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Preferential expression of cystein-rich secretory protein-3 (CRISP-3) in chronic pancreatitis

Q. Liao[1], J. Kleeff[1], Y. Xiao[1], A. Guweidhi[1], A. Schambony[3], E. Töpfer-Petersen[3], A. Zimmermann[2], M.W. Büchler[1] and H. Friess[1]

[1]Department of General Surgery, University of Heidelberg, Heidelberg, Germany, [2]Institute of Pathology, University of Bern, Bern, Switzerland and [3]Institute of Reproductive Medicine, School of Veterinary Medicine Hannover, Hannover, Germany

Summary. Background: Chronic pancreatitis (CP) is a progressive inflammatory process resulting in exocrine and endocrine pancreatic insufficiency in advanced stages. Cysteine-rich secretory protein (CRISP-3) has been identified as a defense-associated molecule with predominant expression in the salivary gland, pancreas and prostate. Aims: In this study, we investigated CRISP-3 expression in normal pancreatic tissues, chronic pancreatitis tissues, pancreatic cancer tissues and pancreatic cancer cell lines, as well as in other gastrointestinal organs. Materials and Methods: 15 normal pancreatic tissues, 14 chronic pancreatitis tissues and 14 pancreatic cancer tissues as well as three pancreatic cancer cell lines were analyzed. Moreover, hepatocellular carcinoma and esophageal, stomach and colon cancers were also analyzed together with the corresponding normal controls. Results: CRISP-3 was expressed at moderate to high levels in chronic pancreatitis tissues and at moderate levels in pancreatic cancer tissues but at low levels in normal pancreatic tissues, and was absent in three pancreatic cancer cell lines. CRISP-3 expression was below the level of detection in all cancerous gastrointestinal tissues and in all normal tissues except 2 of 16 colon tissue samples. CRISP-3 mRNA signals and immunoreactivity were strongly present in the cytoplasm of degenerating acinar cells and in small proliferating ductal cells in CP tissues and CP-like lesions in pancreatic cancer tissues. In contrast, CRISP-3 expression was weak to absent in the cytoplasm of cancer cells as well as in acinar cells and ductal cells in pancreatic cancer tissues and normal pancreatic tissues. Conclusion: These results reveal that the distribution of CRISP-3 in gastrointestinal tissues is predominantly in the pancreas. High levels of CRISP-3 in acinar cells dedifferentiating into small proliferating ductal cells in CP and CP-like lesions in pancreatic cancer suggests a role of this molecule in the pathophysiology of CP.

Key words: CRISP-3, Chronic pancreatitis, Pancreatic cancer

Offprint requests to: Helmut Friess, M.D., Department of General Surgery, University of Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany. Fax: + + 49 6221 56 6903. e-mail: helmut_friess@med.uni-heidelberg.de

Introduction

Chronic pancreatitis (CP) is a progressive inflammatory disease of the pancreas, clinically characterized by recurrent attacks of abdominal pain, and in advanced stages by exocrine and endocrine pancreatic insufficiency. Morphologically and histologically, CP is characterized by acinar cell destruction, dedifferentiation of acinar cells into ductular complexes, duct cell proliferation and hyperplasia, fibrosis, and infiltration of inflammatory cells.

Human cysteine-rich secretory protein 3 (CRISP-3), also named SGP28 (specific granule protein of 28 kDa), is the third member of the cysteine-rich secretory protein family, which shares about 72% amino acid identity with CRISP-2/TPX-1 (Haendler et al., 1993; Kratzschmar et al., 1996). CRISP-3 has been detected in several human tissues, with predominance in the salivary gland, pancreas and prostate, and in less abundance in the epididymis, ovary, thymus and colon (Kratzschmar et al., 1996). Because of its sequence homology to the pathogenesis-related proteins (Pfisterer et al., 1996) and its expression in B lymphocytes, CRISP-3 has been considered a defense-associated molecule in mammals (Pfisterer et al., 1996). Previously, using DNA array technology, we have demonstrated an increase of CRISP-3 mRNA expression in CP in comparison with the normal pancreas and pancreatic cancer (Friess et al., 2001). In the present study, to assess the potential role of CRISP-3 in chronic pancreatitis and pancreatic cancer, we have further investigated CRISP-3 expression and localization in other normal and diseased gastrointestinal tissues, including hepatocellular carcinoma and esophageal, stomach and colon cancer, as well as

inflammatory bowel disease. We now show that CRISP-3 is predominantly expressed in proliferating ductal cells in CP and in CP-like lesions in pancreatic cancer, suggesting a role of this molecule in the pathogenesis of CP.

Materials and methods

Patients and tissue collection

Normal human pancreatic tissues were obtained through an organ donor program from 15 (4 female and 11 male) individuals who were free of any apparent pancreatic disease. The median age of the organ donors was 48 years, with a range of 17 to 73 years. Fourteen (3 female and 11 male) CP tissues and 14 (8 female and 6 male) pancreatic cancer tissues were obtained from patients undergoing pancreatic resection. The median ages of the chronic pancreatitis and pancreatic cancer patients were 50 and 62 years, with a range of 33 to 68 years and 44 to 78 years, respectively. The etiology was alcoholic CP in 12 of 14 patients, hereditary CP in one patient, and acquired pancreatic duct obstructive CP in one patient.

Other gastrointestinal cancer tissues were obtained from 12 hepatocellular carcinoma, 7 esophageal, 10 stomach and 12 colon cancer patients undergoing tumor resection, as well as from 27 patients with inflammatory bowel disease (18 Crohn's disease, 9 ulcerative colitis) undergoing resection. Ten normal liver tissues were obtained from patients undergoing liver resection. Normal esophageal, stomach, and colon tissues were obtained from individuals through an organ donor program (6 esophageal, 10 stomach and 16 colon).

Freshly removed tissue samples were immediately fixed in paraformaldehyde solution for 12 to 24 h and paraffin-embedded for in situ hybridization and immunohistochemical staining. Concomitantly, tissue samples for RNA and protein extraction were immediately snap frozen in liquid nitrogen in the operating room upon surgical removal and maintained at -80 °C until use for Northern blot and Western blot analysis. The Human Subject Committees of the University of Bern, Switzerland and the University of Heidelberg, Germany approved the studies, and written informed consent was obtained from each patient prior to surgery.

Cell culture

Human pancreatic cancer cells were routinely grown in DMEM (PANC-1, MIA-PaCa-2) or RPMI 1640 (T3M4) supplemented with 10% heat-inactivated FCS (fetal calf serum), 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine. Cells were maintained at 37 °C in a humid chamber with 5% CO_2 and 95% air atmosphere. All materials for cell culture were purchased from Biochrom KG (Berlin, Germany).

Probe synthesis for Northern blot analysis

A 210 bp fragment of human CRISP-3 cDNA (GenBank accession number X95240) was amplified by RT-PCR using the following primers: CRISP-3 (forward: 5'-CATGCTGAAGATGGAATGGA -3'; reverse: 5'-GGCCCTACACCAAAGTCAAA -3'). The purified PCR products were subcloned into the pGEM-T Easy vector (Promega Biotechnology, Madison, WI, USA) according to the manufacturer's instructions. The sequence of the cDNA fragment was confirmed by automated sequencing (Microsynth GmbH Sequencing Group, Balgach, Switzerland). A 190 bp fragment of mouse 7S that cross-hybridizes with human 7S was used to verify equivalent RNA loading and transfer in Northern blot analysis. For Northern blot analysis, the probes were radiolabeled with α-^{32}P dCTP (Du Pont International, Regensdorf, Switzerland) using a random primer labeling system (Roche Diagnostic Ltd., Rotkreuz, Switzerland) (Kleeff et al., 1998, di Mola et al., 1999). For in situ hybridization, digoxigenin-labeled CRISP-3 cRNA sense and antisense probes were generated using the Ribomax System (Promega Biotechnology, Madison, WI, USA) and the appropriate polymerases, as described previously (Kleeff et al., 1998; di Mola et al., 1999).

Northern blot analysis

The procedures have been described in detail previously (Kleeff et al., 1998, di Mola et al., 1999). Briefly, following electrophoresis of 20 μg total RNA in 1.2% agarose/1.8M formaldehyde gels, RNA was electrotransferred onto nylon membranes and cross-linked by UV irradiation. The filters were prehybridized for 6 h at 42 °C and then hybridized for 20 h at 42 °C in the presence of the radiolabeled cDNA probes for CRISP-3 (10^6 cpm/ml). Blots were then rinsed twice with 2x SSC at 50 °C, and washed twice with 0.2x SSC/2% SDS at 55 °C for 20 min. All blots were exposed at -80 °C to Kodak BioMax films with Kodak intensifying screens, and the intensity of the radiographic bands was quantified by video image analysis, using the Image-Pro plus software (Media Cybernetics, Silver Spring, MD, USA). To verify equivalent RNA loading on Northern blot membranes, filters were rehybridized with the 7S cDNA probe, as reported previously (Kleeff et al., 1998; di Mola et al., 1999).

In situ hybridization

Tissue sections (4 μm) were deparaffinized, dehydrated, and incubated in 0.2 M HCl for 20 min. The sections were treated with proteinase K (50 μg/ml) for 15 min at 37 °C. Following post-fixation with 4% paraformaldehyde in phosphate buffered saline (PBS) for 5 min, the samples were prehybridized at 60 °C for 2 hours in 50% formamide (v/v), 4x SSC, 2x Denhardt's

solution and 250 μg/ml RNA. Hybridization was performed overnight at 60 ºC in 50% (v/v) formamide, 4x SSC, 2x Denhardt's solution, 500 μg/ml RNA and 10% dextran sulfate (w/v). The final concentrations of the digoxigenin-labeled probes were approximately 0.5 ng/μl. After hybridization, the sections were washed and treated with RNase. The samples were then incubated with an anti-digoxigenin antibody conjugated with alkaline phosphatase (1:500). For color reaction, 5-bromo-4-chloro-3-indolyl phosphate and nitro-blue-tetrazolium were used. For control experiments, the slides were incubated with RNase or with the corresponding sense probes. Pretreatment of the slides with RNase abolished the hybridization signals produced by the antisense probe. Furthermore, incubation with the sense probe failed to produce specific in situ hybridization signals (Kleeff et al., 1998, di Mola et al., 1999). The materials for in situ hybridization were purchased from Roche GmbH, Mannheim, Germany (proteinase K, RNase, AP-Conjugate) or Sigma, Buchs, Switzerland (RNA and NBT).

Western blot analysis

Approximately 200 mg of frozen normal, chronic pancreatitis and pancreatic cancer tissues were powdered and thawed in an ice-cold suspension buffer (10 mM Tris-HCl, pH 7.6, 100 mM NaCl) containing a proteinase inhibitor cocktail (R&D Systems GmbH, Wiesbaden-Nordenstadt, Germany). Cultured pancreatic cancer cells ($1x10^6$) were washed in ice-cold PBS, scraped, centrifuged briefly and lysed in 1 ml of ice-cold lysis buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% SDS), supplemented with the Complete-TM mixture of proteinase inhibitors (Roche Diagnostics, Basel, Switzerland). Tissues and cells were homogenized for 5 min, then centrifuged (14,000 rpm, 30 min at 4 °C), the supernatants were collected, and the protein concentration was measured with the BCA protein assay (Pierce Chemical Co., Rockford, IL, USA). 40 μg protein from each sample was diluted in sample buffer (250 mM Tris-HCl, 4% SDS, 10% glycerol, 0.006% bromophenol blue and 2% ß-mercaptoethanol), boiled for 5 min, cooled on ice for 5 min, and size-fractionated on 12% SDS-polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes at 100 V for 90 min.

Subsequently, membranes were incubated in a blocking solution (5% non-fat milk in 20 mM Tris-HCl, 150 mM NaCl, 0.1% Tween-20: TBS-T), followed by incubation overnight with a polyclonal chicken anti-CRISP-3 antibody (Schambony et al., 1998) in blocking solution (1:10,000) at 4 °C. Membranes were then washed with TBS-T and incubated with a horseradish peroxidase-conjugated anti-chicken IgG (BioRad Laboratories, Hercules, CA, USA, 1:3000 dilution) for 1h at room temperature. Antibody detection was performed with the enhanced chemoluminescence (ECL) Western blot detection system (Amersham Life Science, Amersham, UK). The intensity of the bands was quantified by video image analysis, using the Image-Pro plus software.

Immunohistochemistry

Immunohistochemical analysis was performed with the streptavidin-peroxidase technique as previously reported (Kleeff et al., 1998; di Mola et al., 1999). Briefly, consecutive 3-5 μm paraffin-embedded tissue sections were deparaffinized and dehydrated. Incubating the slides in methanol containing 0.6% hydrogen peroxide blocked endogenous peroxidase activity. Then the sections were incubated for 30 min at room temperature with 10% normal goat serum prior to overnight incubation at 4 °C with the chicken anti-CRISP-3 antibody (Schambony et al., 1998) diluted in 10% normal goat serum (1:1000). Secondary biotinylated goat anti-chicken IgG (Kirkegaard & Perry Laboratories Inc., Gaithersburg, MD, USA) was used. Slides were counterstained with Mayer's hematoxylin. To ensure specificity of the primary antibody, consecutive tissue sections were incubated in the absence of the primary antibody. In this case no immunostaining was detected.

Statistical analysis

Results are expressed as median and mean ± SD. The Mann-Whitney-U test was used for statistical analysis. p values of <0.05 were defined as significant.

Results

CRISP-3 expression in pancreatic tissues and other gastrointestinal tissues

15 normal human pancreas, 14 chronic pancreatitis and 14 human pancreatic cancer samples, as well as three pancreatic cancer cell lines, were investigated by Northern blotting to detect the expression of CRISP-3 mRNA (Fig. 1). The transcript size was approximately 2.2 kb for CRISP-3 mRNA, consistent with the known length of the CRISP-3 cDNA (2124 bp) (Kjeldsen et al., 1996). CRISP-3 mRNA was present at relatively moderate to high levels in chronic pancreatitis samples and at moderate to low levels in pancreatic cancer samples, but at relatively low levels in all normal pancreas samples, and was absent in three pancreatic cancer cell lines (Fig. 1A). Quantification of the mRNA signals revealed that CRISP-3 mRNA levels were 5.3±1.6-fold and 37.3±6.8-fold increased in chronic pancreatitis samples in comparison with pancreatic cancer samples and normal pancreatic samples, respectively ($p<0.05$) (Fig. 1B). In contrast to pancreatic tissues, CRISP-3 mRNA was absent in other gastrointestinal cancer tissues (12 hepatocellular carcinomas and 7 esophageal, 10 stomach and 12 colon cancers). CRISP-3 mRNA was also absent in

inflammatory bowel disease (18 Cohn's disease, 9 ulcerative colitis tissues). In addition, CRISP-3 mRNA expression was absent in normal liver, esophageal, and stomach samples, and detectable only in 2/16 normal colon tissues (Fig. 1C and data not shown).

To determine whether CRISP-3 protein levels were also elevated in chronic pancreatitis, Western blot analysis (Fig. 2) was performed in 6 normal, 8 CP, and 7 pancreatic cancer samples and in 3 pancreatic cancer cell lines, which were also used for Northern blot analysis. This analysis revealed a single immunoreactive band of approximately 28 kDa in accordance with the known size of CRISP-3 (Kjeldsen et al., 1996). CRISP-3 protein levels were high in CP tissues and moderate in pancreatic cancer tissues, but weak to absent in normal tissues (Fig. 2A, B), and were absent in pancreatic cancer cell lines (Fig. 2B). Densitometric analysis (Fig. 2C) of all the autoradiograms (6 normal, 8 CP, 7 pancreatic cancer samples) indicated that there was a 1.8±6.7-fold and 5.6±7.7-fold increase of CRISP-3 protein levels in CP tissues in comparison with pancreatic cancer tissues and normal pancreatic tissues, respectively ($p<0.05$).

To assess the exact site of CRISP-3 mRNA expression in human pancreatic tissues, in situ hybridization analyses were carried out next (Fig. 3). In normal pancreatic tissues, CRISP-3 mRNA signals were weak to absent in the cytoplasm of acinar cells, ductal cells, and islet cells (Fig. 3A). CRISP-3 mRNA signals were weak to absent in the cytoplasm of morphologically normal ductal and acinar cells (Fig. 3B), inflammatory cells and islet cells in CP, but intense in the cytoplasm of degenerating acinar cells and proliferating ductal cells in CP (Fig. 3C) and CP-like lesions in pancreatic cancer tissues. In contrast, CRISP-3 mRNA levels were weak to absent in the cytoplasm of cancer cells (Fig. 3D) as well as in inflammatory cells and islet cells in pancreatic cancer tissues. The control

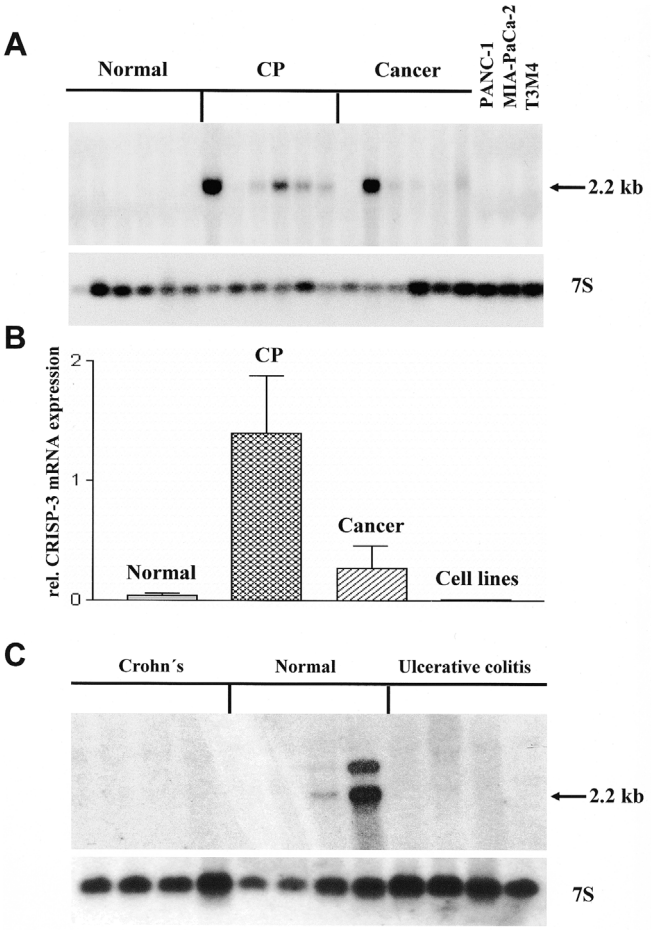

Fig. 1. Northern blot analysis of CRISP-3 mRNA expression. Total RNA (20 μg) isolated from pancreatic tissues and cancer cells is subjected to Northern blot analysis and hybridized with the ^{32}P-labeled CRISP-3 cDNA probe. Blots are subsequently rehybridized with a 7S cDNA probe to verify equivalent RNA loading **(A)**. Relative CRISP-3 mRNA expression is calculated as ODCRISP-3/OD7S for each sample **(B)**. Total RNA (20 μg) isolated from normal colon specimen and inflammatory bowel tissues as indicated is subjected to Northern blot analysis **(C)**.

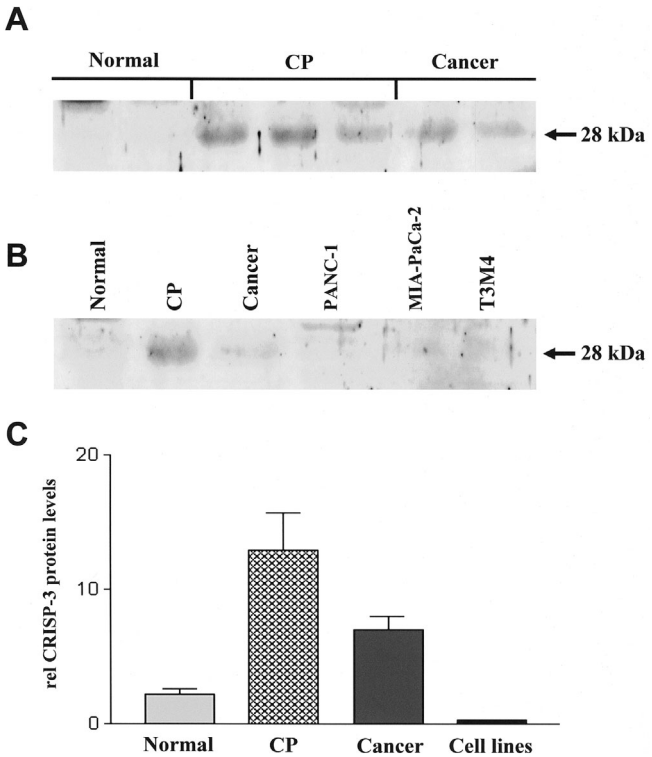

Fig. 2. Western blot analysis of CRISP-3 protein levels in pancreatic tissues and pancreatic cancer cells. Protein from each sample (40 μg is subjected to immunoblot analysis using the anti-CRISP-3 antibody as described in the Methods section. Antibody detection is performed with the enhanced chemoluminescence system **(A, B)**. **A, B:** representative blots are shown. Densitometry of all Western blots **(C)** shows that CRISP-3 protein levels are significantly increased in chronic pancreatitis tissues in comparison with normal pancreatic tissues, pancreatic cancer tissues and pancreatic cancer cells ($p<0.05$).

CRISP-3 mRNA sense probe did not reveal any specific signal (data not shown).

Similar to the in situ hybridization findings, in the normal pancreas, CRISP-3 immunoreactivity was weak to absent in the cytoplasm of acinar cells, ductal cells, and islet cells (Fig. 4A). CRISP-3 immunoreactivity was weak to absent in normal-appearing acinar cells (Fig. 4B) but strongly present in the cytoplasm of degenerating acinar and proliferating ductal cells in CP tissues (Fig. 4C,D) and CP-like lesions in pancreatic cancer tissues (Fig. 4E), but weak to absent in the cytoplasm of cancer cells (Fig. 4F) as well as in the cytoplasm of morphologically normal ductal cells, inflammatory cells and islet cells in pancreatic cancer tissues and CP tissues (data not shown).

Discussion

CP is characterized histomorphologically by progressive development of fibrosis and atrophy and

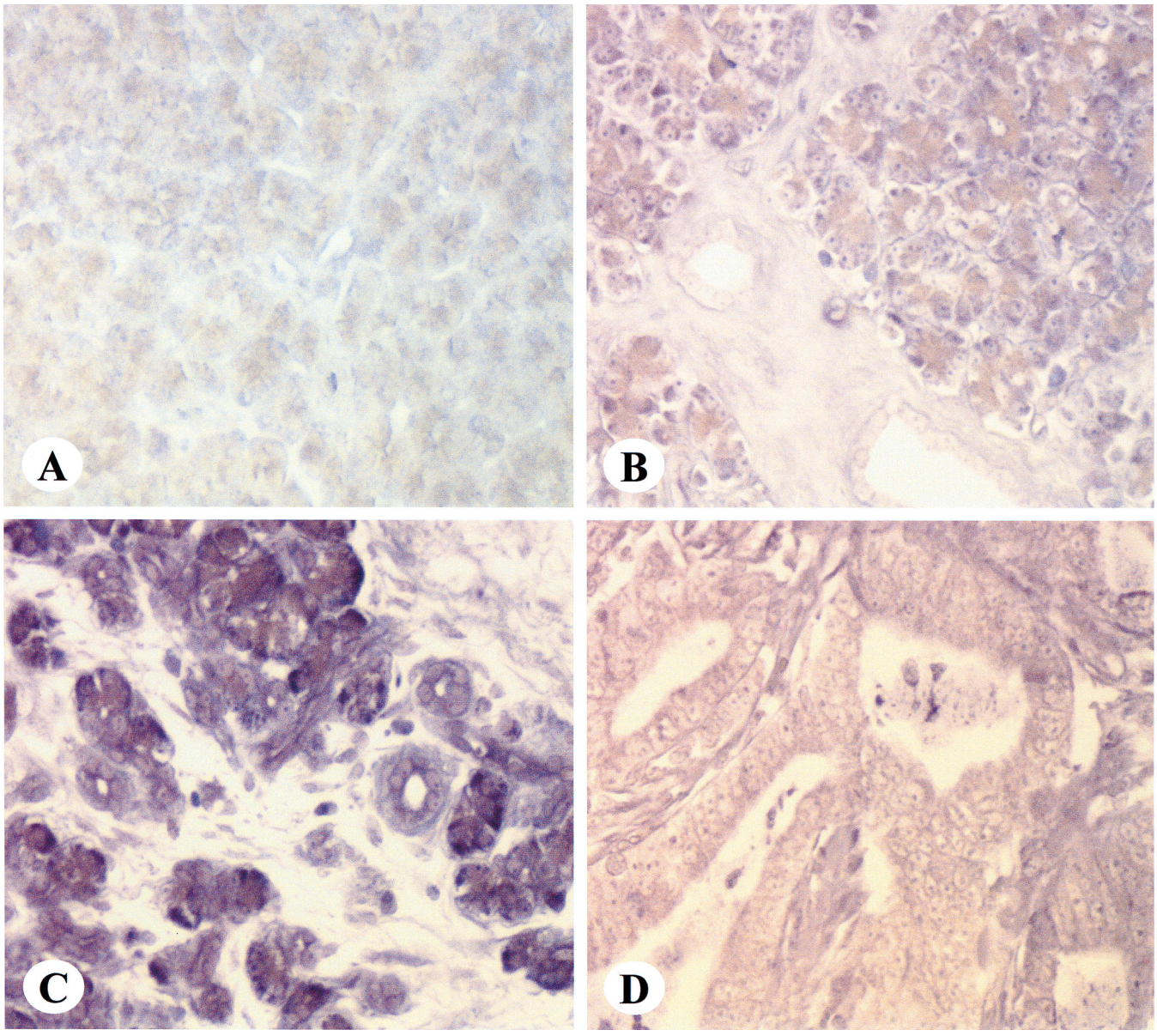

Fig. 3. In situ hybridization of CRISP-3 is carried out with a CRISP-3-specific digoxigenin-labeled antisense probe and anti-digoxigenin antibodies conjugated with alkaline phosphatase as described in the Methods section. CRISP-3 mRNA signals are weak to absent in normal pancreatic tissue **(A)**; weak to absent in the cytoplasm of ductal cells and normal-appearing acinar cells in CP **(B)**, but intense in the cytoplasm of degenerating acinar cells and proliferating ductal cells from acinar cells in CP tissues **(C)**. In contrast, CRISP-3 mRNA signals are weak to absent in the cytoplasm of cancer cells in pancreatic cancer tissues **(D)**. x 200

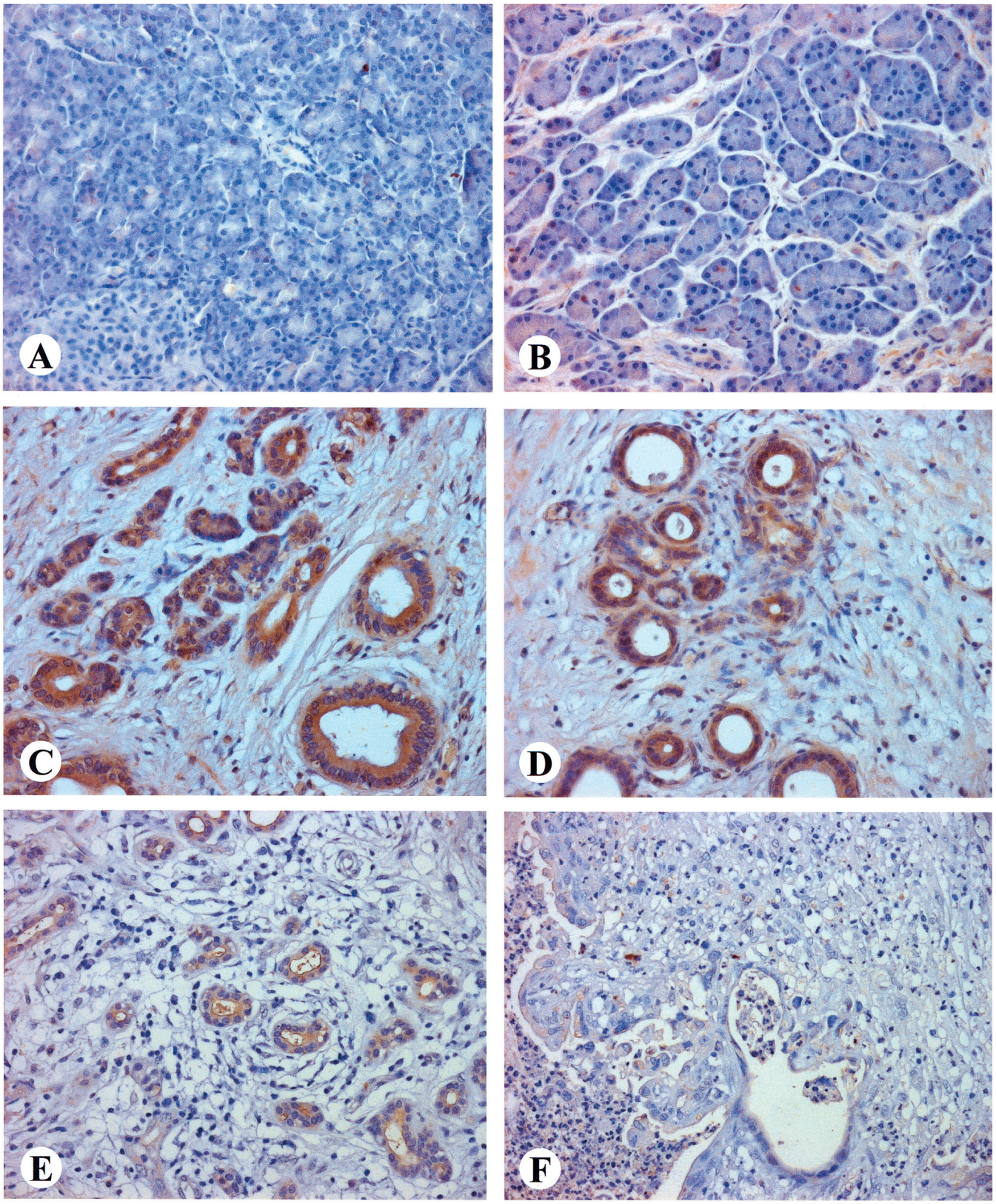

Fig. 4. Immunohistochemical analysis of CRISP-3 is performed with the streptavidin-peroxidase technique using the anti-CRISP-3 antibody as described in the Methods section. CRISP-3 immunoreactivity is weak to absent in the cytoplasm of acinar cells, ductal cells, and islet cells in normal pancreatic tissues **(A)**; weak to absent in normal-appearing acinar cells in CP **(B)**; strongly present in the cytoplasm of degenerating acinar and proliferating ductal cells from acinar cells in CP tissues **(C, D)** and CP-like lesions in pancreatic cancer tissues **(E)**, but weak to absent in the cytoplasm of cancer cells in pancreatic cancer tissues **(F)**. x 200

remodeling of the pancreatic parenchyma, including acinar cell degeneration, ductal cell proliferation and hyperplasia (DiMagno, 1993). Degenerating/ dedifferentiating acinar cells are characterized by widening of acinar lumina and decreased height of acinar cells. These cells then form tubular complexes composed of low cuboidal or flattened cells surrounding a large acinar lumen, with a loss of pancreatic enzyme synthesis, and an increase of cytoskeletal proteins. Although the morphological and molecular changes in CP have been more clearly identified in the past decade, the complex pathogenetic mechanisms of CP have not been completely elucidated (Adler and Schmid, 1997; Friess et al., 1998).

The cysteine-rich secretory protein (CRISP) family was originally described in the mouse, and comprises evolutionarily conserved polypeptides with a potential involvement in the innate immunity (Haendler et al., 1993; Kratzschmar et al., 1996). The growing superfamily of CRISPs is characterized by 16 conserved cysteine residues, 14 of them clustered in the C-terminal half of the protein (17). The CRISP genes are located on human chromosome 6 in close vicinity to the major histocompatability complex region. They are expressed in the specific granules of human neutrophils (Kjeldsen et al., 1996), in murine B cell precursors (Pfisterer et al., 1996), by glands with an exocrine function and by mucosal epithelial surfaces (Mizuki and Kasahara, 1992; Eberspaecher et al., 1995), which suggests a role in non-specific defense reactions, similar to the other antimicrobial peptides such as defensins (Kagan et al., 1994) and magainins (Berkowitz et al., 1990).

Members of the CRISP gene family include CRISP-1, CRISP-2 (also called testis-specific protein1 "Tpx1"), and CRISP-3 (also called specific granule protein of 28 kDa "SGP28") (Kjeldsen et al., 1996; Kratzschmar et al., 1996). Human CRISP-3 is most closely related to human CRISP-2, with an amino acid homology of 72% (Kratzschmar et al., 1996). The tissue distribution of CRISP transcripts shows that the expression of human CRISP-1 and CRISP-2 is confined to the male genital tract (Kratzschmar et al., 1996). Thus, CRISP-1 expression is limited to the epididymis, while CRISP-2 expression is high in the testis and also clearly detectable in the epididymis (Kratzschmar et al., 1996). In contrast, CRISP-3 transcripts are more widely distributed and are abundant in salivary glands, pancreas and prostate, and are less abundant in the epididymis, ovary, thymus and colon (Kratzschmar et al., 1996).

The function of CRISP-3 is unknown, but its expression pattern suggests a specificity for tissues with an exocrine function (Kratzschmar et al., 1996). A direct role in the secretory process itself or in secretions, where CRISP-3 might possibly act as an anti-infectious agent, is therefore possible (Kratzschmar et al., 1996). In this context, it has been shown that CRISP-3 is expressed in the B-lymphoid lineage, specifically at the pre-B cell stage (Pfisterer et al., 1996). In addition, the expression of CRISP-3 in B cells is regulated by Oct2, a transcription factor expressed throughout the B lymphoid lineage which plays an essential role during the terminal phase of B cell differentiation (Pfisterer et al., 1996). Furthermore, CRISP-3 has been identified as a novel early response gene that may participate in the pathophysiology of autoimmune lesions in Sjögren's syndrome (Tapinos et al., 2002).

Previously, we have demonstrated increased levels of CRISP-3 mRNA in CP in comparison with the normal pancreas and pancreatic cancer (Friess et al., 2001). In the present study, Northern blot analysis showed that CRISP-3 mRNA was expressed specifically in diseased pancreatic tissues. Thus, high expression was seen in CP and pancreatic cancer, as compared to normal control tissues. Interestingly, CRISP-3 mRNA expression was below the level of detection in cancerous gastrointestinal tissues, which included hepatocellular carcinoma, esophageal cancer, stomach cancer and colon cancer. CRISP-3 mRNA expression was also below the level of detection in inflammatory bowel diseases. In normal colon samples, CRISP-3 expression was only evident in 2 of 16 tissues. These findings are partly in disagreement with results obtained by Kartzschmar and co-workers, who demonstrated CRISP-3 expression in the normal colon (Kratzschmar et al., 1996). However, in their study, poly-A mRNA was utilized to detect weak CRISP-3 expression in the normal colon, while in the present study total RNA was used for analysis. It is therefore likely that CRISP-3 mRNA levels are too low in most normal colon specimen to be detected by total RNA Northern blot analysis.

In situ hybridization and immunohistochemistry analysis showed that CRISP-3 mRNA and protein were strongly present in the cytoplasm of degenerating acinar cells and proliferating ductal cells in CP and CP-like lesions adjacent to the pancreatic cancer masses, but weak to absent in the cytoplasm of cancer cells in pancreatic cancer. Furthermore, CRISP-3 expression was weak to absent in the cytoplasm of acinar cells, ductal cells and islets in normal pancreatic tissues. Although the utilized CRISP-3 antibody was raised against equine CRISP-3 and might cross-react with CRISP-1 and CRISP-2, three lines of evidence suggest that the observed immunostaining was specific for CRISP-3. First, Western blot analysis revealed a single immunoreactive band of 28 kDa, which is in accordance with the known size of CRISP-3 (Kjeldsen et al., 1996). Second, there was a close correlation between CRISP-3 in situ hybridization and immunohistochemistry results. Third, previous analyses have shown that CRISP-1 expression is epididymis-specific, CRISP-2 expression is mainly observed in the testis and the epididymis, whereas CRISP-3 mRNA expression is more widely distributed and abundant in the pancreas (Kratzschmar et al., 1996). The differences in the observed CRISP-3 expressions levels in the normal pancreas in the aforementioned and our study might be due to different methods of quantification of CRISP-3 expression. Irrespectively, we could confirm that CRISP-3 is

expressed in the normal pancreas yet not (or in less abundance) in other gastrointestinal tissues, which confirms the results of Kratzschmar et al. (1996).

Our results suggest that CRISP-3 expression is not associated with carcinogenesis of pancreatic cancer inasmuch as pancreatic cancer cells exhibited only weak to absent CRISP-3 expression in vitro and in vivo. In contrast, CRISP-3 seems to play an important role in acinar cell degeneration and ductal cell proliferation in the course of the tissue destruction and remodeling in CP. During the progression of CP, the destruction of the pancreatic parenchyma is accompanied by tissue remodeling, with ductal cell proliferation and ductular hyperplasia (Sarles et al., 1989, Adler and Schmid, 1997, Friess et al., 1998, di Mola et al., 1999). CRISP-3, which is expressed specifically by glands with an exocrine function (Kratzschmar et al., 1996), is expressed in proliferating ductal cells but not in cancer cells that have lost their exocrine function.

In vivo studies have shown that CRISP-3 is under androgen control in the salivary gland (Haendler et al., 1993, 1997; Schwidetzky et al., 1995; Schambony et al., 1998). Studies of the androgen profile in patients with CP and pancreatic cancer have revealed that serum testosterone, dihydrotestosterone with testosterone/dihydrotestosterone ratio and androstanediol glucuronide levels were significantly lower in pancreatic cancer patients compared to CP patients (Robles-Diaz et al., 1987; Sperti et al., 1992; Jansa et al., 1996). It is tempting to speculate that this changed androgen profile might contribute to the observed differential expression of CRISP-3 in CP and pancreatic cancer.

CRISP-3 expression was absent in inflammatory cells, such as B-lymphocytes and granulocytes, in CP tissues. Although this observation is somewhat surprising, it might be explained by the absence of precursor B lymphocytes in CP and pancreatic cancer (Emmrich et al., 1998), in which CRISP-3 is known to be expressed. In addition, it has been shown that CRISP-3 is expressed in specific granules of neutrophils in response to pathogen infections (Pfisterer et al., 1996; Haendler et al., 1997), which might also be absent in CP and pancreatic cancer (Emmrich et al., 1998).

In conclusion, the present study revealed that the distribution of CRISP-3 in gastrointestinal tissues is predominantly in the pancreas. High levels of CRISP-3 in acinar cells dedifferentiating into small proliferating ductal cells in CP and CP-like lesions in pancreatic cancer suggests a role of this molecule in the pathophysiology of CP.

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