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A Subgroup of Patients with Chronic Pancreatitis Overexpress the c-erb B-2 Protooncogene

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Objective
Chronic pancreatitis (CP) is a chronic condition associated with pancreatic fibrosis. A small subgroup of patients with CP develop enlargement of the head of the pancreas (EHP). This study examined some of the mechanisms that may lead to the development of EHP.

Summary Background Data
The c-erb B-2 protooncogene encodes a 185-kDa transmembrane growth factor receptor (p185) that regulates cell growth and differentiation.

Methods
The authors analyzed c-erb B-2 expression in samples obtained from the head of the pancreas from 26 patients with CP (5 women, 21 men) using immunohistochemical and molecular techniques. A diagnosis of CP with EHP was made when the vertical pancreatic head diameter was greater than 4 cm (14 patients), as determined by contrast-enhanced computed axial tomography scan. Pancreatic tissues from 15 healthy organ donors served as control subjects.

Results
In all patients without EHP and in the healthy control subjects, p185 immunoreactivity was present at low levels. In contrast, strong p185 immunoreactivity was observed in acinar and ductal cells in all patients with EHP. By *in situ* hybridization, c-erb B-2 messenger ribonucleic acid (mRNA) grains were expressed at high levels in patients with CP with EHP in both ductal and acinar cells. Northern blot analysis demonstrated a 4.5-fold increase ($p < 0.001$) in c-erb B-2 mRNA levels in patients with EHP compared with patients without EHP and healthy control subjects. Southern blot analysis did not reveal c-erb B-2 gene amplification or rearrangement.

Conclusions
These findings indicate the c-erb B-2 is not overexpressed in most patients with CP. However, its overexpression in patients with CP with EHP suggest that c-erb B-2 may contribute to the pathophysiologic processes that lead to pancreatic head enlargement.

Chronic pancreatitis (CP) is an often painful condition of the exocrine pancreas characterized by variable degrees of exocrine and endocrine insufficiency.[1-4] In the western world, approximately 70% of the cases are believed to be caused by chronic alcohol abuse.[1-3] Other predisposing factors include cholelithiasis with pancreatic duct obstruction, hereditary causes, and idiopathic conditions.[1-4] Morphologically, the pancreas in CP is characterized by enlarged pancreatic ducts, loss of parenchyma, and variable amounts of scarring.[5] Histologically, patients with moderate to severe CP exhibit pancreatic acinar cell degeneration, duct cell proliferation, pseudoductular hyperplasia, extensive fibrosis, and infiltration with inflammatory cells.[5,6] For unknown reasons, a limited number of patients with CP develop enlargement of the head of the pancreas (EHP) with a vertical diameter that is greater than 4 cm.[7-10] This enlargement can lead to obstruction of the duodenum, portal vein, main pancreatic duct, and choledochus, often necessitating surgical resection of the pancreatic head.[7-10] To date, it has not been possible to differentiate between CP patients with and without EHP on the basis of histopathologic criteria. Furthermore, the biochemical and molecular mechanisms that dictate whether CP patients will develop enlargement of the head of the pancreas are completely unknown.

Recent studies with transgenic mice indicate that overexpression of transforming growth factor alpha (TGF-α) is associated with pancreatic fibrosis and histologic features that are seen in CP in humans.[11] Furthermore, the pancreas in CP in humans is associated with overexpression of the epidermal growth factor (EGF) receptor, TGF-α, acidic fibroblast growth factor (aFGF), and basic fibroblast growth factor (bFGF), raising the possibility that excessive activation of tyrosine kinase receptors contributes to the pathophysiologic changes observed in CP.[12,13] It is not known, however, whether the expression of tyrosine kinase receptors other than the EGF receptor also is altered in CP. Recently, we determined that a high percentage of pancreatic adenocarcinomas overexpress the EGF receptor and its homologue, the c-erb B-2 protooncogene.[14-16] Furthermore, we found that c-erb B-2 expression is increased in regions adjacent to the cancer cells that exhibit acinar cell degeneration and pseudoductular metaplasia of the kind seen in CP.[16] Therefore, in our study, we studied c-erb B-2 expression in pancreatic tissues from CP patients. Currently, we report that c-erb B-2 messenger ribonucleic acid (mRNA) levels are not increased in CP, except if these patients also exhibit EHP.

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PATIENTS AND METHODS

Pancreatic tissue was obtained from 5 women and 21 men with CP who underwent a duodenum-preserving pancreatic head resection.[7-9] This procedure consists of a subtotal resection of the pancreatic head, sparing the duodenum and the stomach.[7-9] The median age of all the patients was 43 years, with a range of 30 to 59 years. Patients with pancreatic pseudocysts, phlegmon, pancreas divisum, or recent attacks of acute pancreatitis were excluded from the study. Based on the findings of the computed tomography scan, patients were subdivided into one group without EHP and one group with EHP. Pancreatic head enlargement was defined as a vertical pancreatic head diameter of 4 cm or more, as shown by contrast enhanced computed axial tomography scan, that was detectable for at least 12 weeks preoperatively without evidence of regression. These radiologic measurements were carried out independently by radiologists before pancreatic head resection.

In 12 patients with CP (3 women, 9 men) without EHP, surgery was performed because of stenosis of the main pancreatic duct, severe pain, and a history of recurrent episodes of acute pancreatitis. Eleven of these patients had a history of alcohol abuse, with a daily alcohol intake of 132±8.9 g (mean±SE). In 14 patients with CP (2 women, 12 men), surgery was done because of significant EHP, causing local complications such as duodenal obstruction, cholestasis, pancreatic main duct obstruction, and portal hypertension.[7-9] Excessive alcohol consumption was implicated in all 14 patients, who had a daily alcohol intake of 130±3.6 g (mean ± SE). Histologically, the alterations in the pancreas were graded as moderate to severe in the case of all 26 patients with CP. Healthy human pancreatic tissue samples were obtained from 4 women and 11 men through an organ donor program. The median age of the organ donors was 39 years, with a range of 18 to 50 years. All the healthy tissues used in this study also were obtained from the pancreatic head region. In all cases, portions of freshly removed tissue samples were fixed immediately in Bouin's fluid or paraformaldehyde solution for 12 to 24 hours and paraffin-embedded for histologic analysis. Other tissue portions, destined for ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) extraction were immediately frozen in liquid nitrogen after surgical removal, and maintained at −80 C until use. All studies were approved by the Human Subjects Committees of the University of Ulm, Germany, the University of Berne, Switzerland, and the University of California, Irvine.

Immunocytochemistry

A highly specific monoclonal antibody (c-neu; Ab-3; Oncogene Science, Inc., Uniondale, NY) against a 14-amino acid carboxyl domain of the human c-erb B-2 gene product (p185) was used for immunohistochemistry. Serial paraffin-embedded sections (5 μm thick) were subjected to immunostaining, using the streptavidin-peroxidase technique (Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). Endogenous peroxidase activity was blocked by incubating for 30 minutes at room temperature with 0.3% hydrogen peroxide in methanol.[14-16] The sections were then incubated for 30 minutes at 23 C with 10% healthy goat serum before overnight incubation at 4 C with the anti-c-erb B-2 antibody diluted 1:50 in 0.01 mol/L phosphate-buffered saline (PBS). Bound antibody was detected with a biotinylated goat antimouse immunoglobulin G secondary antibody and streptavidin-peroxidase complex, followed by incubation with diaminobenzidine tetrahydrochloride (0.03%) as the substrate.[14-16] Counterstaining was done with Mayer's hematoxylin. To ensure specificity of anti-p185 antibodies, control slides either were incubated in the absence of primary antibody or with an immunoglobulin G antibody against an irrelevant antigen. In both cases, no immunostaining was detected (data not shown). Immunohistochemical analysis was performed by two independent observers blinded to patient status.

In Situ Hybridization

Tissue sections were placed on poly-L-lysine-coated slides, deparaffinized, incubated at 23 C for 10 minutes with 1 μg/mL of proteinase K (Boehringer-Mannheim Biochemicals, Indianapolis, IN), and for 10 minutes with 0.5 × SSC (1 × SSC = 150 mM NaCl, 15 mM Na-citrate, pH 7.0), as previously reported.[14,17] After incubation for 3 hours at 42 C in hybridization solution,[14,17] hybridization was initiated by the addition of fresh hybridization buffer containing 120,000 cpm of the c-erb B-2 antisense riboprobe and 50 μg of yeast transfer RNA (GIBCO BRL, Inc., Gaithersburg, MD) and incubated overnight at 50 C.[14,17] Then the sections were washed at 23 C in 2 × SSC, 10 mM of 2-mercaptoethanol, and 1 mM of edetic acid (EDTA) for 10 minutes (twice each) and digested with RNAse A (20 μg/mL; Sigma Chemical Co., St. Louis, MO) for 30 minutes at 23 C. Then the slides were washed three times at 23 C, twice at 50 C in 0.1 × SSC, 10 mM of 2-mercaptoethanol, and 1 mM of EDTA, and twice in 0.5 × SSC at 23 C.[14,17] The sections were dehydrated by immersion in graded alcohol containing 0.3 mol/L ammonium acetate, dried, coated with NTB2 nuclear emulsion (Eastman Kodak Co., Rochester, NY), and exposed in the dark for 5 to 10 days. After development, the slides were counterstained with Mayer's hematoxylin. Pretreatment of the slides with RNAse abolished the hybridization signal by the antisense probe. Furthermore, sense probe corresponding to the antisense probe failed to produce a signal (data not shown).

Northern Blot Analysis

Total RNA was extracted by the guanidine isothiocyanate method and size-fractionated on a 1.2% agarose/1.8 mol/L formaldehyde gel.[14,16,18] The RNA (20 μg) was electrotransferred onto nylon membranes (GeneScreen, Du Pont, Boston, MA) and cross-linked by ultraviolet irradiation.[14,16,18] The blots then were prehybridized, hybridized, and washed under two high-stringency conditions, depending on whether antisense riboprobes or cDNA probes were used, as described previously.[14,16,18] In the case of antisense riboprobe, the blots were prehybridized overnight at 65 C and then hybridized for 24 hours at 65 C in the presence of 1×10^6 cpm/mL of the labeled antisense riboprobe.[14,16,18] The blots were rehybridized at 42 C for 18 hours in the presence of a 0.19 kb BamHI fragment of mouse 7S cDNA that cross-hybridizes with human RNA, using 1×10^4 cpm/mL of the random labeled cDNA probe.[14,19] The blots were exposed at −80 C to Kodak XAR-5 film with Kodak intensifying screens (Eastman Kodak). 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 (c-erb B-2/7S) was calculated for each sample.

Southern Blot Analysis

Pancreatic tissues were pulverized in liquid nitrogen and incubated for 16 hours at 50 C in a buffer containing 100 mM of NaCl, 10 mM of Tris-HCl, pH 8.0, 25 mM of EDTA, pH 8.0, 0.5% sodium dodecyl sulfate, and 0.1 mg/mL of proteinase K.[20] The DNA was purified by phenol/chloroform extraction, ethanol-precipitated, and digested with the restriction endonuclease EcoRI and BglII.[20] The DNA samples (10 μg) underwent electrophoresis on 0.7% agarose gels and were transferred onto Hybond N Plus nylon membranes (Amersham Corp., Arlington Height, IL) by alkaline capillary transfer.[21] The membranes were prehybridized and hybridized for 16 hours at 42 C in a buffer that contained 50% formamide, 1% SDS, 0.75 mol/L of NaCl, 5 mM of EDTA, 5 × Denhardt's solution, 100 μg/mL of salmon sperm DNA, 10% Dextran sulfate and 50 mM of Na_2PO_4, pH 7.4. For hybridization, 1×10^6 cpm/mL [α-^{32}P]-labeled cDNA probes were used. Washings were done in 0.5 × SSC and 0.1% SDS at 42 C for 20 minutes and two times

in 0.1% × SSC and 0.1% SDS at 42 C for 25 minutes.[14,16] To confirm equivalent DNA loading of all lanes, the blots were rehybridized with a cDNA probe for human β-actin.[22] The blots were exposed at −80 C to Kodak XAR-5 film with Kodak intensifying screens (Eastman Kodak).

Probes

A 0.4-kb BamHI-EcoRI fragment of human pHER2-436-1 cDNA (American Type Culture Collection, Rockville, MD) was subcloned into the pGEM7Zf vector (Promega Corporation, Madison, WI) and used for synthesis of antisense probe, after linearization with BamHI. Sense probe synthesis was carried out after linearization with EcoRI. A 3.2-kb TaqI fragment of human pCER204 cDNA (American Type Culture Collection)[23] was used for Southern blot analysis using a random primer labeling system (Boehringer-Mannheim, Indianapolis, IN).[24] To verify equivalent RNA and DNA loading, filters were rehybridized with a 7S cDNA and a 1.1-kb EcoRI fragment of human pHHCI89 β-actin cDNA (American Type Culture Collection), respectively.[19,22] [$\alpha-^{32}$P]-CTP (Amersham, Arlington Height, IL) and [$\alpha-^{35}$S]-UTP (Amersham) were used for cRNA probe synthesis.[25] [$\alpha-^{32}$P]-dCTP (Amersham) was used for cDNA synthesis.

Video Image Analysis

To obtain quantitative analysis of *in situ* hybridization data, sections were examined with a dark field filter using a Nikon Diaphot microscope and Nikon FX microscopic photometric system (Nikon, Tokyo, Japan) equipped with a video camera and a video image analysis system (Image I Videoimage Analysis System, West Chester, PA). Images were scanned for average pixel intensity and background density, allowing for computer-based correction of any uneven image illumination, using a Compaq computer and the Phoscan program (Compaq Computer Co., Houston, TX).[14] Densitometric analysis of the silver grains was measured under 400 × magnification in 12 randomly selected fields per slide. The number of grains was calculated as a percentage of the area occupied by the silver grains in a total area of each field.

Statistical Analysis

The results were expressed as median and range or as mean ± standard error (SE). For statistical analysis, the Wilcoxon test or the Students t test, with $p < 0.05$ as the level of significance, were used. The relationship between c-erb B-2 mRNA levels and the size of the pancreatic head was determined by means of a linear regression analysis. Significance was defined as $p < 0.05$.[26]

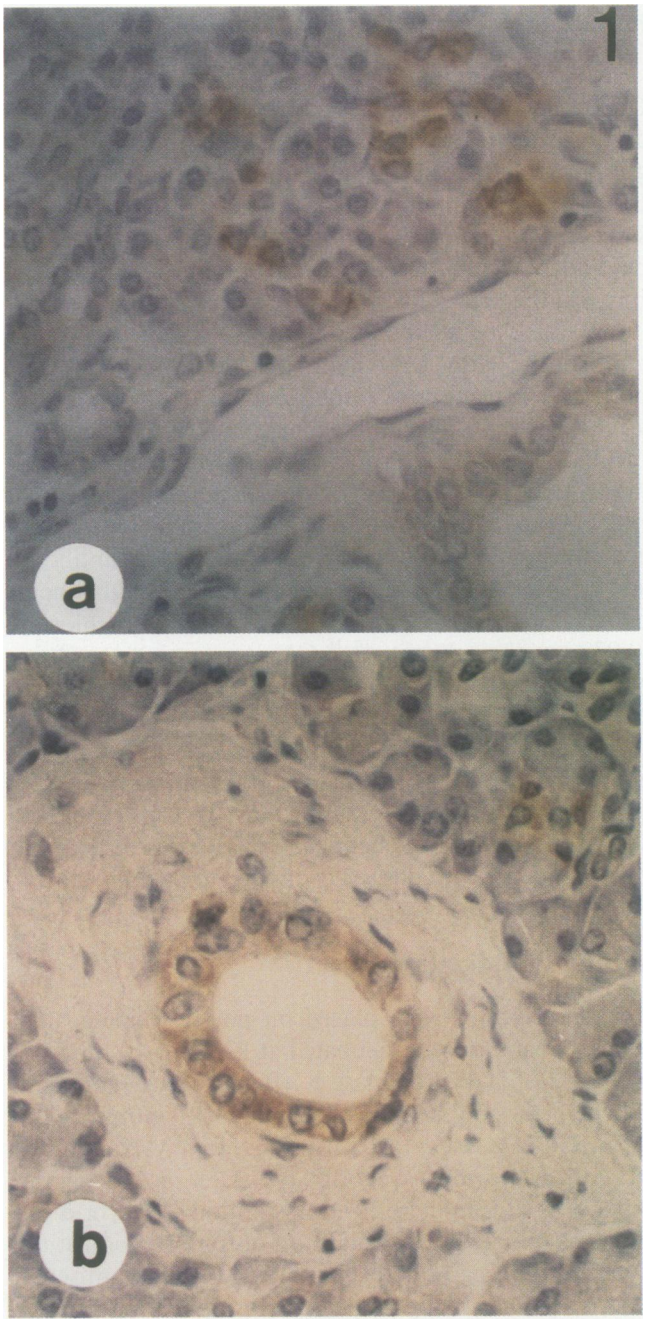


Figure 1. Immunohistochemical staining of c-erb B-2 gene product in the healthy human pancreas. Faint immunoreactivity was present in the cytoplasm of some acinar (A) and ductal cells (B). Original magnification × 100.

RESULTS

Immunocytochemical Studies

In the healthy human pancreas, p185 immunoreactivity was present in a focal pattern in the cytoplasm of

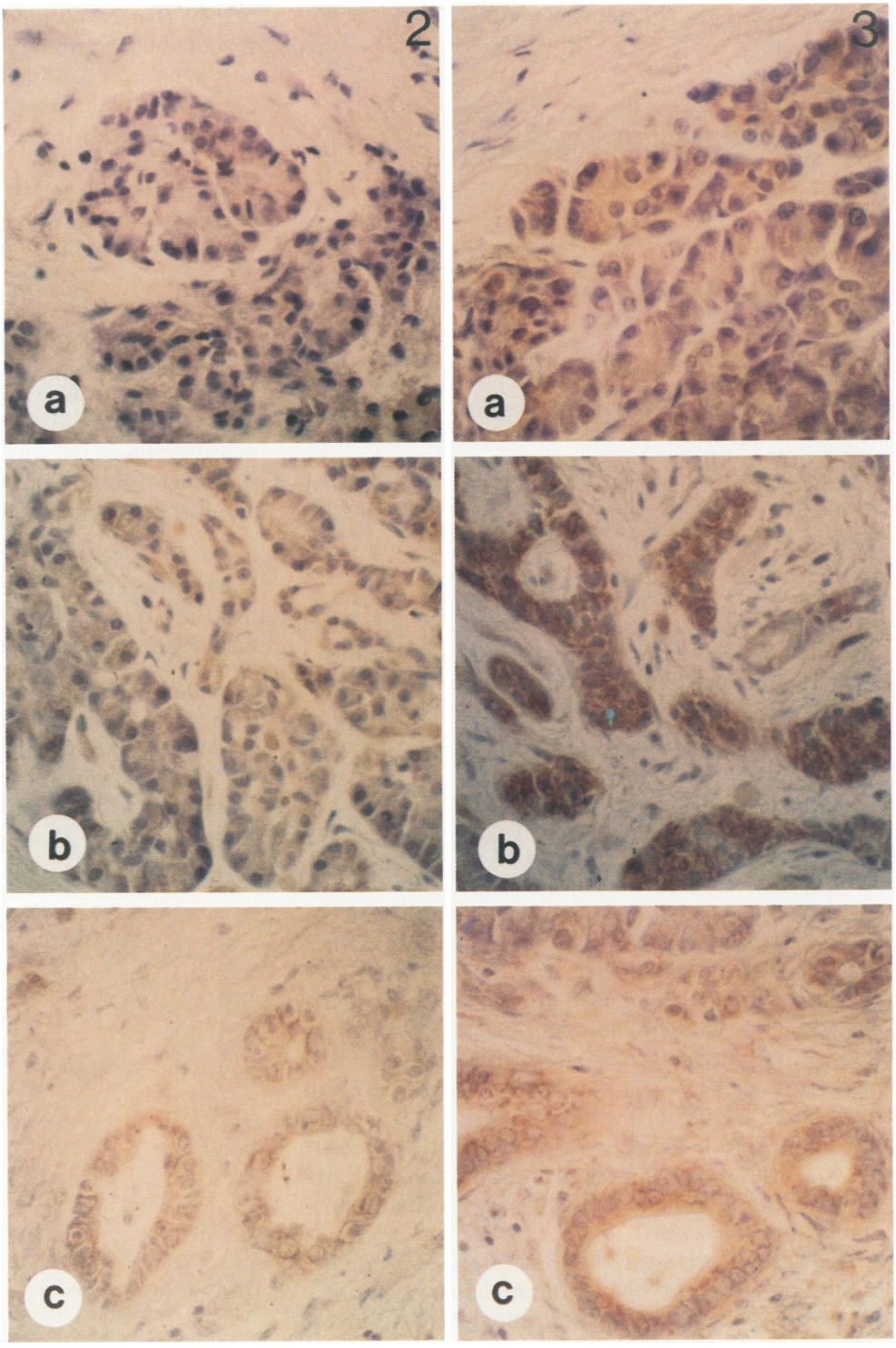


Figure 2. Immunohistochemical staining of c-erb B-2 gene product in CP samples without enlargement of the head of the pancreas. The majority of the acinar cells were devoid of immunoreactivity (A). Pseudoductular structures (B) and ductal cells (C) exhibited moderate p185 immunostaining. Original magnification × 100.

Figure 3. Immunohistochemical staining of c-erb B-2 gene product in CP samples with enlargement of the head of the pancreas. There was intense immunostaining in the acinar cells (A), areas of pseudoductular metaplasia (B), and ductal cells (C). Original magnification × 100.

some acinar cells (Fig. 1A) and ductal cells (Fig. 1B). In CP samples obtained from patients without EHP, p185 immunostaining was similar to that observed in healthy control subjects. Although a few acinar cells exhibited p185 immunostaining, the majority of these cells were devoid of p185 immunoreactivity (Fig. 2A). Faint p185 immunostaining was detectable in the pseudoductular structures (Fig. 2B) and in the ductal cells of most ducts (Fig. 2C). In the CP tissues obtained from patients with EHP, there was a diffuse increase in cytoplasmic p185 immunostaining in the acini (Fig. 3A). Most of the pseudoductular structures exhibited intense p185 immu-

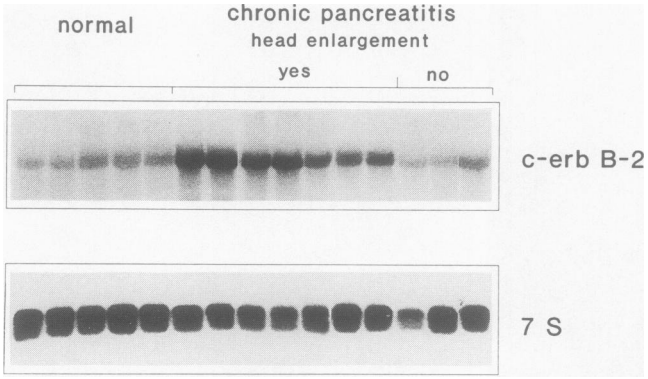


Figure 4. Northern blot analysis. Ribonucleic acid (20 μg/lane) from the pancreas of five healthy organ donors, seven patients with CP and enlargement of the pancreatic head, and three patients with CP without pancreatic head enlargement was size-fractionated. The filter was probed with [α-^{32}P]-labeled c-erb B-2 cRNA probe (1×10^6 cpm/mL; 5-day exposure) and [α-^{32}P]-labeled 7S cDNA probe (5×10^4 cpm/mL; 24-hour exposure). The c-erb B-2 mRNA migrated as a single band of 4.8 kb. 7S RNA, migrating as a 0.4 kb species, was used to assess RNA loading.

nostaining (Fig. 3B), which also was prominent in most ductal cells (Fig. 3C). A few fibroblasts also exhibited p185 immunoreactivity in both CP groups (data not shown).

Molecular Studies

Northern blot analysis of total RNA extracted from the healthy human pancreas revealed the presence of c-erb B-2 mRNA in each sample (Fig. 4). In tissues obtained from CP patients without EHP, c-erb B-2 mRNA levels were comparable to those in the healthy control subjects, whereas in CP patients with EHP, c-erb B-2 mRNA levels were increased (Fig. 4). Densitometric analysis of the Northern blots indicated that, by comparison with healthy control subjects, there was a 4.5-fold increase (range: 1.7–8.7) in c-erb B-2 mRNA levels in tissues from CP patients with EHP ($p < 0.001$) (Fig. 5). In contrast, in the CP patients without EHP, c-erb B-2 mRNA levels were comparable to healthy control subjects ($p = 0.32$). None of the samples revealed any aberrant mRNA transcripts.

To determine the site of synthesis of p185, *in situ* hybridization was carried out with an antisense c-erb B-2 riboprobe. Specific *in situ* hybridization grains were present at low levels in focal areas in pancreatic acinar and ductal cells in the healthy pancreas (Fig. 6A). In patients with CP without EHP, the number of grains was comparable to that observed in healthy control subjects (Fig. 6B). In contrast, a greater number of *in situ* hybridization grains was observed consistently in samples from patients with CP with EHP, which co-localized within the cell types that exhibited increased p185 immunoreactivity (Fig. 6C). Video image analysis revealed that, in tissues obtained from patients with CP with EHP, the *in situ* signal was 4.8-fold greater ($p < 0.001$) than in the pancreas of patients with CP without EHP or in healthy control subjects.

Next, Southern blot analysis was carried out to investigate if the increased c-erb B-2 mRNA levels were caused by amplification of the c-erb B-2 gene. After EcoRI digestion of 10 μg of genomic DNA and hybridization with a 3.2-kb c-erb B-2 cDNA probe, five fragments of the c-erb B-2 gene were detectable (Fig. 7). There were no aberrant bands in tissues of patients with CP. A similar analysis after BglII digestion also did not reveal any aberrant DNA fragments (data not shown). Densitometric analysis and calculation of the optical density ratio of the c-erb B-2 fragments with the corresponding β-actin signals did not reveal c-erb B-2 gene amplification in any of the CP tissue samples.

Correlation of c-erb B-2 Expression with the Size of the Head of the Pancreas and EGF Receptor Expression

In the 14 patients with CP with EHP, the mean vertical diameter of the pancreatic head mass as measured by contrast enhanced computed axial tomography scan was 5.6 ± 0.4 cm (range of 4.1 cm to 8.2 cm), whereas in the 12 patients with CP without EHP, it was 3.3 ± 0.2 cm (range of 2.9 to 3.8 cm). This difference was statistically

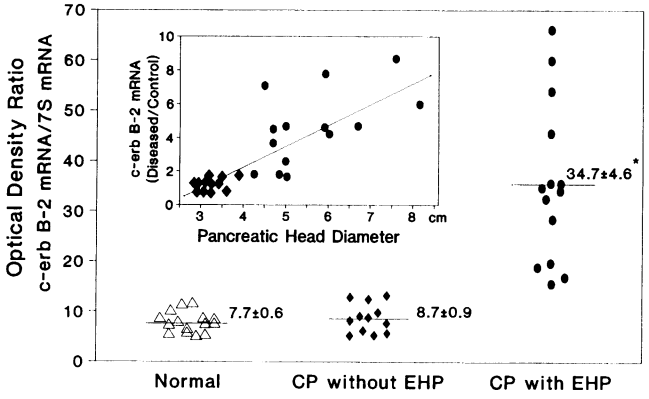
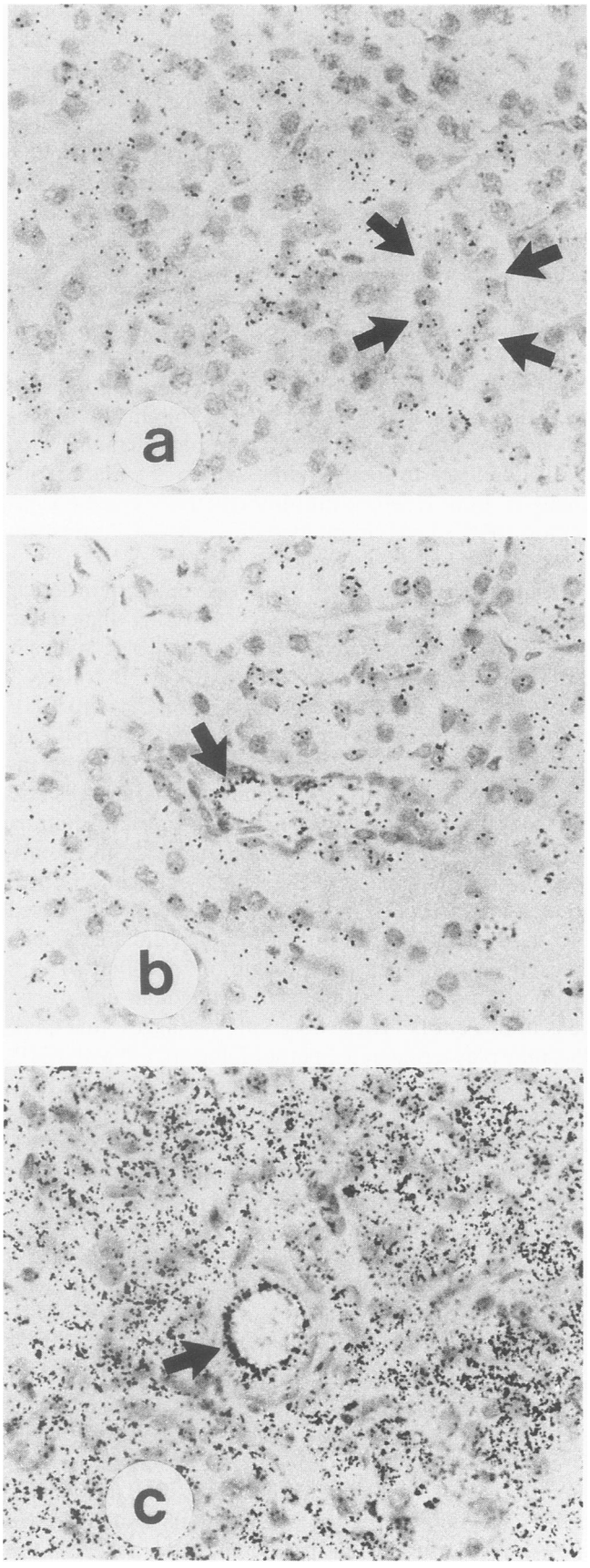


Figure 5. Densitometric analysis. Northern blots from healthy organ donors (△), patients with chronic pancreatitis without pancreatic head enlargement (CP without EHP, ♦), and patients with chronic pancreatitis with enlargement of the head of the pancreas (CP with EHP, ●) were analyzed by densitometry. Ribonucleic acid levels were expressed as the ratio of optical densities of the c-erb B-2 mRNA and 7S mRNA signals. Horizontal lines represent the mean for each group, and the values shown are the means ± SE. *$p < 0.005$ compared with either healthy or CP. **Inset:** Correlation between the vertical pancreatic head diameter and c-erb B-2 mRNA levels. Values were calculated as ratio of c-erb B-2/7S mRNA in diseased pancreatic samples divided by the mean of the ratio c-erb B-2/7S mRNA in healthy control subjects.



significant ($p < 0.001$). The vertical diameters of the pancreatic heads of all pancreases obtained from the organ donors were comparable to those observed in CP patients without EHP. Linear regression analysis (Fig. 5, insert) indicated that there was a positive correlation ($y = -2.92 + 1.31 \times X$, $R = 0.82$) between the vertical diameter of the pancreatic head and c-erb B-2 mRNA levels (Fig. 5, $p < 0.001$).

The level of expression of the EGF receptor was available in 23 of the CP samples.[12] By comparison with the healthy control subjects, EGF receptor mRNA levels were increased in 5 of 12 CP samples without EHP and in 7 of 13 CP samples with EHP (Table 1). Thus, 6 of 13 CP samples with EHP did not exhibit an increase in EGF receptor mRNA levels, indicating that EHP could occur in the absence of EGF receptor overexpression.

DISCUSSION

The pathophysiology of chronic pancreatitis remains controversial. The most widely held concept postulates that CP is caused by qualitative and quantitative changes in the secretion of an antilithogenic protein called lithostatine, which leads to intraductal precipitation of pancreatic proteins.[27,28] Furthermore, histopathologic observations indicate that recurrent attacks of acute pancreatitis may be associated with small areas of pancreatic necrosis followed by fibrotic regeneration.[29] It has been suggested, therefore, that recurrent attacks of acute pancreatitis also play an important role in the pathophysiology of CP.[29] It also has been proposed that increased levels of free radicals caused by decreased hepatic detoxification and direct toxic effects of alcohol on pancreatic acinar and ductal cells are major pathophysiologic factors in this disorder.[30,31] However, the basic cellular and molecular mechanisms that lead to the histologic alterations that occur in CP are not known. Recently, we have reported that a significant number of CP patients overexpress the EGF receptor, its ligand TGF-α,[12] aFGF, and bFGF.[13] Furthermore, transgenic mice overexpressing TGF-α exhibit pancreatic alterations that are similar to those found in human CP.[11] These observations indicate that changes in the expression of growth factor receptors and their ligands may be involved in the pathobiology of CP.

Figure 6. In situ hybridization. Detection of c-erb B-2 mRNA *in situ* hybridization grains in acinar cells and ductal cells (arrows) in the healthy human pancreas (A). In CP tissues of patients without enlargement of the pancreatic head (B), there was a similar density of c-erb B-2 mRNA grains in pancreatic acinar and ductal cells. In contrast, in CP tissues with enlargement of the head of the pancreas (C), there was a marked increase of c-erb B-2 mRNA in both acinar and ductal cells. Original magnification $\times$ 100.

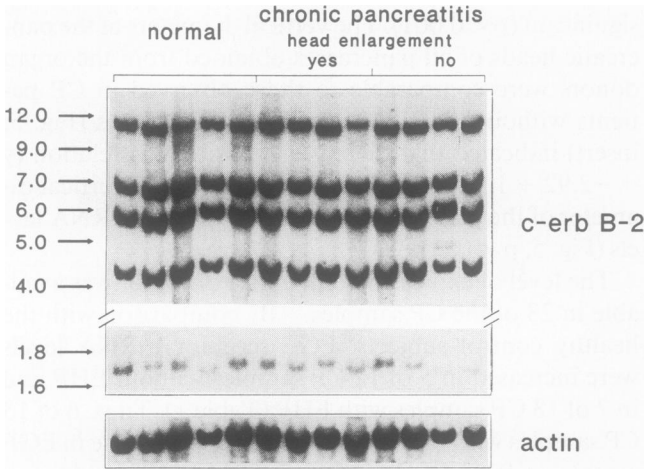


Figure 7. Southern blot analysis. Following EcoRI digestion, genomic DNA (10 μg) was size-fractionated and transferred onto nylon membranes. The filters were hybridized with a [α-^{32}P]-labeled c-erb B-2 cDNA (1×10^6 cpm/mL; 4-day exposure) and a [α-^{32}P]-labeled β-actin cDNA (1×10^6 cpm/mL; 24-hour exposure) to verify equivalent DNA loading. Five bands corresponding to the c-erb B-2 gene were detectable. There was no evidence for gene amplification or gene rearrangement in any of the CP samples.

The c-erb B-2 protooncogene exhibits significant sequence homology with the human EGF receptor and with the *neu* protooncogene, which was originally identified as a transforming oncogene in DNA obtained from rat neuroblastoma cells.[32–34] C-erb B-2 encodes a transmembrane protein that binds a number of ligands, including heregulin, *neu* differentiation factor, and glial growth factors.[35–39] Overexpression of c-erb B-2 in transfection experiments is associated with acquisition of the transformed phenotype.[40,41] Furthermore, several different types of cancers have been shown to overexpress c-erb B-2.[42–46] In breast and gastric carcinomas, enhanced c-erb B-2 expression is associated with greater tumor aggressiveness, indicating that c-erb B-2 may give certain cancer cells a growth advantage.[42–44] In contrast, in pancreatic cancers, c-erb B-2 overexpression occurs more frequently in well-differentiated carcinomas (Grade 1 and 2) than in poorly differentiated carcinomas (Grade 3 and 4), and also is observed in CP-like pseudoductular structures adjacent to the cancer cells.[16] These observations raise the possibility that c-erb B-2 contributes to the morphogenesis of well-differentiated tubular structures in pancreatic cancer and of pseudoductular structures in CP-like lesions that are common in this disorder.[16]

Table 1. COMPARISON OF EGF RECEPTOR AND c-erb B-2 EXPRESSION IN 13 CP PATIENTS WITH EHP AND 10 CP PATIENTS WITHOUT EHP

	Receptor Overexpression		
	EGF Receptor	c-erb B-2	EGF receptor + c-erb B-2
CP (n = 10)	5	0	0
CP + EHP (n = 13)	7	13	7

In the current study, we found low levels of the c-erb B-2 gene product, p185, in acinar and ductal cells in the healthy pancreas and in pancreatic tissues from patients with CP without EHP. Because the vast majority of patients with CP do not exhibit EHP, this observation suggests that c-erb B-2 does not have a role in the pathophysiologic alterations that occur in most patients with CP. In contrast to these findings, there is a marked increase in EGF receptor immunoreactivity and EGF receptor mRNA levels in many patients with CP, irrespective of the presence or absence of EHP.[12]

In CP patients with EHP, p185 was abundant in acinar and ductal cells and in areas exhibiting pancreatic pseudoductular metaplasia. By Northern blot analysis, there was almost a fivefold increase in c-erb B-2 mRNA levels in tissue samples of patients with CP with EHP compared with healthy control subjects and patients with CP without EHP. By *in situ* hybridization, this increased expression was evident in both acinar cells and ductal cells, indicating that enhanced immunostaining was caused by enhanced synthesis of p185. In the absence of evidence for c-erb B-2 gene amplification, these results suggest that c-erb B-2 mRNA levels are increased in CP patients with EHP as a result of enhanced gene transcription or message stabilization, and that p185 is synthesized at increased rates in both acinar and ductal cells in this entity. These observations suggest that c-erb B-2 may play a role in the pathobiology of CP with EHP. In support of this hypothesis, there was a direct and significant correlation between the vertical pancreatic head diameter and c-erb B-2 mRNA levels. To our knowledge, this is the first reported demonstration of significant overexpression of c-erb B-2 in a nonmalignant condition.

It is not readily evident how c-erb B-2 overexpression might lead to pancreatic head enlargement. However, some of the ligands that bind and activate this receptor, such as *neu* differentiation factor, inhibit the growth of certain mammary tumor cell lines and can induce their differentiation into milk-producing cells.[35,36] In other instances, *neu* differentiation factor may be mitogenic.[38] Other ligands that bind to c-erb B-2, such as the glial growth factors, are potent mitogens for fibroblastic cells.[39] The ability of c-erb B-2 to mediate such diverse growth-regulating signals in different cell types raises the

possibility that its overexpression in the subset of patients with CP and EHP may lead to enhanced ductal cell proliferation and pseudoductular metaplasia, acinar cell atrophy and dedifferentiation, and fibroblastic proliferation with consequent pancreatic fibrosis. However, it also is possible that c-erb B-2 expression is increased in CP with EHP as a result of the associated inflammatory changes in this disorder without directly contributing to the observed pathobiology.

Recently, it has been reported that the incidence of pancreatic cancer is increased slightly in patients with CP.[47] According to the multistage hypothesis of carcinogenesis, neoplastic tumors arise as a result of the sequential acquisition of distinct cellular oncogenic alterations.[48] Pancreatic cancers overexpress the EGF receptor, c-erb B-2, the type II transforming growth factor beta receptor, aFGF, bFGF, and the 2-immunoglobulin form of FGF receptor I.[14-16,49-51] These tumors also exhibit a high frequency of mutations in the K-ras oncogene[52] and p53 tumor suppressor gene.[53,54] The current data indicate that c-erb B-2 is not overexpressed in CP, except in patients with EHP. Thus, the potential for malignant transformation in CP with EHP may be increased as a result of c-erb B-2 overexpression. However, it is likely that this requires additional molecular defects in the pancreas, such as the loss of p53 tumor suppression function. Taken together, these observations suggest that specific molecular alterations may help define differences between CP, CP with EHP, and pancreatic cancer.

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