Induction of TFF1 gene expression in pancreas overexpressing transforming growth factor α

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Abstract

***Background/Aims*—Chronic pancreatitis is an inflammatory disease of the exocrine pancreas associated with extensive fibrosis, enlarged pancreatic ducts, acinar cell degeneration, and the formation of tubular complexes. The molecular and biochemical alterations associated with these histological changes are not kown. Generally, the new family of TFF peptides (formerly known as P-domain peptides or trefoil factors) is aberrantly expressed during chronic inflammatory diseases of the gastrointestinal tract.**

***Methods*—Using human pancreatic tissues obtained from patients with chronic pancreatitis and murine pancreatic tissues obtained from transgenic mice overexpressing transforming growth factor α (TGF-α), the expression and cellular distribution of TFF1 was analysed using northern blot analysis, polymerase chain reaction (PCR), and immunohistochemistry.**

***Results*—In the normal human pancreas, TFF1 was scarce, with only a few ducts exhibiting cytoplasmic TFF1 immunoreactivity. In contrast, human chronic pancreatitis tissue specimens exhibited strong TFF1 immunoreactivity in ductal cells, areas of ductal hyperplasia, and tubular complexes. Semiquantitative PCR analysis of TFF1 mRNA levels showed enhanced expression of TFF1 in the pancreas of patients with chronic pancreatitis. Furthermore, TFF1 mRNA levels were detectable in the pancreas in four of five transgenic mice overexpressing TGF-α. In contrast, four of five wild type mice did not exhibit a TFF1 mRNA transcript. In addition, while no specific TFF1 immunoreactivity was present in the pancreas of the wild type mice, ductal epithelial cells and duct-like tubular complexes in the pancreas of the transgenic mice overexpressing TGF-α exhibited pronounced TFF1 immunoreactivity.**

***Conclusions*—Ductal cells and tubular complexes in pancreatic fibrosis express TFF1. As the 5'-flanking region of TFF1 contains an epidermal growth factor responsive enhancer region and the expression of epidermal growth factor and TGF-α is enhanced in pancreatic fibrosis, the enhanced expression of TFF1 in pancreatic fibrosis may be mediated by TGF-α.**

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Keywords: fibrosis; pS2; pancreatitis; expression; transforming growth factor α; TFF peptides

Chronic pancreatitis is an inflammatory disease of the exocrine pancreas, which is characterised by the destruction of pancreatic parenchyma leading to exocrine and endocrine insufficiency.[1 2] The histomorphological changes present in chronic pancreatitis include tubular complexes, enlarged pancreatic ducts, pseudoductular hyperplasia, atrophy, replacement of the functional parenchyma by variable amounts of fibrosis, and infiltration by mononuclear cells.[3 4] The biochemical and molecular mechanisms that underly the pathophysiology of chronic pancreatitis are poorly understood. Release of cytokines from inflammatory cells may be involved in stimulating fibroblast proliferation and collagen biosynthesis.[4] Recent analysis showed overexpression of growth factors and their receptors in the fibrotic areas. The pancreas of patients with chronic pancreatitis overexpresses the epidermal growth factor (EGF) receptor and transforming growth factor α (TGF-α), as well as acidic and basic fibroblast growth factors and platelet derived growth factors.[5–8] These observations suggest that changes in the pattern of growth factor expression may be involved in the pathophysiological processes that occur in chronic pancreatitis. Furthermore, transgenic mice that overexpress TGF-α exhibit marked pancreatic fibrosis and transdifferentiation of acinar cells into tubular structures in the absence of an inflammatory reaction.[9]

Secretory TFF peptides (formerly called trefoil factors or P-domain peptides)[10] constitute a family of three members, TFF1 (formerly named pS2), TFF2 (formerly named human spasmolytic peptide), and TFF3 (formerly named intestinal trefoil factor/hP1.B), which exhibit a characteristic TFF domain containing three conserved disulphide bridges.[11–14] As their expression is greatly enhanced in areas of gastrointestinal damage after peptic ulceration and chronic inflammation, TFF peptides are attributed a key role in mucosal restitution and repair.[11 15] TFF1 consists of a single TFF domain which is cleaved from a precursor.[16] Originally, the TFF1 transcript was identified by differential screening of a cDNA library

Abbreviations used in this paper: TFF, trefoil factor family; EGF, epidermal growth factor; TGF-α, transforming growth factor α; RT-PCR, reverse transcription polymerase chain reaction; GAP, glyceraldehyde-3-phosphate dehydrogenase.

Table 1 Primer sequences used for reverse transcription polymerase chain reaction analysis

Name	*Sense (5'–3')*	*Antisense (5'–3')*	*Size (bp)*
mTFF1	AAACATGTATCATGGCCC	GAATTCGAGGACTAAAAGTCTG	322
mTFF2	TTCCACCCACTTCCAAAC	AATGCTGTGTCTAGCCACTG	310
mTFF3	CAGATTACGTTGGCCTGTCTCC	ATGCTTGCTACCCTTGGACCAC	254
rGAP	GCTGGATCCTTCATTGACCTCAACTAC	CGAGAATTCATACCAGGAAATGAGC	860
hTFF1	GAGAACAAGGTGATCTGCGC	TGGTATTAGGATAGAAGCACC	208

m, mouse; r, rat; h, human.

prepared from MCF-7 breast cancer cells grown in the presence of oestrogen. Transcription of TFF1 is inducible by EGF and phorbol myristate acetate, as well as c-Jun, because of the existence of a complex enhancer region apart from its oestrogen responsive promoter, located 5' upstream of the TFF1 gene on chromosome 21q22.3.[17] This factor was shown to be overexpressed in a variety of epithelial malignancies, such as gastric, pancreatic, breast, and ovarian cancers.[18–21] Furthermore, its expression is highly correlated with the presence of oestrogen receptors in breast and ovarian cancers.[19] Recent analysis points to a role for TFF1 in the promotion of epithelial cell migration, promoting the hypothesis that TFF peptides act as motogens.[22–25] In contrast, inactivation of TFF1 leads to the development of severe hyperplasia and dysplasia, as well as adenomas and carcinomas of the antral and pyloric mucosa in TFF1 null mice, indicating that TFF1 may also be essential for the differentiation of epithelial cells in the gastrointestinal tract.[26]

As the formation of tubular complexes occurs in the inflammatory pancreas[27] and recent data point to the existence of an acinar-ductal-carcinoma sequence arising in the duct-like epithelium of these tubular complexes,[28 29] we sought to assess the expression of TFF1 in the pancreatic parenchyma of patients with chronic pancreatitis and in the pancreas of transgenic mice overexpressing TGF-α which share similar histomorphological changes.[9] Using northern blot analysis, immunohistochemical, semiquantitative and qualitative polymerase chain reaction (PCR) approaches, we analysed the expression of TFF1 in ductal epithelial and acinar cells in chronic pancreatitis, in tubular complexes in the pancreatic parenchyma of patients with chronic pancreatitis, and in the pancreas of transgenic mice overexpressing TGF-α.

Materials and methods

The following products were purchased: Hybond membranes and [α-^{32}P]dCTP (3000 Ci/mmol) from Amersham (Braunschweig, Germany); *Taq* polymerase from Gibco-BRL (Eggenstein, Germany); pGEM3Zf vector from Promega Biotech (Mannheim, Germany); oligonucleotides from MWG-Biotech (Ebersberg, Germany). All other chemicals and reagents were of molecular biology grade and were purchased from Sigma Chemical (Deisenhofen, Germany).

TRANSGENIC MICE

Transgenic mice were obtained from E Sandgren (University of Pennsylvania, Philadelphia, PA, USA). This line was generated by the introduction of the elastase promoter fused to the rat TGF-α cDNA, leading to the overexpression of TGF-α mRNA and protein in the pancreas.[30] Five transgenic animals were used for this study; control animals were of the BL6 strain. All animals were maintained on laboratory chow and tap water. They were housed and treated in accordance with appropriate guidelines. On day 120 they were killed, the pancreas was removed, and tissues were snap frozen in liquid nitrogen. In all transgenic mice the pancreas exhibited characteristic cytological and histological changes, such as fibrosis and transdifferentiation of acinar cells, as the result of the overexpression of TGF-α in the pancreas.[9 30]

TISSUE SAMPLES

Chronic pancreatitis tissues were obtained from patients undergoing pancreatic surgery (three women and seven men). Normal pancreatic tissues were obtained from five men and five women through an organ donor programme. To ensure sampling uniformity, tissue samples from organ donors and patients with chronic pancreatitis were always obtained from the head of the pancreas. The median age of the patients with chronic pancreatitis was 42 years (range 27–50), and that of the organ donors was 42 years (range 14–60). Immediately after surgical removal, all tissue samples were either fixed in Bouin's solution or frozen in liquid nitrogen. All chronic pancreatitis tissue samples were graded independently by a pathologist, and classified histologically as exhibiting moderate to severe disease with areas of fibrosis, acinar cell atrophy, and ductal cell proliferation.[31]

RNA EXTRACTION

Total RNA was extracted from the human pancreatic tissues and the pancreas of the transgenic mice by the acid guanidinium thiocyanate method.[32]

POLYMERASE CHAIN REACTION (PCR)

Two primer sequences, corresponding to the respective mRNA of human and mouse TFF1, mouse TFF2 and TFF3, and rat glyceraldehyde-3-phosphate dehydrogenase (GAP) were used for reverse transcription PCR.[33 34] Table 1 gives all the primer sequences. β-Actin cDNA was amplified as previously described.[8] cDNAs were synthesised from total RNA (1 μg/sample) isolated from human and mouse pancreatic tissues, using oligodeoxythymidylate and reverse transcriptase.[32] After inactivation, 1 μl of the reaction mixture was incubated in buffer containing 0.2 mM concentrations of dATP, dCTP, dGTP, and dTTP, 0.2 μM concentrations of each oligonucleotide

primer, 3 mM $MgCl_2$, and 10 × buffer consisting of 200 mM Tris-HCl (pH 8.0), 500 mM KCl, and 1 U Taq polymerase.[32] Reaction cycles consisting of 45 seconds at 94°C, one minute at 55°C, and 90 seconds at 72°C were repeated 35 times for amplification of human TFF1. Mouse TFF PCR conditions consisted of 40 cycles of 30 seconds at 94°C, 30 seconds at 60°C, and 30 seconds at 72°C. The PCR products were size fractionated on polyacrylamide gels (human TFF1) and 1.2% agarose gels (mouse TFF1–3).[32]

SEMIQUANTITATIVE PCR ANALYSIS

Semiquantitative analysis of TFF1 expression was performed using a protocol previously described by Dallman *et al.*[35] Briefly, after reverse transcription of the extracted total RNA, 1/20th of the cDNA was used for the PCR analysis. Primers were used at final concentrations of 0.2 μM. Primer sequences and PCR conditions are described above. PCR products were analysed by gel electrophoresis and dot blot hybridisation. A 15 μl portion of the amplification mixture was removed every five cycles from 20 to 35 cycles of PCR. The samples were dot-blotted to nylon membranes and probed using a [^{32}P]dCTP-random primed labelled TFF1 cDNA probe.[32 35]

NORTHERN BLOT ANALYSIS

Total RNA was size-fractionated, and blotted on to nylon membranes as previously described.[32] The blots were prehybridised for one hour at 42°C in prehybridisation buffer containing 50% formamide, 0.1% sodium dodecyl sulphate, 5 × SSC (0.15 M NaCl + 0.015 M sodium citrate), 2.5 × Denhardt's, 250 μg/ml salmon sperm DNA, and 50 mM Na_2PO_4, pH 6.5. The blots were hybridised at 42°C for 16 hours with the labelled cDNA probes, and washed as previously described.[34] The blots were exposed at −80°C to Kodak XAR-5 film with Kodak intensifying screens.

CDNA PROBE SYNTHESIS AND LABELLING

A 208 bp cDNA fragment of human TFF1 was amplified by PCR and specific primers as outlined above.[33 34] The PCR product was size fractionated on an agarose gel to determine the correct size. After elution from the agarose gel, the cDNA fragment was sequenced using an automated fluorescence sequencer in order to confirm the sequence of the cDNA fragment. Subsequently the TFF1 cDNA fragment, corresponding to nucleotides 52–260,[33 34] and a 190 bp BamHI fragment of the mouse 7S cDNA,[36] which cross hybridises with human 7S RNA and which was used to verify equivalent RNA loading, were random prime labelled with [^{32}P]dCTP.[32]

IMMUNOHISTOCHEMISTRY

The presence of human TFF1 was assessed using paraffin wax embedded tissue sections (n = 10 for chronic pancreatitis and normal pancreas), and the presence of mouse TFF1 was assessed using frozen tissues obtained from three transgenic mice and three controls. The human tissues were fixed in Bouin's solution and paraffin wax embedded, and the murine tissues were snap frozen. A highly specific polyclonal antibody for TFF1 (pNR-2/pS2) (Dianova, Hamburg, Germany) was applied. This antibody was raised against a synthetic 31 amino acid peptide corresponding to the C-terminus of the human TFF1/pS2 protein[37], KGCCFDDTVRGVPWCFYPNTIDVP-PEEECEF. The specificity of the antibody had been previously confirmed by preadsorption of the primary antiserum with the synthetic peptide and the immunogen and by western blot analysis.[37 38] Paraffin wax sections (4 μm thick) were deparaffinised and rehydrated. The frozen tissues were fixed in acetone (4°C) for five minutes. The antiserum was diluted to 1:120 (paraffin wax) and 1:150 (frozen) in buffer containing 50 ml RPMI 1640, 450 ml distilled water, and 50 ml fetal bovine serum (pH 7.5). For negative controls, the primary antibody was omitted. For a positive control we used gastric tissues and breast cancer tissues from humans and gastric tissues from wild type mice. All sections were subjected to microwave treatment for 3 × 10 minutes in citrate buffer. Endogenous peroxidase activity was inhibited by immersing the sections in 3% H_2O_2 for five minutes. The sections were incubated with the

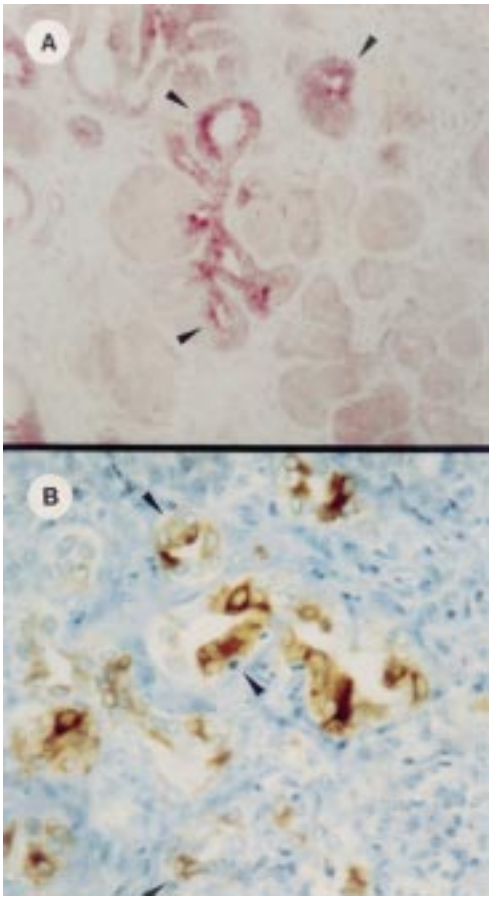

Figure 1 TFF1 immunoreactivity in tubular complexes in pancreatic fibrosis. (A) Transgenic mice overexpressing transforming growth factor α. Strong TFF1 immunoreactivity was observed in the duct-like cells of tubular complexes (arrowheads). (B) Human chronic pancreatitis. TFF1 immunoreactivity was also present at the surface of these duct-like cells forming a central lumen (arrowheads). Original magnifications: A, 120 ×; B, 400 ×.

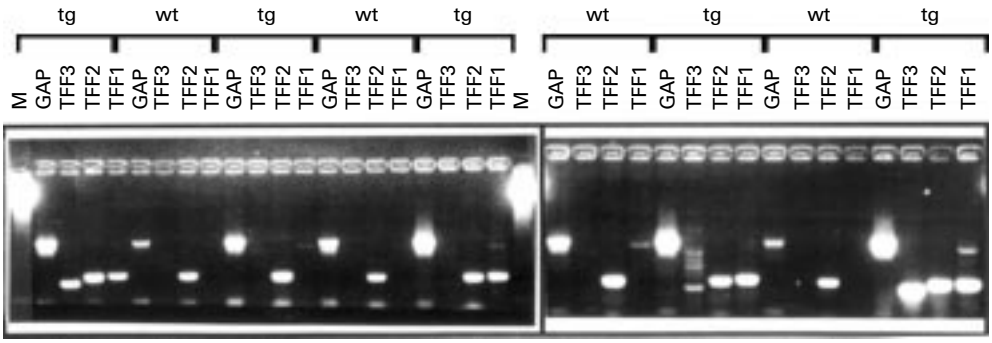

Figure 2 Reverse transcription polymerase chain reaction (PCR) analysis of TFF1 expression in the pancreas of wild type and transgenic mice. With cDNAs obtained from total RNA which had been extracted from the pancreas of wild type mice (wt; n = 4) and mice overexpressing transforming growth factor α (tg; n = 5), a band corresponding to TFF1 was observed in the pancreas in four of five transgenic mice. In contrast, only one wild type mouse exhibited the TFF1 transcript in the pancreas. TFF2 was expressed in all transgenic and wild type mice, whereas TFF3 expression was detectable in three of five transgenic mice. Parallel PCR amplification of the cDNAs using two primers specific for glyceraldehyde-3-phosphate dehydrogenase (GAP) confirmed the integrity of the cDNAs used. M, DNA marker.

antiserum at 37°C for one hour and washed with Tris buffered saline. The reaction was detected using the ABC detection kit labelled with alkaline phosphatase (Vector, Burlingame, California, USA). The analysis was performed according to the manufacturer's recommendations and all reactions were performed at 23°C. Finally, the sections were counterstained with Mayer's haematoxylin.[32]

Results

TFF1 IS LOCATED IN DUCTAL CELLS AND TUBULAR COMPLEXES IN THE PANCREAS OF TRANSGENIC MICE OVEREXPRESSING TGF-α

Whereas the pancreas of wild type mice exhibited no histopathological alterations of pancreatic morphology (not shown), the pancreas of all transgenic mice overexpressing TGF-α exhibited an increase in total organ size, extensive fibrosis, and the formation of tubular complexes[9] (fig 1A). In addition, acinar cell height was decreased in the transgenic mice leading to the formation of duct-like structures. To assess the localisation of TFF1 within the pancreas of the transgenic mice, we performed an immunohistochemical analysis. Acinar cells and fibroblasts did not exhibit TFF1 immunoreactivity. However, TFF1 immunoreactivity was found on the surface and the cytoplasm of ductal cells in the pancreas of the transgenic mice. Furthermore, while acinar cells in pancreatic fibrosis did not exhibit TFF1 immunoreactivity, acinar complexes with decreased acinar cell height and the formation of a lumen exhibited strong TFF1 immunoreactivity which was present at the apical surface towards the lumen of these duct-like structures (fig 1A, arrowheads). These duct-like formations, corresponding to the tubular complexes within the pancreatic fibrosis of the transgenic mice, exhibited strong TFF1 immunoreactivity throughout the pancreas of these transgenic mice (fig 1A).

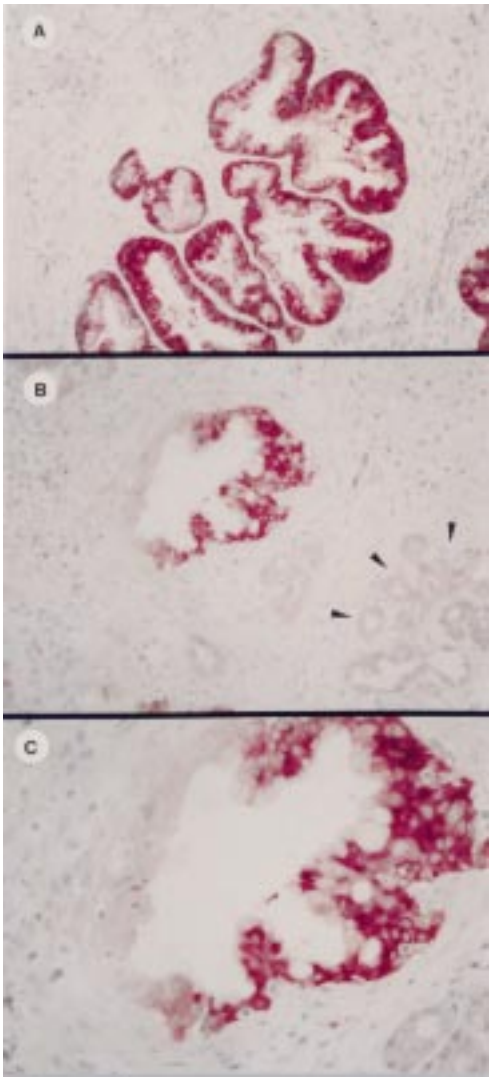

Figure 3 TFF1 immunoreactivity in chronic pancreatitis. Strong TFF1 immunoreactivity was present in the vast majority of pancreatic ducts (A) and areas of ductal hyperplasia also exhibited intense TFF1 immunoreactivity (B,C). However, not all ducts exhibited TFF1 immunoreactivity (B, arrowheads), and acinar and islet cells, as well as fibroblasts and inflammatory cells, were generally devoid of TFF1 immunoreactivity. Original magnifications: A, B, 120 ×; C, 400 ×.

INDUCTION OF TFF 1 mRNA LEVELS IN THE PANCREAS OF THE TRANSGENIC MOUSE OVEREXPRESSING TGF-α

The mRNA transcripts corresponding to the three TFF peptides in the pancreas of transgenic mice overexpressing TGF-α was assessed using the reverse transcription PCR method.

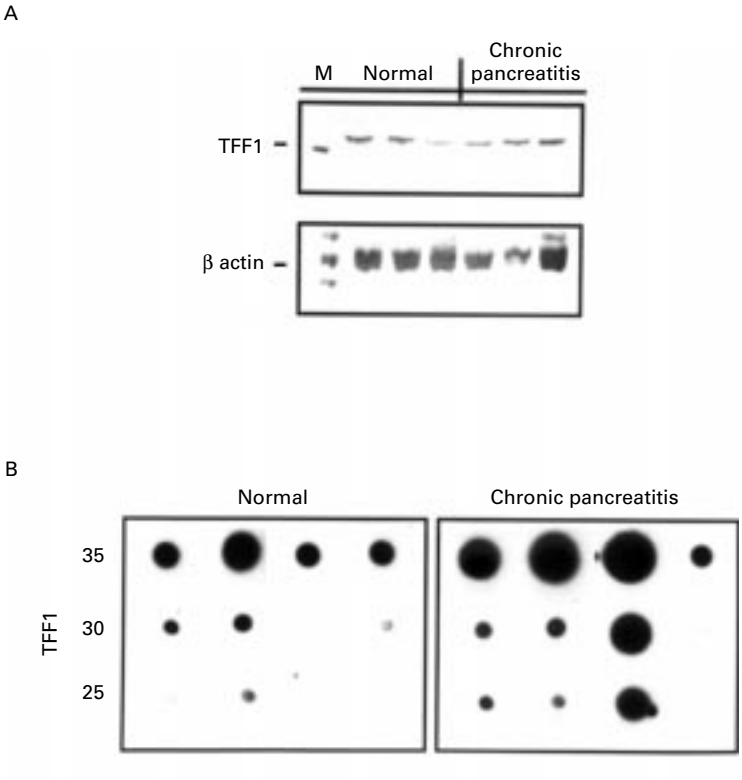

Figure 4 (A) Reverse transcription polymerase chain reaction (PCR) analysis of TFF1 expression in the normal pancreas and in chronic pancreatitis. With cDNA obtained from total RNA extracted from normal pancreatic tissue samples (n = 3) and samples from patients with chronic pancreatitis (n =3), a band corresponding to TFF1 was present in all samples, as detected by polyacrylamide gel electrophoresis. Parallel PCR amplification of the cDNAs using two primers specific for β-actin confirmed the integrity of the cDNAs used. M, DNA marker. (B) Semiquantitative PCR analysis of TFF1 expression in chronic pancreatitis. Total RNA was extracted as previously described and PCR analysis was performed. A 15 µl portion of the amplification mixture was removed every five cycles from cycles 25 to 35. The samples were dot-blotted to Nylon membranes and probed using a [^{32}P]dCTP random prime labelled TFF1 cDNA probe.

Total RNA was extracted from the pancreas of wild type and transgenic mice and subjected to PCR amplification using primers specific for mouse TFF1, TFF2, and TFF3. Whereas we could detect no differences in TFF2 expression between the transgenic and wild type mice, expression of TFF1 and TFF3 in four of five and three of five transgenic mice respectively was observed in two subsequent independent reverse transcription PCR analyses (fig 2). In contrast, only one of five wild type mice showed TFF1 mRNA. To confirm the integrity of the RNA extracted from the pancreas we performed reverse transcription PCR using primers specific for GAP, which showed the presence of GAP in all cases. Northern blot analysis of TFF1 mRNA levels in the pancreas of wild type and transgenic mice, however, failed to detect a specific band corresponding to TFF1 mRNA.[39] The subsequent hybridisation with a 7S probe and ethidium bromide staining of total RNA run on agarose gels, however, confirmed the integrity of the extracted RNA (not shown).

TFF1 peptide is present in ductal cells and tubular complexes in human chronic pancreatitis

In the normal human pancreas, TFF1 immunoreactivity was present in a few ductal cells. Neither acinar nor islet cells exhibited TFF1 immunoreactivity (not shown). In the pancreas of patients with chronic pancreatitis, strong TFF1 immunoreactivity was detectable in the cytoplasm and on the surface of ductal epithelial cells (fig 3). Furthermore, TFF1 was expressed in areas of ductular hyperplasia and tubular complexes (fig 1B). Again, acinar and islet cells, as well as infiltrating mononuclear cells and fibroblasts, were devoid of TFF1 immunoreactivity in chronic pancreatitis (figs 1 and 3).

TFF1 mRNA level is enhanced in chronic pancreatitis

Using total RNA and a cDNA probe specific for the TFF1 transcript, we were unable to detect a band corresponding to TFF1 mRNA in our northern blot analysis. Subsequent hybridisation with a 7S probe and ethidium bromide staining of total RNA run on agarose gels confirmed the integrity of the extracted RNA (not shown). Therefore we performed reverse transcription PCR analysis using two specific TFF1 primers. After polyacrylamide gel electrophoresis, a single band corresponding to the TFF1 transcript was detectable in extracts from normal pancreas and patients with chronic pancreatitis (fig 4A). To assess the levels of TFF1 mRNA in the normal pancreas and in chronic pancreatitis, we performed a semiquantitative PCR analysis of TFF1 expression. Dot blot hybridisation of PCR products obtained after 25, 30, and 35 cycles showed enhanced expression of TFF1 in chronic pancreatitis as compared with the normal pancreas (fig 4B).

Discussion

Chronic pancreatitis is an inflammatory disease of the exocrine pancreas characterised by the replacement of pancreatic parenchyma by fibrosis.[1 2] Apart from fibrosis, the pancreatic parenchyma may also exhibit enlarged ducts, infiltration by inflammatory cells, the formation of duct-like tubular complexes, regions of acinar cell degeneration, and duct cell proliferation.[3] In human acute and chronic pancreatitis, pancreatic cancer, and animal models of acute pancreatitis, duct-like structures, referred to as tubular complexes, have been described.[4 8] Tubular complexes evolve as the acinar cells decrease in height and lose acinar cell specific antigens, paralleled by increased expression of duct cell markers.[9 39]

The molecular alterations underlying the fibrotic changes and the formation of these duct-like complexes are largely unknown. Recently, tyrosine kinase receptors, comprising the large family of growth factor receptors, have been shown to be overexpressed in the pancreas of patients with chronic pancreatitis.[5–8] Thus, growth factors of the EGF, fibroblast growth factor, and platelet derived growth factor family and their respective receptors are aberrantly expressed in chronic pancreatitis.[5–8] Furthermore, immunohistochemical analysis of the pancreatic parenchyma in chronic pancreatitis has shown enhanced expression of these growth factors in areas of ductal hyperplasia and ductal metaplasia or tubular complexes.[5 6 8] The aberrant

expression of growth factors and their tyrosine kinase receptors in focal proliferative lesions, such as ductal hyperplasia and ductal metaplasia or tubular complexes in pancreatic cancer and chronic pancreatitis, has given rise to the hypothesis that tyrosine kinases may contribute to the formation of potentially preneoplastic lesions in pancreatic carcinogenesis.[40] Further evidence for the role of tyrosine kinases in pancreatic carcinogenesis has come from the generation of transgenic mice overexpressing TGF-α.[30] The pancreas of these mice is increased in size, which is accounted for by the increased formation of connective tissue. Furthermore, transdifferentiation of acinar cells leading to the formation of tubular complexes as described above has been reported in the pancreas of these mice.[9 30] In a recent study, the formation of tumours originating from these tubular complexes in the pancreas of TGF-α transgenic mice has been reported,[28] raising the hypothesis that tubular complexes form preneoplastic lesions in pancreatic carcinogenesis and supporting the hypothesis of an acinar-ductal-carcinoma sequence in pancreatic carcinogenesis.[28 29] To assess the role of other factors that may contribute to the formation of these complexes, we analysed the expression of TFF1 in the pancreas of patients with chronic pancreatitis and in the pancreas of transgenic mice overexpressing TGF-α, as the two tissues exhibit comparable histomorphological changes, such as fibrosis and the formation of tubular complexes. We chose to assess the expression of TFF1 in human and experimental pancreatic fibrosis for several reasons: (1) TFF1 has been reported to be overexpressed in pancreatic cancer[20 41]; (2) TFF1 contains a complex enhancer region responsive to EGF, which is overexpressed in chronic pancreatitis and pancreatic cancer[17]; (3) TFF1 may act as a motogen, raising the hypothesis that this factor, upon induction by EGF and/or TGF-α, may be involved in the generation of tubular complexes in pancreatic fibrosis.[22–25] In our study, the expression of TFF1 was enhanced in human and experimental pancreatic fibrosis. Furthermore, TFF1 expression was localised to ductal epithelial cells in human chronic pancreatitis and duct-like cells forming tubular complexes.

Immunohistochemical analysis of the expression of growth factors and TFF peptides in human disease, and experimental models of gastrointestinal mucosal restitution and repair have led to the understanding that TFF peptides play an important role in the process of mucosal repair.[11 12 42] Furthermore, an ulcer associated cell lineage was defined which exhibits abundant TFF1 expression at the surface cells and the upper duct cells, while TFF2 expression is abundant in the lower duct and coexpressed with EGF in the glandular area.[42] This lineage has been identified in the intestine, gall bladder, and pancreatic ducts in chronic pancreatitis.[15 42] As the TFF1 gene is induced by EGF, it was speculated that secretion of EGF in the basal compartment of the ulcer associated cell lineage would induce the expression of TFF1 in the surface cells. Further evidence for a growth factor dependent induction of TFF1 expression resulted from the analysis of a complex enhancer region responsive to oestrogens, EGF, phorbol myristate acetate, and the c-Ha-ras oncoprotein and the c-Jun protein.[17] The induction of TFF1 expression by growth factors of the EGF family, such as EGF and TGF-α, was further supported by analysis of TGF-α knockout and TGF-α transgenic mice.[43 44] The stomach of transgenic mice overexpressing TGF-α has a mucosal phenotype closely resembling Menetrier's disease, and an expansion of the foveolar compartment and decrease in the gastric gland neck and fundic region has been reported.[43] TFF1 expression in the stomach of these mice is observed in the cells deep in the gland covering the entire region of foveolar hyperplasia, raising the hypothesis that TGF-α may induce the expression of genes related to the process of differentiation along the surface cell lineages. In a model of TGF-α knockout mice, expression of TFF2 and TFF3 after gastric ulceration was increased in wild type mice and unchanged in knockout mice, supporting a role for TGF-α in the regulation and induction of gastrointestinal regeneration and differentiation mediated by TFF peptides.[44]

In conclusion, our observation of TFF1 expression in the duct-like tubular complexes in the pancreas of transgenic mice overexpressing TGF-α and in human chronic pancreatitis, in which TGF-α is overexpressed in the pancreatic parenchyma, along with the reported induction of TFF1 by TGF-α in other transgenic models, raises the hypothesis that TFF1 may be involved in the formation of tubular complexes upon induction by TGF-α and thus points to a role for TFF peptides as differentiation factors.

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1 Korc M, Schmiegel W. Chronic pancreatitis. *Curr Opin Gastroenterol* 1994;**10**:502–6.
2 DiMagno EP, Layer P, Clain JE. Chronic pancreatitis. In: Go VLW, ed. *The pancreas, pathology, and disease*. New York: Raven Press, 1993:665–706.
3 Oertel JE, Heffess CS, Oertel YC. Pancreas. In: Sternberg SS, ed. *Diagnostic surgical pathology.* New York: Raven Press, 1993:57–93.
4 Elsässer HP, Adler G, Kern HF. Fibroblast structure and function during regeneration from hormone-induced acute pancreatitis in the rat. *Pancreas* 1989;**4**:169–78.
5 Friess H, Yamanaka Y, Büchler M, *et al.* Increased expression of acidic and basic fibroblast growth factors in chronic pancreatitis. *Am J Pathol* 1994;**144**:117–28.
6 Korc M, Friess H, Yamanaka Y, *et al*. Chronic pancreatitis is associated with increased levels of the epidermal growth factor receptor, TGF-α, and phospholipase-C gamma. *Gut* 1994;**35**:1468–73.
7 Friess H, Yamanaka Y, Büchler M, *et al.* A subgroup of patients with chronic pancreatitis overexpress the c-erb B-2 protooncogene. *Ann Surg* 1994;**220**:183–92.
8 Ebert M, Kasper HU, Hernberg S, *et al.* Overexpression of platelet-derived growth factor (PDGF) B chain and type β PDGF receptor in human chronic pancreatitis. *Dig Dis Sci* 1998;**43**:567–74.
9 Bockman DE, Merlino G. Cytological changes in the pancreas of transgenic mice overexpressing transforming growth factor-α. *Gastroenterology* 1992;**103**:1883–92.
10 Wright NA, Hoffmann W, Otto WR, *et al.* Rolling in the clover: trefoil factor family (TFF)-domain peptides, cell migration and cancer. *FEBS Lett* 1997;**408**:121–3.
11 Hoffmann W, Hauser F. The P-domain or trefoil motif: a role in renewal and pathology of mucous epithelia? *Trends Biochem Sci* 1993;**18**:239–43.
12 Williams GR, Wright NA. Trefoil factor family domain peptides. *Virchows Arch* 1997;**431**:299–304.
13 Thim L. Trefoil peptides: from structure to function. *Cellular and Molecular Life Sciences* 1997;**53**:888–903.
14 Sands BE, Podolsky DK. The trefoil peptide family. *Annu Rev Physiol* 1996;**58**:253–73.

15 Wright N, Poulsom R, Stamp GWH, *et al*. Epidermal growth factor (EGF/URO) induces expression of regulatory peptides in damaged human gastrointestinal tissues. *J Pathol* 1990;**162**:279–84.
16 Jakowlew SB, Breathnach R, Jeltsch JM, *et al*. Sequence of the pS2 mRNA induced by estrogen in the human breast cancer cell line MCF-7. *Nucleic Acids Res* 1984;**12**:2861–78.
17 Nunez AM, Berry M, Imler JL, *et al*. The 5' flanking region of the pS2 gene contains a complex enhancer region responsive to oestrogens, epidermal growth factor, a tumor promotor (TPA), the c-Ha-ras oncoprotein and the c-jun protein. *EMBO J* 1989;**8**:823–9.
18 Dante R, Ribieras S, Baldasini S, *et al*. Expression of an estrogen-induced breast cancer-associated protein (pS2) in benign and malignant human ovarian cysts. *Lab Invest* 1994;**71**:188–92.
19 Henry JA, Piggott NH, Mallick UK, *et al*. pNR-2/pS2 immunohistochemical staining in breast cancer: correlation with prognostic factors and endocrine response. *Br J Cancer* 1991;**63**:615–22.
20 Collier JD, Bennett MK, Bassendine MF, *et al*. Immunolocalization of pS2, a putative growth factor, in pancreatic carcinoma. *J Gastroenterol Hepatol* 1995;**10**:396–400.
21 Wright NA. The expression of growth factors in the pancreas in chronic pancreatitis. *Digestion Surgery* 1994;**11**: 143–6.
22 Dignass A, Lynch-Devaney K, Kindon H, *et al*. Trefoil peptides promote epithelial migration through a transforming growth factor-β independent pathway. *J Clin Invest* 1994;**94**:376–83.
23 Playford RJ, Marchbank T, Chinery R, *et al*. Human spasmolytic polypeptide is a cytoprotective agent that stimulates cell migration. *Gastroenterology* 1995;**108**:108–16.
24 Chinery R, Playford RJ. Combined intestinal trefoil factor and epidermal growth factor is prophylactic against indomethacin-induced gastric damage in the rat. *Clin Sci* 1995;**88**:401–3.
25 Marchbank T, Westley BR, May FEB, *et al*. Cys^{58} of human pS2 plays a key role in the stimulation of healing in vitro and in vivo [abstract]. *Gut* 1997;**41**:A45.
26 Lefebvre O, Chenard MP, Masson R, *et al*. Gastric mucosa abnormalities and the tumorigenesis in mice lacking the pS2 trefoil protein. *Science* 1996;**274**:259–62.
27 Bockman DE, Boydston WR, Anderson MC. Origin of tubular complexes in human chronic pancreatitis. *Am J Surg* 1982;**144**:243–9.
28 Wagner M, Lührs H, Klöppel G, *et al*. Malignant transformation of duct-like cells originating from acini in TGFα transgenic mice. *Gastroenterology* 1998;**115**:1254–62.
29 Parsa I, Longnecker DS, Scarpelli DG, *et al*. Ductal metaplasia of human exocrine pancreas and its association with carcinoma. *Cancer Res* 1985;**45**:1285–90.
30 Sandgren EP, Leuttecke NC, Palmiter RD, *et al*. Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* 1990;**61**:1121–35.
31 Klöppel G, Maillet B. Pathology of acute and chronic pancreatitis. *Pancreas* 1993;**8**:659–70.
32 Ebert M, Yokoyama M, Kobrin MS, *et al*. Induction and expression of amphiregulin in human pancreatic cancer. *Cancer Res* 1994;**54**:3959–62.
33 Jeltsch JM, Roberts M, Schatz C, *et al*. Structure of the human oestrogen-responsive gene ps2. *Nucleic Acids Res* 1987;**15**:1401–14.
34 Lefebvre O, Wolf C, Kedinger M, *et al*. The mouse one P-domain (pS2) and two P-domain (mSP) genes exhibit distinct patterns of expression. *J Cell Biol* 1993;**122**:191–8.
35 Dallman MJ, Larsen CP, Morris PJ. Cytokine gene transcription in vascularised organ grafts: analysis using semiquantitative polymerase chain reaction. *J Exp Med* 1991;**174**:493–6.
36 Balmain A, Krumlauf R, Vass JK, *et al*. Cloning and characterization of the abundant cytoplasmic 7S RNA from mouse cells. *Nucleic Acids Res* 1982;**10**:4259–77.
37 Piggott NH, Henry JA, May FEB, *et al*. Antipeptide antibodies against the pNR-2 oestrogen-regulated protein of human breast cancer cells and detection of pNR-2 expression in normal tissues by immunohistochemsitry. *J Pathol* 1991;**163**:95–104.
38 Henry PA, Bennett MK, Piggott NH, *et al*. Expression of the pNR-2/pS2 protein in diverse human epithelial tumours. *Br J Cancer* 1991;**64**:677–82.
39 Arias AE, Bendayan M. Differentiation of pancreatic acinar cells into duct-like cells in vitro. *Lab Invest* 1993;**69**:518–30.
40 Korc M. Role of growth factors in pancreatic cancer. *Surg Oncol Clin North Am* 1998;**7**:25–41.
41 Welter C, Theisinger B, Seitz G, *et al*. Association of the spasmolytic polypeptide and an estrogen-induced breast cancer protein (pS2) with human pancreatic carcinoma. *Lab Invest* 1992;**66**:187–92.
42 Wright NA, Pike C, Elia G. Induction of a novel epidermal growth factor-secreting cell lineage by mucosal ulceration in human gastrointestinal stem cells. *Nature* 1990;**343**:82–5.
43 Goldenring JR, Poulsom R, Ray GS, *et al*. Expression of trefoil peptides in the gastric mucosa of transgenic mice overexpressing transforming growth factor-α. *Growth Factors* 1996;**13**:111–19.
44 Cook GA, Yeomans ND, Giraud AS. Temporal expression of trefoil peptides in the TGF-alpha knockout mouse after gastric ulceration. *Am J Physiol* 1997;**272**:G1540–9.