Cell. Mol. Life Sci. 62 (2005) 2916–2920
1420-682X/05/242916-5
DOI 10.1007/s00018-005-5479-3

Cellular and Molecular Life Sciences

Pleiotropic effects of Trefoil Factor 1 deficiency

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Online First 5 December 2005

Abstract. Trefoil Factor 1 (TFF1), the first member of the trefoil factor family, is normally expressed in the stomach mucosa. Ectopic expression is also observed in various human pathological conditions, notably in numerous carcinomas and gastrointestinal acute inflammatory disorders. In vivo experimental data using TFF1-deficient mice highlight the pleiotropic functions of TFF1: (i) it is a gastric tumor suppressor gene involved in gastric ontogenesis and homeostasis; (ii) it protects gut mucosa from aggression; (iii) it participates in folding secreted proteins inside the endoplasmic reticulum. At the cellular level, it limits cell proliferation and apoptosis, and favors cell differentiation. Collectively, these data suggest that TFF1 may provide an alternative pharmacological tool for the prevention and treatment of human gastrointestinal diseases.

Key words. TFF1; pS2; gastrointestinal tract; ontogenesis; inflammation; homeostasis; tumor suppressor; cell differentiation; TFF1 deficiency.

Introduction

Trefoil Factor 1 (TFF1, previously named pS2) is a small secreted protein that was first identified in the human breast cancer cell line MCF7 [1]. It belongs to the TFF protein family, characterized by a clover leaf-like disulphide structure named the TFF domain [2–4]. In mammals, two other TFFs have been identified: TFF2 (Trefoil Factor 2/SP/Spasmolytic Polypeptide) and TFF3 (Trefoil Factor 3/ITF/Intestinal Trefoil Factor). There is a syntenic conservation between human and mouse TFF genes that form a cluster (TFF1-TFF2-TFF3 order) on chromosome 21 [5] and 17 [6], respectively. TFF1 gene and protein structure and regulation have been previously reviewed [3].

In man and mouse, TFF1 is expressed from the body to the pyloric sphincter of the stomach mucosa (fig 1A). In addition, TFF1 is also expressed by the pancreas in the mouse. In the stomach body, TFF1 is located in epithelial cells of the upper part of the pits, an area where cells undergo commitment and differentiation to give rise to a functional secreting mucosa. TFF1 is secreted in the gastric juice. It is observed in the cytoplasm with a preferential perinuclear accumulation, a subcellular localization appropriate for a secreted protein [7].

Abnormal TFF1 expression occurs in acute inflammatory disorders of the entire human gastrointestinal tract such as duodenal ulceration or Crohn disease at the time of tissue renewal, in cells of regenerating tissues surrounding the areas of damage. Thus, TFF1 is involved in the protection and repair of gastrointestinal mucosa integrity [8, 9]. Consistently, transgenic mice overexpressing human TFF1 in their jejunum show a lower rate of induced ulceration [10].

TFF1 is also ectopically expressed in most human carcinomas, including mammary gland, pancreas, large bowel, biliary tract, lung, oesophagus, bladder, prostate and mucinous-subtype ovarian tumors, as well as their associated metastases [3]. In all TFF1-positive carcinomas, the protein is expressed in the cytoplasm of the malignant epithelial cells, as it is in normal stomach cells.

The clinical relevance of TFF1 has been established for breast carcinomas. About 50 % of breast primary tumors express TFF1. It is also found in lymph node and distal

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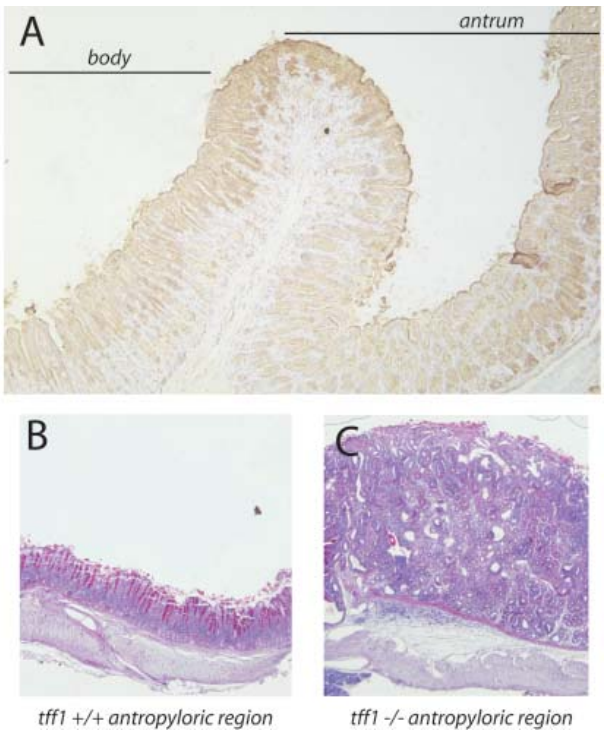

Figure 1. TFF1 is expressed in the gastric mucosa and is essential for the ontogeny of the antro-pyloric region. (*A*) TFF1 immunostaining of a gastric section spanning the body and the antral regions of the stomach from a wild-type mouse. In the body region TFF1 is mainly expressed by pit cells, while in the antrum it is expressed both by pit and gland cells. (*B* and *C*) Histological analysis of the antro-pyloric region of a wild-type mouse and a TFF1-deficient mouse, respectively. Images were taken at the same magnification. In *C*, note the high-grade dysplasia of the epithelial cells lining the pits.

metastases. TFF1 is a factor of good prognosis, being estradiol-regulated via an estrogen-responsive element present in its promoter [11]. Accordingly, the presence of TFF1 is significantly associated with a positive response to hormone therapy. Thus, TFF1 is useful in the characterization of human breast tumors and the identification of patients likely to respond to endocrine therapy [3]. Finally, circulating TFF1 might be useful for patient follow-up [12].

TFF1 has been proposed to be a growth factor or an oncogene since it is widely expressed in malignant tissues which are normally TFF1-negative. However, such proposed functions were discarded when transgenic mice expressing TFF1 in their mammary glands [13] or ileon [10] did not develop tumors or show differences in the number and size of mammary ducts and intestinal villi, respectively. Furthermore, animals fed with milk containing TFF1 from transgenic mice exhibited no alteration of their gastrointestinal tract [13].

To further investigate TFF1 function, transgenic mice deficient for the TFF1 gene were developed [14]. The present review is dedicated to the functional lessons drawn from these animals which allowed the characterization of TFF1 as a pleiotropic factor.

TFF1: a gastric tumor suppressor

TFF1-deficient mice develop gastric adenomas and carcinomas

TFF1-deficient mice [14] are fertile and apparently normal. However, the antro-pyloric mucosal thickness regularly increases with age, giving rise to adenomas and pyloric stenosis in 1-year-old animals (fig 1B, C). The phenotype is fully penetrant. Regardless of age, hypertrophic pits occupy the entire mucosa. The epithelial cells show severe hyperplasia and high-grade dysplasia. Moreover these cells do not reach their correct differentiation and are non-functional since they are almost entirely devoid of mucus. In the body of the stomach, TFF2 expression is defective. Occasionally, in the pyloric antrum, invading cells are detected across the muscularis mucosa [14]. No metastatic dissemination is observed in the lungs or liver of these animals.

TFF1 deficiency alters the commitment program of pit and parietal cell lineages

During embryonic development [15], TFF1 begins to be expressed at day 17 in the epithelial cells lining the surface and the nascent stomach pits and glands. TFF1 deficiency affects the differentiation pathways of distinct gastric progenitors. While neck and zymogenic cells are not altered by the lack of TFF1, both pit and parietal cells are affected. In the compartmentalized gastric glands (day 21) the pit cell population is amplified and the number of parietal cells per gland decreases. The role and mechanisms of TFF1 action in this process remain unknown. TFF1 might control the dual differentiation program of pre-pit cells into mature pit and neck cells and be necessary for the commitment of some pre-pit cells into parietal cells [15]. Accordingly, Li et al. [16] showed that, in addition to its expression in pit cell region, the TFF1 gene is expressed in the progenitor cell zone of the mouse oxyntic mucosa.

TFF1 expression is altered in human gastric carcinomas

Thus TFF1, whose loss of activity leads to an accumulation of large numbers of undifferentiated gastric epithelial cells which can serve as precursors of fully malignant cells, can be considered as a gastric-specific tumor suppressor gene. Data from human gastric carcinomas strongly support such a function: TFF1 expression is lost in 40–60% of gastric tumors while normal adjacent tis-

sues remain positive [3, 17]; like TFF1-deficient mouse tumors, this loss is accompanied by an alteration in TFF2 expression; the chromosome 21q22 region is commonly deleted [18, 19]; the TFF1 gene itself shows mutations and deletions [20, 21] as well as silencing via promoter hypermethylation [22]. In this context, it has been shown that the tissue-specific expression of TFFs along the gastrointestinal tract is dependent on the methylation status of their proximal promoter/enhancer regions and in the stomach, TFF1 is not methylated [5].

Collectively, these data indicate that TFF1 is a gastric tumor suppressor. It controls gastric ontogenesis via regulation of the commitment program of pit and parietal cell lineages, and the antro-pyloric homeostasis.

TFF1, a factor of gastrointestinal cell differentiation

TFF1 delays G1-S phase transition of the cell cycle

Tumor suppressors can either lower cell proliferation, increase cell death or favor cell differentiation. Treatment with recombinant human TFF1 reduces IEC18, HCT116 and AGS human gastrointestinal cell proliferation [23]. Similarly, transfected HCT116 cells synthesizing constitutive or doxycycline-induced human TFF1 show reduced growth [24, 25]. TFF1 acts on the cell cycle by preventing S phase entry [25]. Consistent with the higher frequency of cells in G1 phase, the percentage of cells positive for cyclin D1 is increased, while cells positive for PCNA (S phase) and cyclin B1 (G2 phase) are decreased. Moreover, the TFF1-induced G1-S transition delay results from upregulation of both INK4 (p15 and p16) and CIP (p21 and p27) cyclin-dependent kinase (cdk) inhibitors (fig. 2). TFF1 leads to increased retinoblastoma protein (pRb) expression that is associated with a two-fold decrease in E2F activity. Thus, the effector of the anti-mitotic function of TFF1 is the pRb/E2F pathway [25].

TFF1 reduces gastrointestinal cell induced-apoptosis

Although reduced in number, TFF1-treated or -expressing IEC18, HCT116 and AGS cells appear healthy, with no morphological evidence of cell death. Moreover, induced cell apoptosis using various apoptotic stimuli (chemical-, Bad- or anchorage-free-induced) is reduced by the simultaneous addition of recombinant human TFF1 (0.1 µM to 10 µM). The anti-apoptotic function of TFF1 occurs via the decrease of caspase-3, -6, -8 and -9 activities. Caspase-1, -2 and -4/5 activities are not affected. Finally, TFF1 does not inhibit the processing of the inactive pro-caspase-9 via the apoptosome but directly targets the active form of caspase-9 (fig. 2), probably through specific inhibitor(s) of the active form of caspase-9, such as inhibitors of apoptosis (IAPs) and related proteins. This anti-apoptotic effect is not observed for the non-gastrointestinal HeLa and Jurkat cells [25].

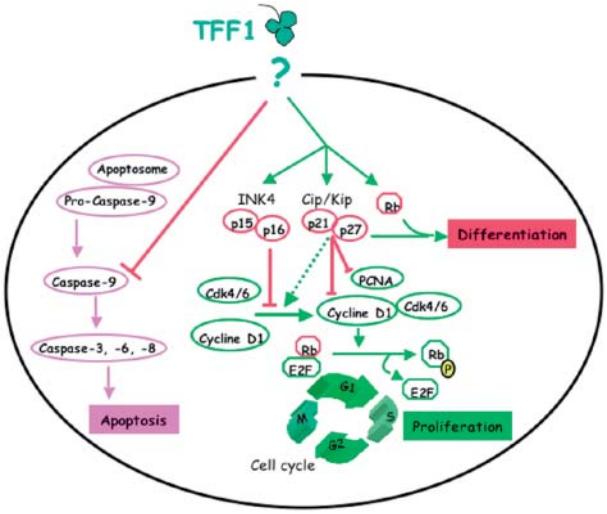

Figure 2. Scheme of the signalling pathways mediating gut cell differentiation via TFF1 antiproliferative and antiapoptotic functions. First, TFF1 delays cell cycle commitment. It increases levels of INK4 and Cip/Kip cyclin-dependent kinase (Cdk) inhibitors. This leads to retinoblastoma protein (Rb)/E2F complexes and to the reduction of E2F transcriptional activity, preventing entry of cells through the G1-S transition. Second, TFF1 reduces cell apoptosis by inhibiting Caspase-9 activity. The molecular mechanism involved in transduction of the external TFF1 stimulus remains unknown (?).

Collectively these data indicate that TFF1 is a factor for gastrointestinal cell differentiation. Similar dual and paradoxical anti-proliferative and anti-apoptotic functions have already been reported for tumor suppressor genes involved in cell differentiation such as pRb, which is essential for correct retina ontogenesis, and whose deficiency induces tumors.

TFF1: a gut protective factor

TFF1 deficiency leads to small intestine inflammatory disorders

No obvious small intestine alterations are observed in 3-week-old TFF1-deficient mice. However, adult mice exhibit enlarged villi with increased lymphoid tissue in both the epithelial and lamina propria compartments, while the lining epithelial cells are normal and functional. Expanded lamina propria contains mixed inflammatory cells, consisting of lymphocytes, plasmocytes and macrophages [14]. The numbers of intraepithelial B and T lymphocytes are also increased. Among T cells, the ratio of CD4/CD8 is altered, and the relative percentage of the CD8 subtype is clearly increased. Moreover, in wild-type mice, the CD4 and CD8 subtypes are principally observed in the core and periphery of the lamina propria, re-

spectively, whereas in deficient mice, they are mixed [our unpublished data]. This inflammatory response might be subsequent to a mucus barrier defect, and therefore to lower protection from physical and chemical aggression. In fact, TFF1, a constituent of the gastrointestinal juice [7], is involved in mucus polymerization. Its TFF domain can directly interact with the von Willebrand factor C cysteine-rich domains of MUC2, MUC5AC and/or MUC6 mucins [26].

Celecoxib treatment cures TFF1-deficient gastric tumors

Cyclooxygenase-2 (Cox-2) is a target of nonsteroid anti-inflammatory drugs (NSAIDs), which inhibit conversion of arachidonic acid to prostanoids. Cox-2 expression is elevated in human gastric pre-cancerous lesions and carcinomas, and serves as a prognostic marker. Similarly, Cox-2 is strongly expressed in antro-pyloric tumors of TFF1-deficient mice while its levels are low or nondetectable in the rest of the gastrointestinal tract, similar to the wild-type gut [27]. In contrast to human gastric dysplasias, in TFF1-deficient mice Cox-2 expression localizes exclusively to the stromal cells.

Treatment of TFF1-deficient mice with the Cox-2 selective inhibitor Celecoxib (1600 ppm per os for 3 months) causes ulceration and inflammation (mainly mononuclear) that is restricted to the tumor areas. Thus, similar to other animal models of carcinogenesis, Cox-2 inhibition suppresses TFF1-deficient tumor growth.

Thus, in addition to its function in gut repair in acute inflammatory disorders [8–10], TFF1 exerts a gut protective function.

TFF1, an intra-cellular protein folding factor

TFF1 deficiency permanently activates the unfolded protein response

A set of genes encoding proteins related to the endoplasmic reticulum (ER) machinery is upregulated in TFF1-deficient tumors regardless of tumor stage (8 weeks or 1 year) [28]. Among them are the (ER)-resident GRP78/BiP, ERp72 and p58IPK chaperone proteins involved in the unfolded protein response (UPR). This process is activated when malfolded, underglycosylated or unassembled proteins accumulate in the ER, the site of folding, assembly and degradation of secretory (membrane and secreted) proteins. In addition, two non-ER resident proteins, CHOP10/GADD153 and Clusterin, also known to be involved in protein folding, are overexpressed in TFF1-deficient tumors. Consistent with an ER-related TFF1 function, the rough ER of TFF1-deficient tumor cells is enlarged, containing dense material. The nature of the accumulating proteins remains to be established. Mucins may be among them since both TFF1 and mucins are secreted and transit through the ER. Thus, in vivo TFF1/mucin binding could occur inside the ER, favoring correct mucin folding and/or secretion. Accordingly, TFF1 is co-packaged and co-secreted in mucous granules [29].

Thus, TFF1 exhibits an intracellular function and favors intra-ER protein folding and/or secretion.

Conclusion

From all the studies performed using TFF1-deficient mice, it is clear that TFF1 is a pleiotropic factor that is mainly active in the gut. First, TFF1 is a gastric tumor suppressor gene controlling gastric mucosa ontogenesis and homeostasis through epithelial cell growth arrest and differentiation. TFF1-deficient mice therefore represent a well-characterized genetic model for further gastric neoplasia studies. Second, TFF1 acts both extracellularly and intracellularly to protect gastrointestinal epithelium by participating in the mucous barrier and favoring correct protein folding.

How TFF1 works at the molecular level is elusive. Since it is active extracellularly as a paracrine or autocrine factor, as well as intracellularly, it can be hypothesized that TFF1 acts through several signalling pathway(s). However, the nature of the specific binding proteins, receptors and downstream effectors remains unknown. To date, with the exeption of TFF1 and TFF3, only two types of TFF1 binding proteins have been identified. One is mucins [26], but it remains to be seen whether mucins are able to transduce TFF1 signalling. The other is the recently identified Trefoil Factor Interactions(z) 1 (TFIZ1) which heterodimerizes with TFF1 [30].

Collectively, these data point to a possible clinical interest of TFF1 in gut disorders. Thus, in addition to its use as a prognosis marker in breast carcinomas to identify and follow patients suitable for endocrine therapy, TFF1 may represent a valuable pharmacological tool for the prevention and/or healing of gastrointestinal diseases.

Acknowledgments. We thank S. Chan for critical reading of the manuscript and S. M. Karam, M. P. Chenard and C. Bossenmeyer-Pourie for illustrations. I would like to thank all our colleagues and collaborators who have participated in this work. This work was supported by funds from the Institut National de la Santé et de la Recherche Médicale, the Centre National de la Recherche Scientifique, the Hôpital Universitaire de Strasbourg, the Bristol-Myers Squibb Pharmaceutical Research Institute, the Association pour la Recherche sur le Cancer, the Fondation de France, and the Ligue Nationale Française contre le Cancer and the Comités du Haut-Rhin et du Bas-Rhin (équipe labellisée).

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