Review article

Trefoil Factor Family (TFF): Peptides with Numerous Functions

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Abstract

The trefoil factor family (TFF) consists of a group of small peptides and is highly expressed in tissue that contain mucus-producing cells, predominantly in the mucosa that lines the gastrointestinal tract. Those peptides, which are highly important for epithelial restitution, may act in ways other than using the usual factors responsible for restitution. It was observed that several mechanisms are involved in the TFFs' promotion of restitution. In addition to that, peptides have other functions as well, e.g. they interact with the immune system. Although the TFFs' therapeutic effects have been studied, it is uncertain which of the TFFs' in vitro properties are directly involved when it comes to their in vivo engagement. Observing mice with genetic deletion of TFF peptides can help us discover the function of the peptides that could be indicated by the deletion of the target protein or by adaptive regulation of some other protein that is affected by the deleted gene product. At the very least, a subset of functional networks controlled by a TFF isoform and its downstream effectors can be identified by observing such mice. The discoveries related to the signaling mechanisms of the TFF family leave much to discover about the distinct and shared pathways among those protective peptides.

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Biological Properties of TFF

The trefoil factor family (TFF) includes a group of small peptides, the first member of which was discovered about thirty years ago (1). TFF1 (formerly known as breast cancer-associated peptide pS2), TFF2 (formerly spasmolytic polypeptide SP) and TFF3 (formerly intestinal trefoil factor) are three members of the trefoil factor family (TFF) known in mammals. These three proteins are small compact peptides that have one or two trefoil domains. While TFF1 and TFF3 contain only one trefoil domain, TFF2 contains two trefoil domains. The basic elements of a trefoil domain are 42–43 amino-acid residues. Six cysteine residues form three disulfide bonds, creating a characteristic three-leafed structure (2).

There are several studies that suggest that TFFs can be regulated by cytokines and transcription factors (especially NF-kB) related to the immune system and that TFFs can regulate them in return, but there is also data suggesting otherwise (3-6).

TFFs have been extensively studied in vivo and in vitro, with most data suggesting that these small peptides improve epithelial repair in the gastrointestinal (GI) tract and other body systems (7).

TFF's Role in Mucosal Protection and Repair

Due to their compact structure, TFFs are relatively resistant to proteolytic degradation in the stomach and small intestine (8). TFFs can mostly be found as secreted molecules in the mucus covering normal epithelium. The predominant site of TFF synthesis is the mucin-producing or goblet cells dispersed in epithelia. All three TFFs are expressed in the stomach, being localized to the surface gastric mucosal cells (9). Their mRNA has been found in the brain, lungs, trachea, thyroid gland, salivary glands, prostate, uterus and other organs (10). The genes that code them are located on chromosome 21 (11).

Despite the fact that they have been found in almost all tissue containing mucus-secreting cells, TFFs are predominantly expressed in the gastrointestinal tract. Considering their appearance in mucosal tissue, it can be concluded that their functioning might be related to that of mucins. Nevertheless, TFF2 expression is not so common and there is a possibility that different TFFs have different roles in the protection of the epithelium, which is corroborated by the complementary expression of TFFs in the GI tract and by the simultaneous occurrence of each of them with their unique mucin type (MUC); TFF1 appears with MUC5AC, TFF2 with MUC6, and TFF3 with MUC2 (12-14), although gastric and ocular co-localization of TFF3 with MUC5AC also occurs (15, 16). It is speculated that mucosal defense is improved by direct interaction of TFF peptides and mucins.

The role of TFF peptides in cell migration was observed in several studies (17-20), predominantly as a consequence of the response of a damaged epithelium that strives to restore its continuity. In cases of small discontinuity of the epithelium, where cell proliferation is not required, restitution of the epithelium takes place soon after the injury, with coordination of the removal of damaged cells and the migration of healthy epithelial cells into the injured location (Figure 1). The importance of efficient restitution from the physiological viewpoint is high, as the loss of fluids and electrolytes has to be stopped and the luminal antigens and bacteria have to be prevented from entering the tissue and immune cells of the host. Proliferation occurs instead of restitution when tissue is more severely damaged.

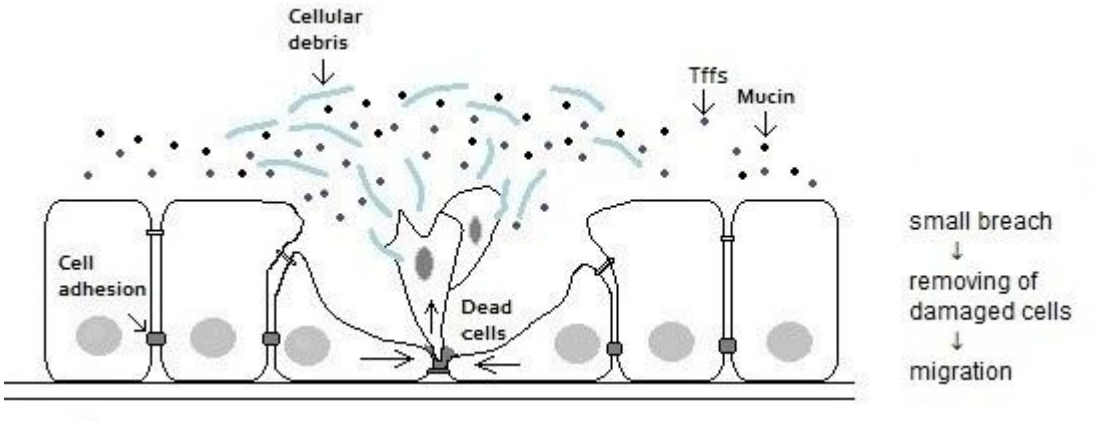

Figure 1. The role of TFF peptides in epithelial restitution

TFF and Energy Metabolism

The influence of trefoil factor proteins on energy metabolism can be observed in mice with TFF3 gene knockout (TFF3-/- mice). The TFF3 knockout mice have a different expression of miRNA associated with the glycolysis and gluconeogenesis metabolic pathways compared to wild-type mice. The TFF3 knockout mice have a significantly lower body weight compared to the wild type (21). A change in the body mass of mice did not occur in the research with increased expression of the TFF3 gene (22). The fatty changes in the liver of mice have been connected with the change of expression of the TFF3 gene (23). Research has shown that the TFF3 protein participates in glucose metabolism. Similarly, a study showed that hepatic TFF3 expression levels were lower in obese (ob/ob) and high-fat-diet-induced obese mice. Cellular glucose output in mice decreased as a consequence of overexpression of TFF3 in primary mouse hepatocytes, which inhibited the expression of gluconeogenic genes. Experiments using the glucose tolerance test and insulin tolerance test showed that adenovirus-mediated overexpression of TFF3 in diabetic or obese mice improved glucose tolerance and insulin sensitivity. The results also showed that TFF3 peptides are a factor in glucose homeostasis and insulin sensitivity. Consequently, it was concluded that said peptide might be a part of successful modern therapies aimed at metabolic disorders related to type 2 diabetes mellitus (24). Increasing concentrations of glucose and insulin treatment boosted the expression of TFF3 in intestinal epithelial cells. In addition to that, insulin treatment caused the upregulation of human sodium/glucose cotransporter 1 (hSGLT1), which additionally increased intracellular glucose levels. Downregulation of TFF3 was observed in diabetes mellitus type 1 patients, but the values were modified by insulin treatment. It was discovered that insulin signaling was important for the optimal expression of TFF3 in intestinal epithelial cells, as it elevates intracellular glucose levels and mediates gene expression (25).

Aberrant energy metabolism in the liver promotes insulin resistance, diabetes, and nonalcoholic fatty liver diseases (26). It was recently shown that liver triglyceride accumulation does not cause cellular injury in the liver; the primary causes of liver injury via increased oxidative stress are free fatty acids or their metabolites (27). Changes in lipid metabolism, especially the increase of saturated fatty acids, are associated with increased endoplasmic reticulum (ER) stress, oxidative stress and liver injury in the course of development of fatty liver disease (28). Sirtuin 1 (SIRT1) plays a key role in metabolic regulation, adaptation and oxidative stress. Acting as a nuclear metabolic sensor and deacetylating a wide range of targets, it leads to epigenetic modifications of histones and modulation of transcription factors or metabolic enzymes (29). In addition to SIRT1, peroxisome proliferator-

activated receptors (PPARs) also have an important role in cell metabolism (30).
In case of TFF3 deficiency, the profile and accumulation of fatty acids (FAs) in the liver are affected (Table 1), with no obvious oxidative stress increase, although the expression/activity of monitored enzymes changes, as does the level of SIRT1 and PPARg protein. Due to the strong downregulation of hepatic TFF3 in diabetic/obese mice, its presence in circulation and its regulation by food/insulin, TFF3 represents an interesting new candidate for research in metabolic relevant conditions (31).

Table 1. Fatty acids in liver of Tff3 -/- mice compared to wild type (elevated ↑, decreased ↓)

Fatty acids	Tff3 -/-
SATURATED	
C14:0 myristic acid	↓
C18:0 stearic acid	↑
C20:0 arachidic acid	↑
MONOUNSATURATED	
C16:1 palmitoleic acid (ω-9)	↓
C18:1 oleic acid (ω-9)	↓
C18:1 vaccenic acid (ω-7)	↓
C20:1 eicosenoic (gondoic acid) (ω-9)	↓
POLYUNSATURATED	
C20:2 eicosadienoic acid (ω-6)	↑
C20:4 (AA) arachidonic acid (ω-6)	↑
C18:3 (ALA) alpha linolenic (ω-3)	↓
C22:6 (DHA) docosahexaenoic (ω-3)	↑
RATIO ω-3/ω-6	↑

TFF's Participation in Defense Against Harmful Agents

Another role of the TFF3 protein is the defense of the organism against harmful agents. Mice that cannot synthesize enough TFF3 protein in their liver are deprived of the protective effect of the TFF3 protein in the serum after myocardial (32) and brain ischemia, which consequentially leads to greater tissue damage. Thus, in such mice, a significantly higher activity of caspase 3 and a higher level of cell death in the ischemic cerebral lesion were observed, together with a larger fraction of cerebral infarcts and a smaller fraction of injuries in the cerebral hemisphere, accompanied by more severe forelimb motor deficits. Since the mice were TFF3-deficient, recombinant TFF3 was administered intravenously and it reversed changes in cerebral injury and forelimb motor function, pointing at the existence of an endocrine neuroprotective mechanism that uses TFF3 from the liver in experimental cerebral ischemia/reperfusion injury (33). TFF3-/- mice have difficulties with regeneration of the mucous membrane of the gastrointestinal tract (34).

High-salt diet (HS) causes endothelial dysfunction and vitiates vascular reactivity to various stimuli. In a recent study, transgenic TFF3-/- mice were introduced as a new model, characterized by a favorable ratio of ω-6/ω-3 free fatty acids and modified metabolism of arachidonic acid (AA). The results showed that acute HS intake has a much smaller impact on FIR (flow-induced response) in TFF3-/- mice compared to the wild type (WT) (35). The study

showed that HS intake does not affect NO production in TFF3-/- mice (36).

According to another study, although the TFF3 peptide is not expressed in an intact corneal epithelium, its expression is extensively upregulated following an epithelial injury. In addition to that, corneal injuries in TFF3-/- mice take much more time to re-epithelialize compared to similar injuries in wild-type mice. In case of alkali-induced corneal wounds, external application of recombinant TFF3 to the wounds speeds up the in vivo and combined in vivo/in vitro model wound healing in both wild-type and TFF3 /- mice. This proves that TFF3 has a key role in the mechanism of corneal wound healing, which opens a possibility of creating new ways of coping with non-healing wounds (37).

TFF in the Respiratory System, Pregnancy and Tumorigenesis

A study (38) describing a murine asthma model found that trans-differentiating Clara cells specifically express TFF1 which is stored in a specific subset of secretory granules. This is proof that TFF1 is an autocrine factor for the trans-differentiation of Clara cells into goblet cells. Another study (39) showed that TFFs play such a role in the differentiation of the airways as well, showing the induction of TFF3 synthesis with the differentiation in in vivo humanized tracheal xenograft and in vitro air-liquid interface culture models. In addition to that, exogenous TFF3 promoted differentiation of ciliated cells in an EGF-receptor-dependent manner. Both studies implied that TFFs may have important roles in different processes of differentiation of airways, making them promising new targets in treatment of severe chronic and acute airway diseases.

Dynamic changes of trefoil factor proteins in a pregnant woman's serum point at their importance in embryogenesis (40). The presence of the TFF3 protein in the cartilage of mice fetuses during endochondral ossification has been identified, while the exclusion of the TFF3 gene causes changes to the histomorphological structure of cancellous bone, as well as hearing disorders and accelerated presbycusis, which indicates that it has a role in morphogenesis of organs (41-43).

The TFF3 gene participates in the proliferation of pancreatic cells – decreased expression of the TFF3 gene leads to decreased proliferation of pancreatic -cells, while increased expression leads to increased proliferation of pancreatic -cells, having no influence on their function (44). The TFF3 protein is also related to angiogenesis, which makes it an important factor in tumor pathogenesis (45). Research has identified increased expression of the TFF3 gene in gastrointestinal and lung tumors, advanced prostate cancer, hepatocellular carcinoma and other tumors (46-49). Expression of the TFF3 gene has a predictive role in breast tumors (50) and is simultaneously identified as a valuable and easily detected biomarker in screening for stomach cancers. Moreover, serum TFF3 might predict gastric cancer more efficiently than the PG test, while the combined testing of serum PG (pepsinogen test) and TFF3 could make gastric cancer screening even more efficient (51).

Conclusions

Despite the fact that not much is known about the TFF signaling pathways, some straightforward benefits of TFF peptides for healthy and damaged tissue have been discovered. TFFs are pivotal for mucosal protection and repair of epithelial surfaces, and they also have a role in cancer development and progression. Trefoil factors can be used as prognostic markers for different types of carcinoma. However, their biological effects are still unknown. Considering that there are not many studies on the influence of the TFF peptides on vascular reactivity, it would be interesting to find out more about their role in it.

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