Gurgaon, Haryana, India
3. Reader, Department of Periodontology, Faculty of Dental Sciences, SGT University, Budhera, Gurgaon, Haryana, India

Corresponding author*

Dr. Prateek Gupta, Post Graduate Student, Department of Periodontology, Faculty of Dental Sciences, SGT University, Budhera, Gurgaon.

E-mail- prateek.22.88@gmail.com

ABSTRACT

Trefoil factors (TFFs) are secreted molecules - detected in saliva and oral tissues, which are involved in cytoprotection against tissue damage and the immune response. They interfere with crucial biological processes such as cell proliferation, differentiation, apoptosis and angiogenesis. The objective of this review is to summarize and discuss the mammalian trefoil factors and their relationship with the oral tissues.

Keywords: Trefoil factors, oral tissues, saliva, oral squamous cell carcinoma, periodontal disease.

INTRODUCTION

Trefoil peptides entered into the scientific stage more than 30 years ago when in the early 1980s, Jorgensen and co-workers reported their PSP (pancreatic spasmolytic peptide) and Westley and Rochefort published an unknown 46-kD estrogen-inducible protein for which Masiakowski isolated and characterized the corresponding complementary DNA (cDNA) sequence (pS2).[1,2,3]Due to the different laboratories and experimental methods, a heterogeneous collection of names were introduced for the three mammalian genes and their peptides.[4]

Trefoil factors are expressed and secreted by epithelial cells that line mucus membranes. They are therefore usually expressed in association with mucins, the major protein component of a mucus gel. They are frequently referred to as gastrointestinal factors, thus expressed widely, notably in the bronchial and urogenitary tracts.

The mammalian trefoil factor family (TFF) contains three members; TFF1, TFF2 and TFF3. Individual trefoil factors are expressed by different cells, for instance TFF1 is expressed principally by the superficial cells of the gastric mucosa, whereas TFF2 expression is largely restricted to cells of the basal gastric glands and in Brunner's glands of the duodenum. Trefoil factors are also expressed, sometimes at high levels and sometimes apparently ectopically, in many human adenocarcinomas.[5,6]

The "Trefoil Factor Family (TFF) domain" represents a unique cysteine-rich shuffled module found in a cluster of

secretory peptides as well as in certain mosaic proteins. The amino acid sequence of the first member, human TFF1 was deduced in 1984.[7,8,9]The primary structure of porcine TFF2 (formerly: [pancreatic] spasmolytic polypeptide/[P]SP) was reported in 1985. The high similarity between TFF1 and TFF2 was first recognized 1988 while analyzing the TFF domains from the frog integumentary mucin FIM-A.1 (formerly: spasmolysin)[10,11]TFF3 (formerly: intestinal trefoil factor/ITF/P1.B) is the latest mammalian member of this family detected in 1991 in rats. In humans, the three corresponding TFF genes are clustered on chromosome 21q22.3. The situation in amphibia is much more complex because there are additional TFF peptides existing which have no known ortholog in mammals.[7,12,13]

STRUCTURE OF TFF

The TFF encompasses a group of low molecular weight, soluble proteins that share a common feature - a three-looped trefoil-like structure formed throughinter-chain disulphide bonding which is the basis for the extraordinary resistance of these peptides to hydrolysis and proteolysis.[14,15]Their structure allows them to form dimers, either with themselves or with other trefoil proteins. Homo- or heterodimers of TFFs show different effects through different activation status.[16,17] All three TFF genes are clustered in a tandemly oriented fashion at the genomic region 21q22.3 and have a very similar exonic structure. Moreover, the 5′-untranslated region of all TFFsis very similar, sharing several regulatory sequence motifs, thus suggesting a common or concerted regulation. These motifs are consensus sequences for several transcription factors.[18,19]

EXPRESSION OF TFFs IN NORMAL TISSUE

In normal human tissues, TFFs are mainly expressed in gastrointestinal epithelial cells, where they are co-packaged in the Golgi apparatus into mucus granules and secreted with mucins into the protective layer covering the mucosa. [20,21,22]

The main site of expression of TFF1 is the stomach, where it is abundantly expressed in the superficial and foveolar epithelium.[20,23,24,25,26]A high level of expression of TFF1 is described in upper ducts and surface cells of Brunner's glands in the duodenum. The small intestine generally appears not to express TFF1, although some staining on the tips of villi in the ileum and jejunum has been reported.[27,28]In the normal large intestine, TFF1 expression is demonstrated in some goblet cells, particularly in the distal region. In the pancreas only a few cells in large ducts appear positive, and in gall bladder some patchy epithelial expression has been described.[29,30,31]Outside of the gastrointestinal tract, TFF1 expression has been observed in the respiratory epithelium and focally in duct luminal cells of normal breast.[32,33]

The expression of TFF2 appears to be highly correlated with that of TFF1 in terms of organ specificity. In normal gastric mucosa, TFF2 expression is observed in mucous glands of body and antrum. In duodenum, TFF2 expression is present in Brunner's glands acini and distal ducts. Some focal expression is also observed in duct epithelium of pancreas and in gall bladder epithelium.[20,26,28,31]

Unlike TFF1 and TFF2, TFF3 is majorly expressed in the intestine - goblet cells, in gland acini and distal ducts of Brunner's glands. In contrast to the apparent gastrointestinal specificity of TFF1 and TFF2, TFF3 expression is observed in human uterus, normal breast, some regions of the hypothalamus and in the pituitary gland. Together with TFF1 it is also present in the respiratory epithelium.[19,32]

BIOLOGICAL EFFECTS

Interaction with mucins

TFF domains are integral constituents of certain frog skin mucins and TFF peptides are typical components of the protective mucous gels where they probably interact with mucins. This mucus is often a laminated structure composed of alternating types of mucin/TFF peptide layers, e.g. in the stomach.[7,8,34]

Direct interaction has been reported between TFF1 and the C-termini of mouse Muc2 and Muc5AC clearly indicating a protein-protein interaction, but does not exclude the

possibility of additional protein-sugar interactions.[35]

Furthermore, TFFs affect the viscoelastic properties of mucin preparations. Interaction of TFFs and mucins might also play a role in the packaging and secretion of mucins because TFF1 deficiency leads to accumulation of misfolded proteins in the endoplasmic reticulum.[13,36,37]

Modulation of wound healing, inflammation, differentiation, and cancer progression

All TFF peptides were shown to enhance migration of various cells in vitro ("motogenic effect"). Furthermore, TFF peptides reduce cell–cell and cell–matrix interactions and enhance cell scattering leading to the rapid repair of mucous epithelia by cell migration, a process called "restitution".[7]

Inflammatory processes often accompany mucosal repair. Here, TFFs acts as natural modulators since they are able to enhance tumor necrosis factor-alpha induced interleukin-6 (IL-6) and IL-8 secretion.[38]

Furthermore, the motogenic pathway would be ideally supported by the anti-apoptotic effect of TFF peptides as well as by the ability of TFFs to repress cell cycle progression from G1 towards the S phase. The latter effect is expected to regulate cell differentiation processes (for example, during continuous regeneration of mucous epithelia from stem cells). Dysregulation of this sensitive equilibrium may easily contribute to cancer progression, which would be further enhanced by the pro-angiogenic activity of TFF peptides.[13,39,40]

TFF peptides also play a major role in wound healing responses as well as in pathological processes since they are ectopically expressed in response to mucosal damage as well as during a wide range of chronic inflammatory diseases, various types of metaplasia, and also many tumors. A unique glandular structure - the ulcer-associated cell lineage (UACL) is a hallmark of various chronic inflammatory conditions and is a prominent site of synthesis for all three TFFs as well as epidermal growth factor (EGF). This points to a natural synergy of TFF peptides and EGF for mucosal repair because the UACL is thought to play an important role in ulcer healing.[6]

Neural effects

Besides their synthesis in mucous epithelia, TFFs are also neuropeptides. TFF1 appears to be expressed by glial cells; whereas TFF3 is of neuronal origin. TFF3 is a neuropeptide of oxytocinergic neurons of the hypothalamus and is now also reported to induce Fos expression in these neurons. Furthermore, it showed fear-modulating activities when injected into the rat amygdala. However, the major neural expression site of murine TFF3 is the cerebellum which is developmentally regulated. Consequently, TFF3 would be well suited to support the complex migration and differentiation program of the developing cerebellum.[7,8,13]

PHYSICOCHEMICAL PROPERTIES OF TREFOIL FACTORS

The physicochemical properties of trefoil factors have been studied mainly with the human molecules. Clearly the amino acid residue substitutions between different orthologues of the individual trefoil factors will affect the chemical masses, charges and hydrodynamic properties of the molecules, but it is anticipated that the principal findings of the studies with the human trefoil factors will be applicable to trefoil factors from other species.[41]

Hydrodynamic properties

TFF2 varies from the dimeric forms of TFF1 and TFF3. It has two domains that are linked by a disulphide bond, between Cys6 to Cys104, and additionally by a peptide chain from Leu50 to Asp56.TFF2 is expected to be more compact than the dimer forms of TFF1 and TFF3 because of this additional link between the two domains.[41]

Overall charge

Comparison of the theoretical isoelectric points of human TFF1 and TFF3 monomers shows that they are both acidic.[41,42]

Charge distribution

Greater numbers of acidic residues of TFF1 are present in the termini than in the trefoil domain, whereas TFF3 has the same

number of acidic residues in both areas. The trefoil domain area of TFF3 has more charged residues which are basic in nature than in TFF1.Thus, TFF1 has a greater number of acidic residues than TFF3 and the additional acidic residues are outside the trefoil domain. The different orientations of the side chains of the charged amino acid residues in the molecules exacerbate the greater polarization of TFF1.[42]

Human TFF2 has not been studied in detail, but there is at least one basic residue in each of the amino-terminus, the link region and the carboxy-terminus, which suggests that the uneven distribution of charged residues characteristic of human TFF1 and to a lesser extent human TFF3 is not an important feature of human TFF2 function.[41]

Susceptibility to proteolytic degradation

The protease stability of porcine TFF2 has been investigated by incubation with trypsin, which cleaves after basic residues, and chymotrypsin, which has a broader specificity. Using 2% (w/w) trypsin or 0.5% (w/w) chymotrypsin and incubation at neutral pH for up to 1 h, no degradation was observed, as judged by isoelectric focusing, immunoreactivity and N-terminal sequence analysis.[43]

Similar results have been obtained for rat and human TFF3 incubated with carboxypeptidase, trypsin, chymotrypsin and pepsin. Interestingly, rat but not human TFF3 seems to be degraded by a cocktail of mixed bacterial proteases.[41]

TREFOIL FACTORS AND ORAL CORRELATIONS

In Oral Keratinocytes

Trefoil factor family 3 mRNA was detected by real-time qPCR in keratinocyte cultures from five out of eight donors examined. The mRNA levels were lower in keratinocyte cultures than in Caco-2 intestinal epithelial cells, and the levels of expression of mRNA varied between the different donors. Expression of TFF3 peptide was detected by western blotting of extracts from three cultures of normal oral keratinocytes. In biopsies taken from oral mucosa, the stratified epithelium showed weak staining. In some specimens, there was a tendency for a gradient in the expression, with the strongest staining occurring in the basal and spinous layers, but this was not consistent among the biopsies studied. Many of the nuclei in the basal cell layers of the epithelium displayed nuclei with stained spots. [44]

In Salivary Secretions

TFF3 peptide was detected by western blotting in secretions from all glands. Only one out of five donors displayed detectable amounts of TFF3 in the parotid gland. All mixed secretions from the submandibular and the sublingual glands contained TFF3.[45]

Four of five donors displayed detectable amounts of TFF3 in samples from the minor salivary glands. Specificity of the primary antibody was determined by competitive inhibition with denatured TFF3. Incubation of antibody and denatured TFF3 peptide overnight, and subsequent use of the solution as primary antibody, eliminated the reactive bands of 13 and 7 kDa.[44]

In Periodontal diseases

Studies have demonstrated reduction of TFF3 expression in the saliva and gingival tissues of patients with Chronic Periodontitis. Salivary TFF3 concentration levels were negatively correlated with levels of P. gingivalis and T. forsythia and periodontal pathology. However, the interaction among biologic functions of TFF3, periodontopathic bacterial burden, and development of periodontal diseases is yet to be researched thoroughly. Thus, additional investigations on immune responses of gingival epithelial cells to these pathogens, signaling transduction that regulates TFF expression and mechanisms at the molecular level which regulate TFF3 in periodontal diseases would be of importance.[46]

In Oral Cancer

Reports of altered expression of TFF peptides in patients with various cancers have been seen. Some studies have provided additional information of reduced TFF2 and TFF3 expression in oral mucosal tissues from patients with Oral Squamous Cell Carcinoma (OSCC). Further investigations at

a molecular level are needed to clarify mechanisms regulating TFF expression in the oral compartment and the role of TFFs in the development of oral cancer in order to determine their clinical significance in OSCC.[47]

CONCLUSION

Sufficient data has been collected on the role of TFFs in mucosal protection, but the answers gained have often raised more questions that need to be resolved. This small and enigmatic family of three-leaved proteins has much left to tell us. The upcoming new data shows their involvement in the immune response and the likely physiological interaction with specific binding proteins. Thus, we are still at the beginning of a long and interesting scientific journey in deciphering the biology of the trefoil factors.

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