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Increased Expression of Acidic and Basic Fibroblast Growth Factors in Chronic Pancreatitis

Helmut Friess,* Yoichiro Yamanaka,* Markus Büchler,† Hans G. Beger,‡ David A. Do,* Michael S. Kobrin,* and Murray Korc*

From the Division of Endocrinology and Metabolism, Department of Medicine and Biological Chemistry, University of California, Irvine, California; Department of Visceral and Transplantation Surgery,† University of Bern, Bern, Switzerland; and Department of Surgery,‡ University of Ulm, Ulm, Germany*

Acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) belong to a family of mitogenic polypeptides that are involved in cellular proliferation and differentiation. In this study we investigated the potential role of aFGF and bFGF in chronic pancreatitis (CP), a fibrotic condition associated with acinar cell dedifferentiation and atrophy, and fibroblastic proliferation. By immunohistochemistry, aFGF and bFGF were abundant in pancreatic ductal and acinar cells in pancreatic tissues from CP patients. Immunoblotting with the same highly specific monoclonal antibodies demonstrated a marked increase in aFGF and bFGF in pancreatic homogenates from CP patients by comparison with the normal pancreas. Northern blot analysis indicated that, by comparison with normal controls, 16 of 21 CP tissues exhibited a 14-fold increase in aFGF mRNA levels, and 19 of 21 CP tissues exhibited a 15-fold increase in bFGF mRNA levels. **In situ** ***hybridization confirmed that this overexpression occurred in ductal and acinar cells, and indicated that both mRNA moieties colocalized with their respective proteins. These findings suggest that aFGF and bFGF may either be involved in the pathobiological mechanisms that occur in CP, or that their overexpression may be the consequence of other perturbations that occur in this disorder. (Am J Pathol 1994, 144: 117–128)***

Chronic pancreatitis (CP) is an inflammatory, often painful disease of the exocrine pancreas, which leads to exocrine insufficiency.[1,2] The histomorphological changes that occur in CP include enlarged pancreatic ducts, pseudoductular hyperplasia with redifferentiation of acinar cells, encroachment of periductular and intralobular connective tissue, atrophy, loss of acinar cells, replacement of the functional parenchyma by variable amounts of fibrosis, and infiltration by inflammatory cells.[3] The biochemical and molecular mechanisms that underlie the pathophysiology of CP are poorly understood. It has been proposed that the release of cytokines from inflammatory cells may be involved in stimulating fibroblast proliferation and collagen biosynthesis.[4] However, transgenic mice that overexpress transforming growth factor-α (TGF-α) exhibit marked pancreatic fibrosis and redifferentiation of acinar cells into tubular structures in the absence of inflammatory infiltrates.[5] Furthermore, pancreatic exocrine cells of patients with CP overexpress the epidermal growth factor (EGF) receptor and TGF-α.[6] These observations suggest that changes in the pattern of growth factor expression may be involved in the pathophysiological processes that occur in CP.

The fibroblast growth factor (FGF) gene family consists of a group of homologous growth-promoting polypeptides, that include acidic (aFGF) and basic (bFGF) fibroblast growth factors (also known as FGF-1 and FGF-2, respectively), *int-2* (FGF-3), and the gene products of *hst* (Kaposi FGF or FGF-4), FGF-5, FGF-6, keratinocyte growth factor (FGF-7), FGF-8 (androgen-induced growth factor), and FGF-9.[7,8] aFGF and bFGF are closely related prototypes of this family.[7,8] Both factors are chemotactic toward fibroblasts, promote angiogenesis, and participate in tissue repair.[7,9] Recent studies have implicated the excessive production of bFGF as potentially contributing to fibroblastic proliferation and fibrosis in a number of conditions, including Dupuytren's disease, be-

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Address reprint requests to Dr. Murray Korc, Division of Endocrinology and Metabolism, Medical Sciences 1, C240, University of California, Irvine, CA 92717.

nign prostatic hyperplasia, and endometriosis.[10–12] Furthermore, rat pancreatic AR42J cells that were retrovirally transfected to concomitantly overproduce authentic bFGF and high molecular forms of bFGF were found to exhibit fibroblastic changes in culture, and to be associated with an increased fibroblastic reaction after subcutaneous injection in nude mice *in vivo*.[13] These observations raise the possibility that aberrant FGF production may contribute to some of the histological changes observed in CP. It is not known, however, whether and in which cell types FGFs are expressed in the pancreas in CP. Therefore, in this study, we used immunohistochemical, immunoblotting, Northern blotting and *in situ* hybridization techniques to analyze the expression of aFGF and bFGF in the pancreas of patients with CP. We now report that both aFGF and bFGF are overexpressed in the pancreas of many of these patients and that this overexpression occurs in both acinar and ductal cells.

Materials and Methods

Pancreatic tissues were obtained from 4 female and 17 male patients undergoing a duodenum-preserving pancreatic head resection for CP.[14] The median age of the CP patients was 41.5 years, with a range of 30 to 56 years. Normal human pancreatic tissue samples were obtained from 3 female and 12 male individuals that were free of disease through an organ donor program. The median age of the organ donors was 38.5 years with a range of 18 to 54 years. The tissue samples used for analysis in this control group were also obtained from the head of the pancreas. Freshly removed tissue samples were fixed in Bouin solution or 10% formaldehyde solution for 18 to 24 hours and paraffin embedded for histological analysis. Concomitantly, tissues destined for RNA extraction were frozen in liquid nitrogen and maintained at −80 C until use. Histologically, the CP was graded as moderate to severe in all the patients. All studies were approved by the Human Subjects Committees of the University of Ulm, Germany, and the University of California, Irvine.

Immunocytochemistry

Two highly specific monoclonal antibodies (antibovine aFGF and antibovine bFGF, Upstate Biotechnology Inc., Lake Placid, NY) that recognize human aFGF and bFGF, respectively, were used for immunohistochemical analysis. Consecutive paraffin-embedded sections (5-μm thick) were subjected to immunostaining using a streptavidin-peroxidase technique (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD), as previously reported.[15,16] Optimal results for both antibodies were obtained at an antibody dilution of 1:100. Bound antibody was detected with a biotinylated anti-mouse IgG secondary antibody and streptavidin-peroxidase complex, followed by incubation with diaminobenzidine tetrahydrochloride as the substrate.[15,16] The slides were counterstained with Mayer's hematoxylin. To ensure specificity of the anti-aFGF and anti-bFGF antibodies, consecutive sections were either incubated in the absence of the primary antibody or with a non-immunized mouse IgG antibody. In both cases no immunostaining was detected. Incubation of anti-aFGF antibodies with aFGF but not bFGF abolished the aFGF signal. Furthermore, incubation of anti-bFGF antibodies with bFGF but not aFGF completely abolished the bFGF signal.

Immunoblotting

Pancreatic homogenates from three normal organ donors and three patients with CP were subjected to heparin-Sepharose column chromatography.[17] aFGF and bFGF were eluded with 1.0 and 2.0 M NaCl, respectively.[17] Aliquots of the eluents (100 μg protein/lane) were subjected to 12.5% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, NH).[18] The filters were incubated for 1 hour at 23 C with 5% nonfat milk, and for 18 hours at 4 C with the respective primary antibody (aFGF, 1:400; bFGF, 1:800 dilution). The filters were then washed with buffer containing 20 mM Tris (pH 7.5), 500 mM NaCl, and 0.05% volume/volume Tween 20, and incubated sequentially with an anti-mouse IgG (1:2500 dilution) and ^{125}I-protein A (0.33 μCi/ml). After washing, the blots were exposed for 4 days at −80 C to Kodak XAR-5 film with intensifying screens.

In Situ *Hybridization*

Tissue sections were placed on poly-L-lysine-coated slides then digested with 1 μg/ml proteinase K (Boehringer Mannheim Biochemicals, Indianapolis, IN) and washed with 0.5× SSC (1× SSC = 150 mM NaCl, 15 mM Na citrate, pH 7.0), as previously reported.[15,16,19] After prehybridization for 3 hours, hybridization was initiated by adding the ^{35}S-labeled aFGF or bFGF antisense riboprobe and 50 μg of yeast tRNA (GIBCO BRL, Inc., Gaithersburg, MD).[15,16] The sections were then washed, digested

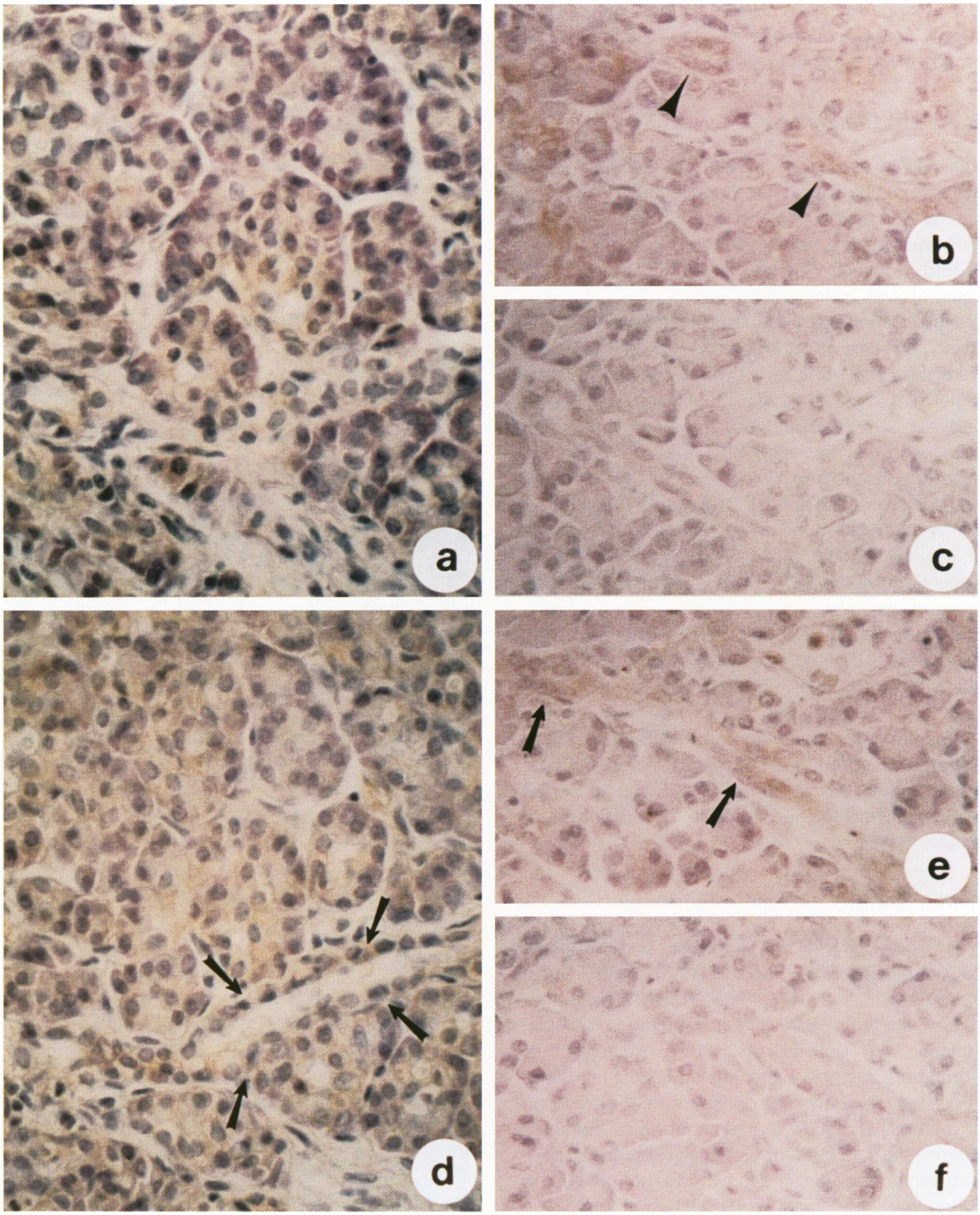

Figure 1. *Immunostaining in the normal human pancreas. aFGF* (**A, B**) *and bFGF* (**D, E**) *immunoreactivity was present in acinar and ductal cells. Arrowheads denote ductal cells exhibiting aFGF immunoreactivity. Arrows denote ductal cells exhibiting bFGF immunoreactivity. Specificity of anti-aFGF and anti-bFGF antibodies was confirmed by incubating consecutive sections with the respective antibodies that were preincubated with aFGF* (**C**) *or bFGF* (**F**), *which completely abolished the respective signals. Original magnification,* ×*100.*

with RNAse A (20 μg/ml; Sigma Chemical Co., St. Louis), coated with NTB2 nuclear emulsion (Eastman Kodak Co.), and exposed in the dark for 8 days.[15,16,19] After development, the slides were counterstained with Mayer's hematoxylin. Pretreatment of the slides with RNAse abolished the hybrid-

ization signal by the antisense probe. Furthermore, sense probes corresponding to the antisense probes failed to produce a signal.

Northern Blot Analysis

Total RNA was extracted by the guanidine isothiocyanate method and size fractionated on a 1.2% agarose/1.8 M formaldehyde gel.[15,20,21] Poly(A)$^+$ RNA was prepared after isolation of total RNA using oligo(dT) column chromatography.[22] The RNA (20 µg/lane) was electrotransferred onto nylon membranes (GeneScreen, Du Pont, Boston) and cross-linked by ultraviolet irradiation.[15,21,22] The blots were then prehybridized, hybridized, and washed under two high stringency conditions, depending on whether antisense riboprobes or cDNA probes were used, as previously described.[15,20–22] In the case of antisense riboprobe, the blots were prehybridized overnight at 65 C and then hybridized for 18 hours at 65 C in the presence of 1×10^6 cpm/ml of the ^{32}P-labeled antisense riboprobe.[15,20–22] To assess equivalent RNA loading, all blots were rehybridized with a mouse 7S cDNA, which cross hybridizes with human 7S RNA.[15,21,23] The blots were exposed at −80 C to Kodak XAR-5 film with Kodak intensifying screens. The intensity of the radiographic bands was quantified by laser densitometry (Ultrascan XL, Pharmacia LKB Biotechnology, Uppsala, Sweden), and the ratio of the optical densities of the RNA levels (aFGF/7S and bFGF/7S) was calculated for each sample, as previously reported.[15,21,22]

cRNA and cDNA Probe Synthesis

A 417-bp *Bam*HI/*Eco*RI fragment of the human pJC3–5 aFGF cDNA[24] and a 219-bp *Bam*HI/*Eco*RI fragment of human pHFL1–7 bFGF cDNA[25] were subcloned into the pGEM3Zf vector (Promega Biotech., Madison, WI). Both cDNAs were a gift from Dr. J. Abraham (California Biotechnology, Mountain View, CA). Probe synthesis was conducted in the presence of either [α-^{32}P]CTP (Amersham Inc., Arlington Heights, IL) for Northern blot analysis or [α-^{35}S]UTP (Amersham Inc.) for *in situ* hybridization. A 190-bp *Bam*HI fragment of mouse 7S cDNA was random labeled with [α-^{32}P]dCTP (Amersham Inc.), as previously reported.[15,21]

Statistical Analysis

The results are expressed as median and range or as mean ± SE. For statistical analysis, the Student's

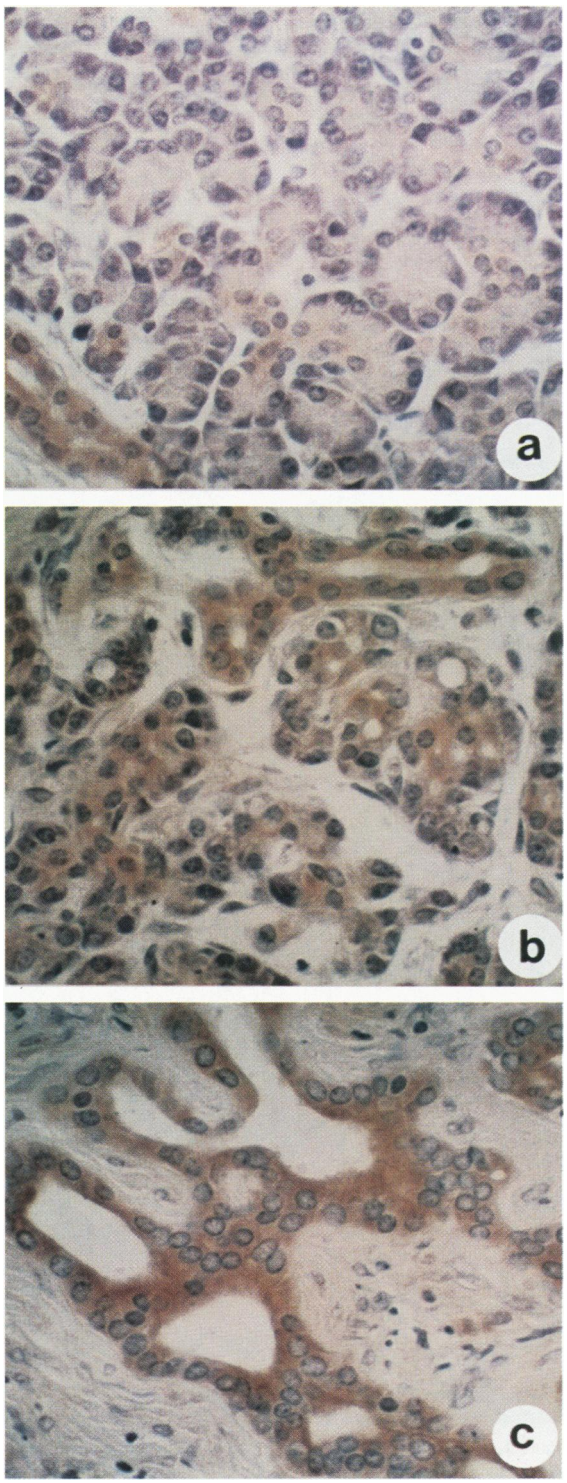

Figure 2. *Immunostaining of aFGF in the pancreas of CP patients. aFGF immunoreactivity was present in acinar and ductal cells in areas of the pancreas that appeared relatively normal* (**A**). *Intense immunoreactivity was present in atrophic acinar cells* (**B**) *and in pseudoductular structures* (**C**). *Original magnification,* ×*100.*

t-test and linear regression analysis were used. Significance was defined as $P < 0.05$.[26]

Results

Immunohistochemical and Protein Analysis

In the normal pancreas, faint aFGF (Figure 1A) and bFGF (Figure 1C) immunostaining was present in the cytoplasm of acinar and ductal cells. Incubation of anti-aFGF antibodies with aFGF abolished the aFGF signal (Figure 1B). Furthermore, incubation of anti-bFGF antibodies with bFGF abolished the bFGF signal (Figure 1D). Both proteins often colocalized as confirmed by immunostaining of consecutive sections (Figure 1A and C). However, aFGF was present more frequently in ductal cells, whereas bFGF was present more frequently in acinar cells. In pancreatic tissues from CP patients, aFGF and bFGF immunoreactivity was markedly increased. The intensity of the immunostaining was only slightly increased in the normal acinar and ductal cells in CP tissues (Figure 2A and 3A). In contrast, there was intense immunostaining for both factors in the degenerating acinar and ductal cells (Figures 2B and 3B), and in regions exhibiting pseudoductular metaplasia (Figures 2C and 3C). The ductal cells of the larger interlobular ducts also exhibited intense immunostaining for both aFGF and bFGF (Figure 4A and B), which was especially prominent at the apical aspect of the cells. Analysis of consecutive sections indicated that aFGF and bFGF immunoreactivity colocalized throughout the pancreatic parenchyma of the CP tissues, and that neither growth factor was evident in the connective tissue and stromal fibroblasts (Figures 2 to 5). As in the case of the normal pancreas, incubation of anti-aFGF antibodies with aFGF abolished the aFGF signal (Figure 5B), whereas incubation of anti-bFGF antibodies with bFGF abolished the bFGF signal (Figure 5D).

To confirm that the increased immunohistochemical staining observed in the CP samples was due to increased levels of aFGF and bFGF, immunoblotting of pancreatic extracts was conducted next. Immunoblotting with the same highly specific monoclonal antibodies that were used for immunohistochemical analysis demonstrated the presence of aFGF (16.5-kd band) and bFGF in the normal human pancreas. The latter migrated as a major 18-kd band and a minor 24-kd band (Figure 6). In the case of bFGF, the CP samples also exhibited a

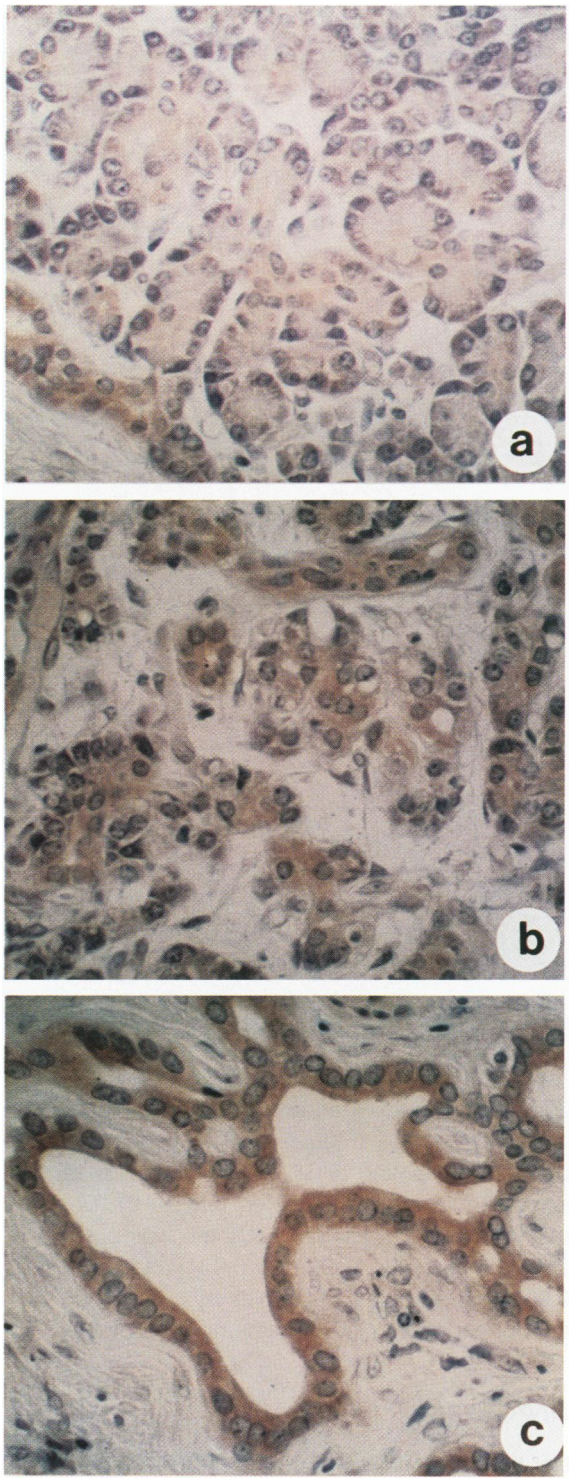

Figure 3. *Immunostaining of bFGF in the pancreas of CP patients. Consecutive sections corresponding to the panels in Figure 2 were stained with anti-bFGF antibodies. bFGF immunoreactivity was present in acinar and ductal cells in areas of the pancreas that appeared relatively normal* (A). *Intense immunostaining was evident in atrophic acinar cells* (B) *and in the pseudoductular structures* (C). *Original magnification,* ×100.

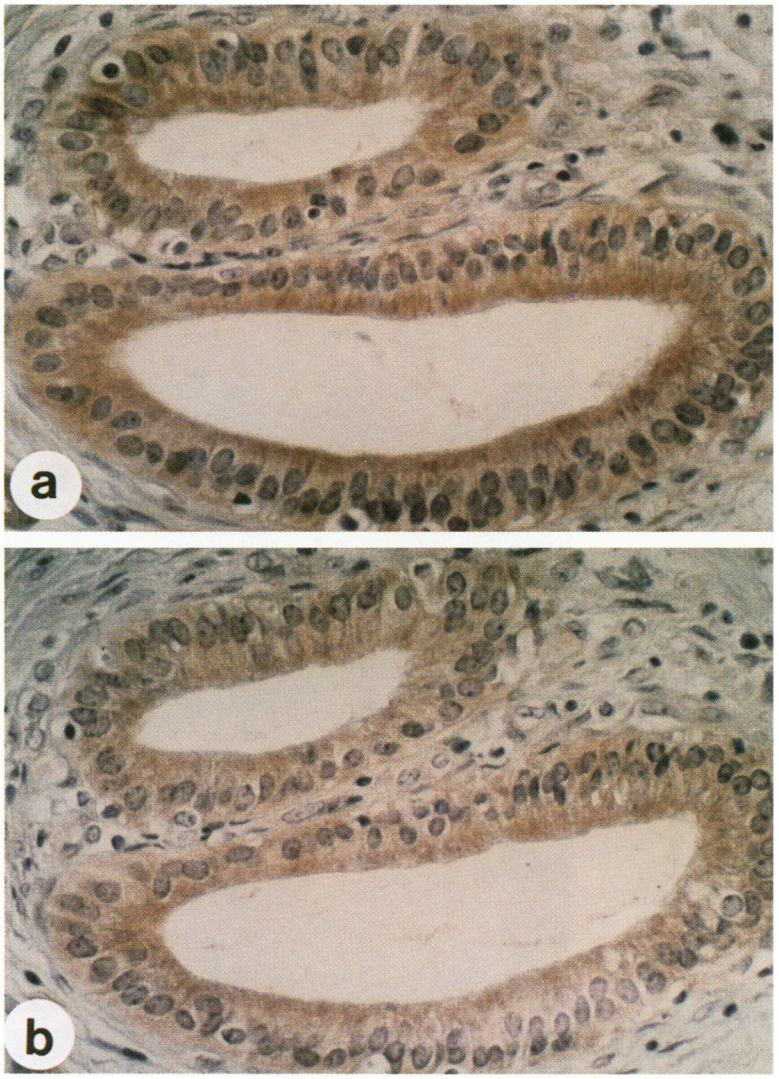

Figure 4. *Immunostaining in ductal cells of large ducts in the pancreas of CP patients. aFGF* (**A**) *and bFGF* (**B**) *colocalized in the ductal cells of the larger ducts. Immunostaining was distributed in the cytoplasm of the cells, and was especially prominent at the apical aspect. Original magnification, ×100.*

29-kd band, as well as two small bands in the 15- and 16-kd range (Figure 6). The intensity of the bands representing aFGF and bFGF was markedly increased in the CP samples (Figure 6). Densitometric analysis indicated that there was a 13- and 28-fold increase in aFGF ($P < 0.001$) and bFGF ($P < 0.001$) levels in the CP samples by comparison with the corresponding levels in the normal samples.

RNA Analysis

Northern blot analysis of poly $(A)^+$ RNA isolated from the normal pancreas revealed a single band (approximately 4.8 kb) in the case of aFGF (Figure 7). In contrast, in the case of bFGF, two major bands (approximately 7.0 and 3.7 kb) were readily evident (Figure 7). In addition, there were two smaller bFGF RNA transcripts (approximately 2.2 and 1.2 kb), which were faint and photographed poorly (Figure 7). Northern blot analysis of total pancreatic RNA revealed a single aFGF transcript (4.8 kb) in both the normal pancreas and the CP samples (Figure 7). Sixteen of the 21 CP samples exhibited an increase in aFGF mRNA levels by comparison with the corresponding levels in the normal pancreas. In the case of bFGF, only the 7.0-kb band was evident in the normal samples, and this band was quite faint (Figure 7). With the exception of two cases, both major bFGF bands were readily visible in the CP samples (Figure 7). Furthermore, the CP samples often exhibited the two smaller bFGF RNA transcripts (2.2 and 1.2 kb). Overall, only two CP samples failed to demonstrate an increase in the levels of either aFGF or bFGF mRNA levels.

Densitometric analysis of all the Northern blots indicated that there was a 10-fold ($P < 0.001$) and a

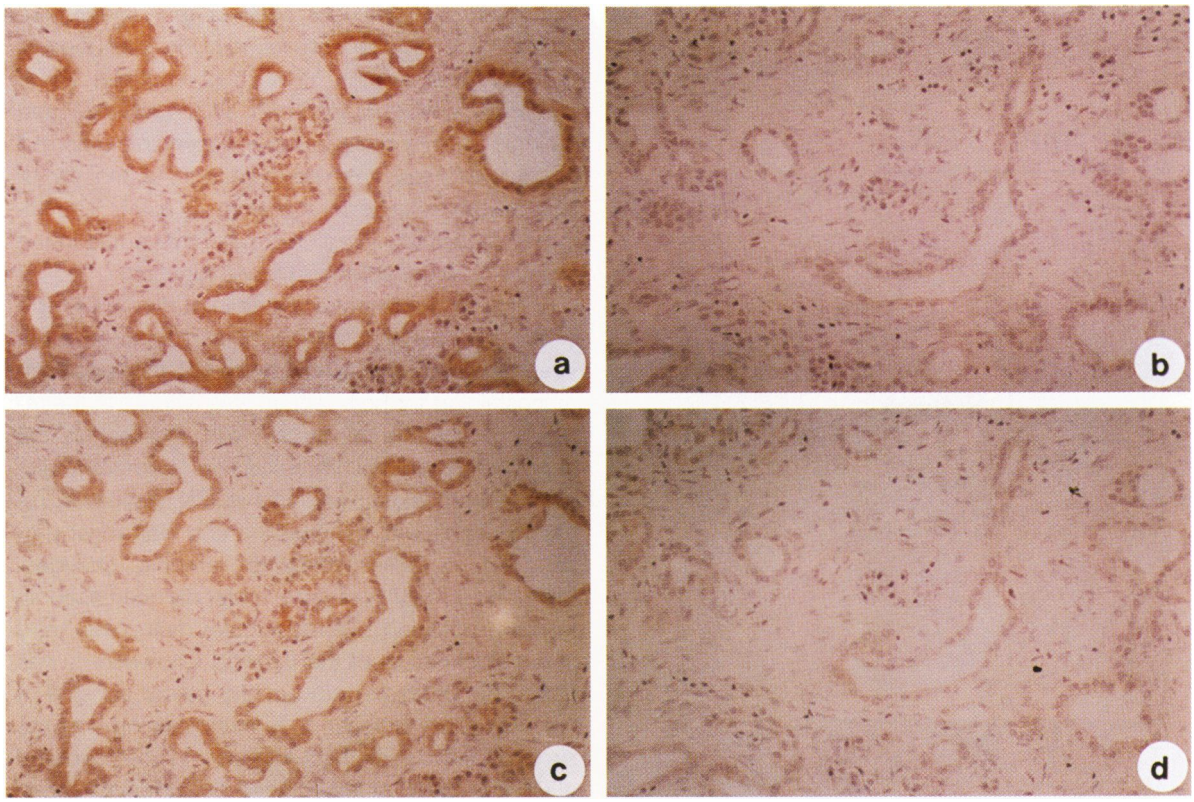

Figure 5. *Colocalization of aFGF and bFGF. Immunostaining of consecutive tissue sections in the pancreas of CP patients revealed the presence of intense aFGF* (A) *and bFGF* (C) *immunoreactivity in atrophic acinar cells and in the pseudoductular structures. Incubation of consecutive sections with anti-aFGF antibodies that have been preincubated with aFGF* (B) *or anti-bFGF antibodies that have been preincubated with bFGF* (D) *abolished the respective signals. Original magnification, ×50.*

14-fold increase ($P < 0.001$) in aFGF and bFGF mRNA levels in the CP samples, respectively, by comparison with the corresponding levels in the normal pancreas (Table I). When only the CP tissues that exhibited increased mRNA levels were included in the calculation, there was a 14-fold increase in aFGF mRNA levels (16 of 21 samples) and a 15-fold increase in bFGF mRNA levels (19 of 21 samples) (Table 1). Linear regression analysis of the fold increase above control in aFGF and bFGF mRNA levels of the individual CP samples indicated that there was a significant positive correlation between the fold increase for each factor ($r = 0.88$, $P < 0.001$). Thus, there was a strong tendency for their concomitant overexpression.

To determine the exact cell type that expressed aFGF and bFGF mRNA, *in situ* hybridization was performed next. aFGF and bFGF mRNA *in situ* hybridization grains were present at low levels in both acinar and ductal cells in the normal human pancreas (Figure 8A and C). In the CP samples that exhibited overexpression by Northern blot analysis, there was a marked increase in the *in situ* hybridization signal in many acinar and ductal cells, especially when these cells exhibited atrophic changes (Figure 8B and D). In contrast, *in situ* hybridization grains were only slightly increased in the surrounding stroma.

Discussion

aFGF and bFGF belong to a family of growth factors that show a high affinity for heparin. aFGF and bFGF are mitogenic polypeptides that regulate numerous biological functions in various cells, including cellular differentiation, migration, and angiogenesis.[7,8] In addition, bFGF increases the production of collagen and plasminogen activator, which are thought to be critical events in tissue remodeling.[27,28] aFGF and bFGF bind to high affinity transmembrane receptors that contain an intracellular tyrosine kinase domain that is separated into two contiguous regions and an extracellular domain that has a two and three immunoglobulinlike region.[29,30] The binding of aFGF and bFGF to their high affinity receptors may require the presence of heparin sulfate proteoglycans, which are present on the cell surface and in the extracel-

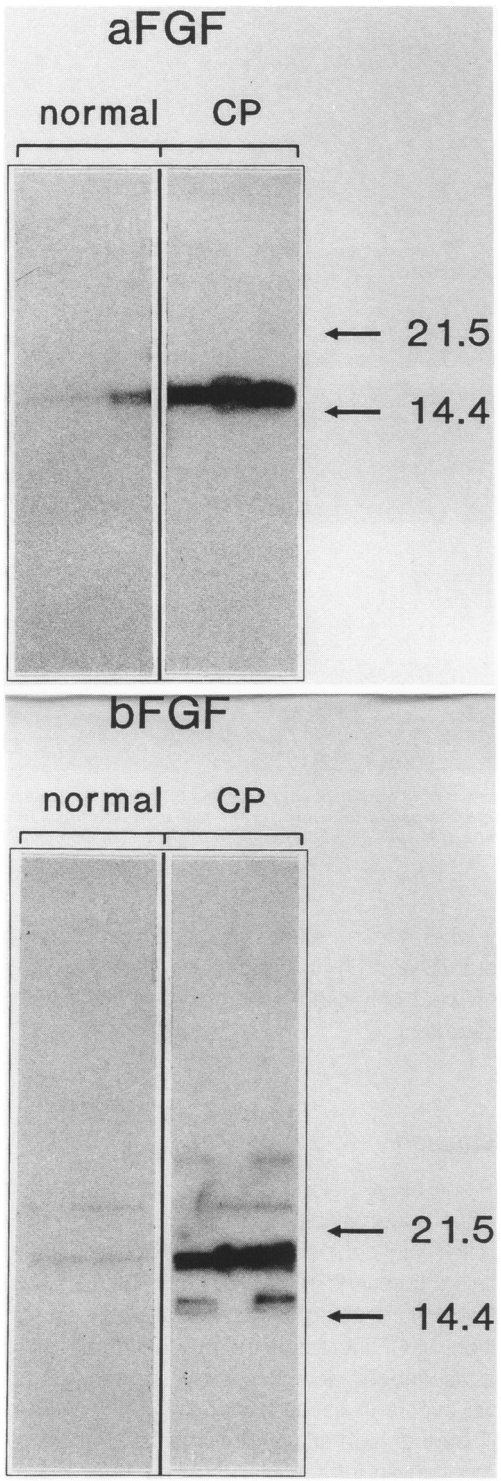

Figure 6. *Immunoblotting. Extracts from three normal human pancreatic tissues and three pancreatic tissues obtained from CP patients were subjected to heparin-sepharose chromatography. Eluents were subjected to electrophoresis and transferred to nitrocellulose membranes for immunoblotting using anti-aFGF antibodies (top) and anti-bFGF antibodies (bottom). The autoradiographs were exposed for 4.5 days. The molecular sizes (kd) of the standards are indicated on the right.*

lular matrix.[31,32] Although aFGF is abundant in neural tissues, it has also been found in the heart, kidney, prostate, and liver.[11,12,29,33,34] In contrast, bFGF seems to be more widespread. aFGF and bFGF and their high affinity receptors are present in the normal human pancreas.[35] Furthermore, both factors stimulate amylase release in rat pancreatic acini,[36,37] raising the possibility that FGFs may have a role in the regulation of pancreatic exocrine function.

In this study we have determined that aFGF and bFGF are overexpressed in human pancreatic tissues obtained from patients with CP. Increased immunostaining for both factors was evident in the remaining acinar and ductal cells, but not in the surrounding stromal fibroblasts or connective tissue. In both the acinar and ductal cells, the immunostaining was cytoplasmic and was most pronounced in cells exhibiting atrophic changes. Immunoreactivity was also markedly increased in regions exhibiting pseudoductular metaplasia, and in the ductal cells of the larger ducts where it was especially prominent toward the apical aspects of the cells. This increase in immunostaining was associated with a marked increase in the levels of aFGF and bFGF as determined by immunoblotting.

In the case of bFGF, immunoblotting revealed the presence of two small (15 and 16 kd) proteins in the CP samples, which most likely represent breakdown products of bFGF.[17] In addition, there was an increase in the intensity of the 24-kd protein and the appearance of a 29-kd protein in the CP samples. Similar high molecular weight forms of bFGF have been previously described in a number of cells, including mammary epithelial cells and neonatal fibroblasts[38,39] and are attributed to the initiation of translation at three CUG start codons that are upstream of the classical AUG initiation site for the 18-kd protein.[40]

Northern blot analysis indicated that aFGF and bFGF mRNA levels were markedly increased in the CP tissues by comparison with the levels observed in the normal pancreas. Most likely this overexpression occurs as a result of enhanced transcription of the respective genes and/or increased mRNA stability. By *in situ* hybridization, both mRNA moieties were expressed at high levels in the remaining acinar and ductal cells, especially in regions exhibiting either cellular atrophy or pseudoductular metaplasia. In contrast, a few *in situ* hybridization grains were present in the surrounding stromal fibroblasts. Taken together with the immunostaining data, these observations suggest that the exocrine cells in CP tissues express high levels of aFGF and bFGF as a

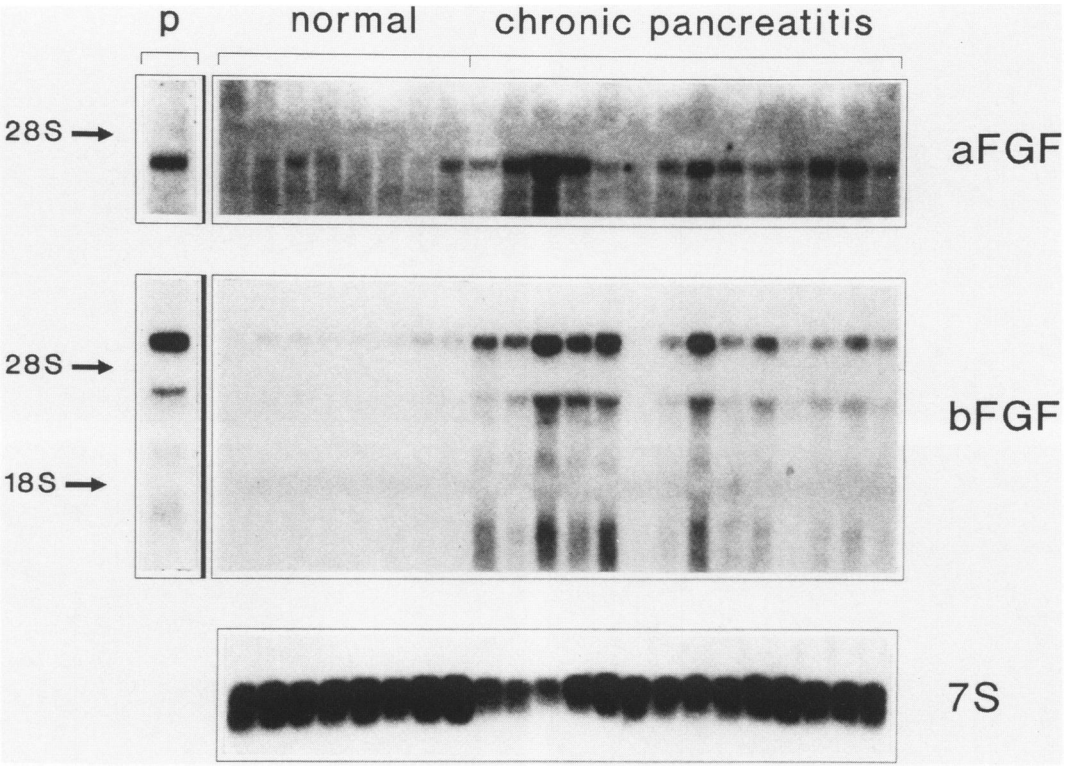

Figure 7. *Northern blot analysis. Poly(A)$^+$ RNA (20 μg, lane p) from the normal human pancreas, and total RNA (20 μg/lane) from the normal human pancreas and pancreatic tissues obtained from CP patients were size fractionated on agarose gels and electrotransferred to a nylon membrane. The filters were probed with [α-^{32}P]-labeled aFGF riboprobe (1 × 10^6 cpm/ml, exposure time 6 days), bFGF riboprobe (1 × 10^6 cpm/ml, exposure time 5 days), and 7S cDNA (1 × 10^5 cpm/ml, exposure time 18 hours). In all cases, aFGF migrated as a single (approximately 4.2 kb) transcript. Analysis of poly(A)$^+$ RNA indicated that four bFGF mRNA transcripts (approximately 7.2, 3.8, 2.2, and 1.2 kb) were expressed in the normal pancreas. aFGF and bFGF were overexpressed in most of the CP samples. The cytoplasmic 7S mRNA, migrating as a 0.4-kb species, was used to assess RNA loading. The migration position of 28S and 18S ribosomal subunits are shown on the left.*

Table 1. *Densitometric Analysis*

	Relative Optical Density	
	aFGF/7S	bFGF/7S
Normal	26.2 ± 5.8 (15)	13.8 ± 2.0 (15)
All CP	268.2 ± 20.5* (21)	183.3 ± 12.5* (21)
CP with increased RNA levels	359.4 ± 15.8* (16)	211.5 ± 9.6* (19)

Northern blots were analyzed by densitometry. Values are the means ± SE from the number of normal and CP samples indicated in parentheses. The ratio of aFGF to the 7S signal (aFGF/7S) and the ratio of bFGF to the 7S signal (bFGF/7S) was calculated for each sample and the resulting normalized values were used for statistical analysis.

* $P < 0.01$, when compared with respective controls.

result of an increase in the synthesis of both factors within these cell types.

aFGF and bFGF are potent mitogens and differentiation factors for mesoderm-derived cells including fibroblasts.[7,9,29] In addition, they exert chemotactic effects on fibroblasts and enhance collagen production by these cells.[7,29,41] The abundance of aFGF and bFGF in the exocrine cells of the CP tissues suggests that they may enhance fibrosis through these mechanisms. In support of this hypothesis, rat pancreatic AR42J cells that overexpress bFGF acquire fibroblastic characteristics in culture.[13] After subcutaneous injection of these cells in nude mice, the resulting tumors exhibit an increased fibroblastic response by comparison with AR42J cells, which do not express bFGF.[13] However, both aFGF and bFGF lack a signal peptide that is a feature of secreted proteins, and their exact mode of release from cells has yet to be elucidated. It is possible that bFGF is spontaneously released from cells and then acts in an autocrine and paracrine manner.[42] It has also been proposed that cell injury and death results in the release of stored aFGF and bFGF,[43] thereby allowing the growth factors to exert paracrine effects on cell growth, differentiation, and migration. Conceivably, the atrophic acinar and ductal cells in the CP tissues may degenerate and release their stored factors via this mechanism. Both factors may also act via intracrine mechanisms before being released from their cell of origin.[44]

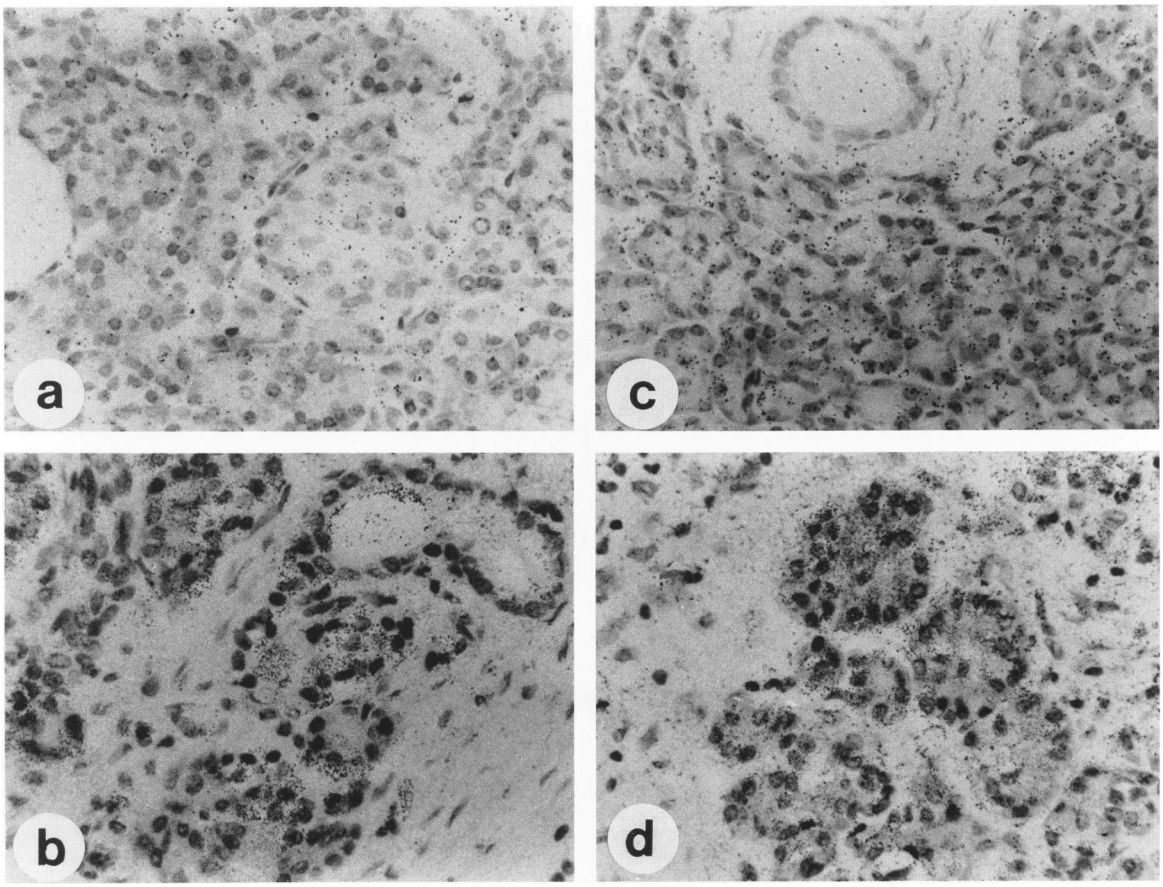

Figure 8. In situ *hybridization. Tissue sections from the normal human pancreas* (**A, C**) *and pancreatic tissues obtained from CP patients* (**B, D**) *were subjected to* in situ *hybridization. Grains corresponding to aFGF mRNA* (**A, B**) *and bFGF mRNA* (**C, D**) *were present at low levels in both acinar and ductal cells in the normal pancreas* (**A, C**), *and were present at increased levels in the tissue samples obtained from CP patients* (**B, D**). *Exposure time, 8 days. Original magnification, ×100.*

The exact role of FGFs in fibrotic disorders is poorly understood. In Dupuytren's contracture, prostatic hyperplasia, rheumatoid arthritis, granulomas, and endometriosis show increased bFGF levels in these disorders.[10–12] Furthermore, in hepatic cirrhosis, the hepatocytes exhibited bFGF immunoreactivity in degenerating fibrotic regions.[12] In contrast, in the normal liver tissue, bFGF was not detectable.[12] To our knowledge, the present findings are the first to indicate that a fibrotic condition may be associated with concomitant overexpression of aFGF and bFGF using both immunohistochemical and molecular techniques. It is not clear, however, whether this overexpression contributes directly to the pathobiological changes that occur in CP, or is a secondary manifestation of these alterations. Nonetheless, we have recently determined that both growth factors are overexpressed in pancreatic ductal adenocarcinomas.[45] In these tumors, there are foci of pancreatic parenchyma that exhibit CP-like alterations. These regions manifest increased aFGF and bFGF immunoreactivity, even in the absence of any inflammatory cells.[45] Furthermore, in this study aFGF and bFGF expression in the CP tissues was often increased in the absence of any inflammatory cells. These observations indicate that aFGF and bFGF may be overexpressed in pancreatic acinar and ductal cells in the absence of inflammation.

Interestingly, five CP samples did not exhibit an increase in aFGF mRNA expression. Two of these samples also failed to exhibit elevated bFGF mRNA levels. The reasons why FGF expression was not altered in these samples are not readily evident. Analysis of the clinical data of these patients did not reveal any difference regarding etiology, clinical history, or course of the disease. It is possible that factors other than aFGF and bFGF may also contribute to the changes that occur in CP. Conceivably, such additional factors may include other members of the FGF family, members of the TGF-β family,[46–49] and TGF-α.[5] Together, these observations suggest that

excessive growth factor expression may initiate autocrine and paracrine pathways that contribute to the pathophysiological changes that occur in this important disease.

Acknowledgments

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