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Trefoil factors: from ulceration to neoplasia

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Abstract. There is convincing evidence that trefoil factors (TFFs) do play an important role in tumourigenesis. However, their specific roles in cancer are not yet clear. Recently, TFFs have been shown to interfere with crucial biological processes such as cell proliferation, differentiation, apoptosis and angiogenesis. Research on the function of TFFs and its relationship with specific signal transduction pathways has also advanced significantly. As a consequence, some ideas about the role of TFFs in cancer have started to take shape. The objective of this review is to summarize and discuss current knowledge on the relationship between TFFs and cancer.

Key words. Cancer; trefoil factors; TFF; ulcer; epithelial restitution; wound healing.

Introduction

The history of research on trefoil factors (TFFs) is tightly connected to cancer. In fact, the first TFF to be identified was the *TFF1* gene as an oestrogen-responsive gene in a breast cancer cell line. In the following years extensive research was conducted on the association between TFFs and cancer, making it clear that along with neoplastic transformation of several epithelial tissues, there are important and reproducible changes in the expression of these peptides.
As research on the function of TFFs progressed, it became increasingly clear that one of the main functions of these peptides is in mucosal protection and healing. On the other hand, impaired healing, when it leads to chronic inflammation-related injury, has long been recognized as a key event in tumourigenesis. Research on the relationship between TFFs and several signal transduction pathways has provided a plethora of information about the putative role of TFFs in crucial biological processes, such as cell proliferation, differentiation and apoptosis. The possibility of understanding the role of TFFs in cancer by linking these areas has been shaping research on this field in the recent years.
The objective of this review is to summarize and discuss current knowledge on the relationship between TFFs and cancer.

TFF structure and expression in normal tissues

The TFF family encompasses a group of small molecular weight, soluble proteins that share as a common feature a three-looped trefoil-like structure formed through interchain disulphide bonding [1]. This trefoil domain is extremely stable and is the basis for the extraordinary resistance of these peptides to hydrolysis and proteolysis [2]. Their structure allows them to form dimers, either with themselves or with other trefoil proteins. Homo- or heterodimers of TFFs were shown to lead to different effects through different activation status [3, 4].
Three human TFFs have been identified to date: TFF1 (formerly pS2) and TFF3 (formerly hITF), with one trefoil domain each, and TFF2 (formerly hSP), with two trefoil domains. TFF1 was originally identified in 1982 as the product of an oestrogen-responsive gene in the breast

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cancer cell line MCF-7 [5]; TFF2 was discovered in 1990 as a human homologue of a peptide isolated from porcine pancreas, the porcine TFF2 (formerly PSP) [6, 7]; and TFF3 was identified in 1993 as a human homologue of rat TFF3 [8–10].

All three TFF genes are clustered in a tandemly oriented fashion at the genomic region 21q22.3, and have a very similar exonic structure [11]. Moreover, the 5′-untranslated region of all *TFFs* is very similar, sharing several regulatory sequence motifs, thus suggesting a common or concerted regulation [11]. These motifs are consensus sequences for several transcription factors. Among these, NFkB [12, 13], GATA-6, USF [14, 15] and C/EBPbeta [13, 16, 17] were already shown to regulate the expression of *TFFs* in response to several stimuli.

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 [18–20].

The main site of expression of TFF1 is the stomach, where it is abundantly expressed in the superficial and foveolar epithelium [18, 21–24]. A high level of expression of TFF1 has also been described in upper ducts and surface cells of Brunner's glands in the duodenum [25]. 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 [26]. In the normal large intestine, TFF1 expression has been demonstrated in some goblet cells, particularly in the distal region [27]. In the pancreas only a few cells in large ducts appear positive [28], and in gall bladder some patchy epithelial expression has been described [29]. Salivary glands were reported as weakly immunopositive [21]. Outside of the gastrointestinal tract, TFF1 expression has been observed in the respiratory epithelium [30] and focally in duct luminal cells of normal breast [31].

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 [18, 24, 32]. In duodenum, TFF2 expression is present in Brunner's glands acini and distal ducts [26]. Some focal expression is also observed in duct epithelium of pancreas [28] and in gall bladder epithelium [29].

Whereas TFF1 and TFF2 are mainly expressed in the stomach, the major site of expression for TFF3 is the intestine. TFF3 is expressed in goblet cells throughout the intestine and in gland acini and distal ducts of Brunner's glands [8-10, 33]. In contrast to the apparent gastrointestinal specificity of TFF1 and TFF2, TFF3 expression has been observed in human uterus [8], normal breast [31], some regions of the hypothalamus and in the pituitary gland [34]. Together with TFF1 it is also present in the respiratory epithelium [30].

Association between TFFs and cancer

Since its discovery, the expression of TFFs has been widely studied in tumours of a variety of organs. TFF1, for example, can be detected in more than 50% of human breast cancers, and has been associated with oestrogen receptor expression [35–37], responsiveness to hormone therapy [38] and favourable prognosis [39, 40] in this type of tumour. In gastric cancer, loss of TFF1 expression has been associated with the intestinal and atypical histological sub-types and with loss of differentiation [22–24, 32, 41–44]. Other tumours found to express TFF1 include cancers of pancreas, lung, endometrium, ovary, prostate, bladder, billiary tract, colorectal, oesophagus and skin [29, 41, 45–52]. Although less extensively studied, there are also numerous reports on the expression of TFF2 and TFF3 in human cancer [18, 31, 42, 45, 51–61].

Despite all the evidence pointing to an association between altered TFF expression and cancer development, the involvement of these proteins in tumourigenesis remained unclear for some time. Experimental evidence for a role in tumour suppression first came from studies using knockout mice lacking the *TFF1* gene [62]. Homozygous animals showed decreased and dysfunctional gastric mucin production with marked antral hyperplasia and dysplasia; all such animals developed antral adenomas, and 30% developed multifocal intramucosal cancers [62]. Later on, mutations in the *TFF1* gene were described in 16,3% of gastric cancers, accompanied by loss of heterozygosity (LOH) in 16.7% of the cases [63]. These results suggested *TFF1* as a gastric-specific tumour suppressor gene. However, other authors, despite finding LOH in 28,6% of the cases, failed to find any mutations in a series of 90 gastric cancer cases [64]. Recently, promoter hypermethylation was suggested to be responsible for silencing of the *TFF1* gene in human gastric cancer [64, 65].

The *TFF2* gene was also found to be downregulated in gastric cancer [59]. Yet, despite the observation of increased gastric proliferation in *TFF2* knockout mice [66], no genetic or epigenetic alterations where found to back-up a tumour suppressor role for *TFF2* [60]. In contrast, *TFF3* seems to be upregulated in pre-neoplastic lesions, especially intestinal metaplasia [59] and in at least a subset of gastric cancers [60].

Functional role of TFFs in cancer

As discussed in the previous section, there is convincing evidence that TFFs do play a role in tumourigenesis. However, most of the data on record are associative in nature, and there are many gaps in our knowledge about the functional role of TFFs in cancer development and progression. In recent years, research on the function of

TFFs and its relationship with signal transduction has advanced significantly. As a consequence, some ideas about the role of TFFs in cancer have started to take shape.

One of the key areas is related with the involvement of TFFs in mucosal defence and reconstitution [67–71]. Impaired healing and its association with chronic inflammation-related injury has long been recognized has a key event in tumourigenesis. Thus the hypothesis that disturbance of the function of TFFs in mucosal healing exacerbates the risk of tumourigenesis in chronic inflammatory conditions constitutes a very appealing model for the involvement of TFFs in cancer. Data on record demonstrates that the expression of TFFs is upregulated in epithelial cells adjacent to ulcerative conditions of the gastrointestinal tract, and in epithelial cells undergoing migration across the base of such lesions (UACL, ulcer associated cell lineage) [28, 67, 72]. Further support for the involvement of TFFs in wound healing came from experimental data showing that TFFs are able to promote epithelial cell migration in vitro and in vivo [68, 73], and to protect against induced gastrointestinal damage in vivo [69, 71]. Moreover, a transgenic mouse model lacking TFF3 has been shown to have impaired mucosal healing [70]. More recently, it has also been shown that TFF2 messenger RNA (mRNA) levels increase within minutes following gastric ulceration in rats [74], and TFF2-deficient mice present a larger number of non steroidal anti-inflammatory drug (NSAID)-induced ulcers when compared with wild-type animals [66].

Another interesting possibility results from the demonstration that TFF1 overexpression can confer on *Helicobacter pylori* an enhanced ability to colonize the gastric mucosa [75]. *H. pylori* infection first induces chronic superficial (non-atrophic) gastritis, which can progress through chronic atrophic gastritis, intestinal metaplasia and dysplasia toward gastric cancer [76, 77]. The inflammatory response to *H. pylori* leads to activation of several signalling pathways which not only help fight the infection but also promote mucosal restitution and wound healing. *H. pylori* infection was shown to upregulate TFF1 in AGS and T84 cells [75, 78], a result that is in accordance with the known function of TFFs in mucosal healing. Interestingly, *H. pylori* was found to bind the dimeric form of TFF1 [75], and *H. pylori* is known to preferentially colonize the surface mucous gel layer of the stomach, which is constituted mainly by MUC5AC and TFF1 [24, 32]. Such interaction between *H. pylori* and TFF1 expression would thus constitute a sort of double-edged sword: on the one hand, *H. pylori*-induced expression of TFF1 would be beneficial owing to its effect on mucosal healing; on the other hand, more TFF1 would lead to increased survival of *H. pylori* in the human stomach.

Regarding research on the relationship between TFFs and signal transduction, one of the most promising results was the demonstration that TFFs may be regulated by the interleukin (IL)6/GP130 pathway [79]. This pathway was shown to have a pivotal role in the immune response of the gut mucosa, and also to be involved in early wound-healing regulation. The dimerization of IL6 receptor (IL6R) and GP130 can lead to two different signalling pathways, according to the cellular context and the kind of GP130 residues that are phosphorylated. It can signal through Janus kinase (JAK) and the transcription factor STAT3, or alternatively through SHP2, Ras, mitogen-activated protein (MAP) kinases and the transcription factor C/EBPbeta. In a very elegant experiment Tebbutt and colleagues [79] demonstrated that mutations in the *GP130* gene that abrogate JAK/STAT signalling lead to impaired mucosal repair in a way that resembles the *TFF3* knockout mouse. In the same model the authors showed that mice lacking GP130-related SHP2-RAS-C/EBPbeta signalling develop gastric adenomas and cancer in a way that mimics the *TFF1* knockout mouse [79, 80]. Notably, Dossinger and colleagues had previously shown that C/EBPbeta downregulates TFF1 [13]. Altogether, these results suggest that disruption of the balance between the two alternative pathways of IL6 signalling may be connected with impaired TFF function and thereby play an important role in gastric carcinogenesis.

In oestrogen-responsive breast cancer, TFF1 and TFF3 were suggested to contribute to breast malignancies by aiding tumour cell dissemination [81]. In support of this hypothesis, stably transfected TFF1 was shown to induce an invasive phenotype in the HCT-8 colon cell line in a RhoA-dependent way. RhoA is a GTPase which mediates growth factor induced reorganization of the cytoskeleton, which is crucial for cell motility [82]. Changes in the expression and/or function of E-cadherin may also account for the ability of cancer cells to detach from the parental tumour cells and invade locally [83]. TFF3 was shown to induce tyrosine phosphorylation of catenins, and to reduce cell-cell adhesion in the HT29 colon cancer cell line through disruption of catenin/cadherin complexes [84, 85].

The ability to escape programmed cell death is crucial for the survival of tumour cells. It was demonstrated that TFF3 induces cell survival via phosphoinositol 3-kinase (Pi3K) and P53 in colon cancer cell lines HT-29 and HCT116, respectively. TFF1 was also able to induce reduced apoptosis in gastrointestinal cells by targeting the active form of caspase-9 [86]. TFF2, on the other hand, was shown to act as a morphogen and promotes cell survival in the MCF-7 breast cancer cell line [87]. Hence, and though this is an area that clearly needs further investigation, an anti-apoptotic effect of TFFs may also be a way of explaining its involvement in cancer progression.

An angiogenesis-promoting role was also described for TFFs. Hypoxia is known to constitute a powerful stimu-

lus for angiogenesis in growing tumours. It leads to the expression of genes and factors that promote the formation of new blood vessels, which is a prerequisite for tumour growth and dissemination. One of the main effectors of hypoxia pathways is hypoxia inducible factor one (HIF-1), which induces the transcription of angiogenic factors. In the same way, TFF3 mRNA and protein have been shown to be induced by HIF-1 [88]. The angiogenic effect of TFFs was shown to be comparable to the classical vascular endothelial growth factor signalling [89].

Conclusions

Changes in the expression of TFFs are a common feature of many types of tumours. In most models it was not experimentally proven whether TFFs are actively involved in cancer onset and progression or whether they constitute a sort of innocent bystander.

The obvious exception to this is TFF1 and gastric cancer. In this particular cancer model there is a large body of experimental evidence showing that impaired function of TFF1 is intimately associated with neoplastic transformation of the gastric mucosa.

The specific roles of TFFs in cancer are not yet clear. TFFs have been shown or suggested to interfere with crucial biological processes such as cell proliferation, differentiation, apoptosis and angiogenesis. Theoretically, TFFs could make their way to tumourigenesis through any of the aforementioned processes. In tumours outside the gastrointestinal tract, TFFs are usually observed in a state of overexpression. Hence, even when TFFs do not play a key role in the molecular genetic pathways that underlie these cancer models, tumours are likely to take advantage of the expression of TFFs.

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