Characterization of Keratinocyte Growth Factor and Receptor Expression in Human Pancreatic Cancer

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Keratinocyte growth factor (KGF) is an angiogenic and mitogenic polypeptide that has been implicated in cancer growth and tissue development and repair. Its actions are dependent on its binding to a specific cell-surface KGF receptor (KGFR), which is encoded by the fibroblast growth factor (FGF) receptor type II (FGFR-2) gene. In the present study, we compared the immunohistochemical localization of KGF and KGFR/FGFR-2 in the normal and cancerous pancreas using specific antibodies that recognize KGF and KGFR/FGFR-2 and examined the expression of KGF, KGFR, and FGFR-2 in human pancreatic cancer by *in situ* hybridization with the corresponding riboprobes. In the normal pancreas, KGF immunoreactivity was present principally in the islet cells, whereas KGFR/FGFR-2 immunoreactivity was present both in the islet and ductal cells. In the pancreatic cancers, moderate KGF and moderate to strong KGFR/FGFR-2 immunoreactivity was present in many of the cancer cells. Furthermore, the ductal and acinar cells adjacent to the cancer cells exhibited moderate to strong KGF and KGFR/FGFR-2 immunoreactivity. By *in situ* hybridization, KGF, KGFR, and FGFR-2 were overexpressed and co-localized in the cancer cells within the pancreatic tumor mass but were even more abundant in the acinar and ductal cells adjacent to the cancer cells. These findings indicate that KGF, KGFR, and FGFR-2 are overexpressed in both the cancer cells and the adjacent pancreatic parenchyma and raise the possibility that KGF may act in an autocrine and paracrine manner to enhance pancreatic cancer cell growth *in vivo*. (Am J Pathol 1998, 153:213–222)

Pancreatic ductal adenocarcinoma is the fifth leading cause of cancer death in the Western world with an overall 5-year survival rate of less than 1% and a median survival after diagnosis of 4 months.^{1,2} Histologically, the cancer cells exhibit well to poorly differentiated ductal-like structures, often surrounded by an extensive desmo-

plastic reaction and infiltration with inflammatory cells.³ The adjacent pancreatic parenchyma harbors regions of acinar cell degeneration and ductal cell proliferation.⁴ A high percentage of these cancers overexpress a number of growth factors and their receptors, including the epidermal growth factor (EGF) receptor, EGF, transforming growth factor (TGF)- α , CRIPTO, TGF- β 1, basic fibroblast growth factor (bFGF), acidic FGF (aFGF), and FGF-5.^{5–10} The overexpression of these mitogenic growth factors may contribute to the biological aggressiveness of pancreatic cancers and to the formation of the abundant stroma that is characteristic of this malignancy.^{7,9}

Keratinocyte growth factor (KGF) is a member of the FGF group of heparin-binding polypeptides that was originally isolated from human embryonic lung fibroblasts.^{11,12} It shares 30 to 70% amino acid sequence homology with other FGFs. In addition to KGF, which is also known as FGF-7, this family includes aFGF, or FGF-1; bFGF, or FGF-2; int-2 (FGF-3); hst/K-FGF (FGF-4); FGF-5; FGF-6; androgen-induced growth factor (AIGF, or FGF-8); glia activating factor (GAF, or FGF-9); FGF-10; and FGF-like molecules termed FGF-11–14.^{11–14} KGF actions are dependent on its binding to a specific cell-surface KGF receptor (KGFR).¹⁵ This receptor possesses intrinsic tyrosine kinase activity and binds KGF and aFGF with high affinity but does not bind bFGF.¹⁵ The extracellular domain of KGFR consists of two or three immunoglobulin-like (Ig-like) regions, whereas its intracellular domain contains a tyrosine kinase region that is interrupted by a nonkinase intervening sequence.¹⁶ KGFR is encoded by the FGF receptor type II (FGFR-2) gene.¹⁶ Because FGFR-2 and KGFR derive from the same gene, the two receptors are homologous in their intracellular domains and most of their extracellular domains. However, they differ from each other in the carboxyl-terminal half of the third Ig-like region of the extracellular domain, as a consequence of alternative mRNA splicing.¹⁶

KGF mRNA levels are elevated in human pancreatic cancers.¹⁷ It is not known, however, whether the cancer cells within the pancreatic tumor mass express KGF, KGFR, or FGFR-2. Therefore, in the present study, we

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examined the expression of KGF, KGFR, and FGFR-2 in the normal human pancreas and in human pancreatic cancers. We now report that KGF, KGFR, and FGFR-2 are overexpressed in pancreatic cancer and that this overexpression occurs to a variable degree in the cancer cells and in the adjoining acinar, ductal, and islet cells.

Materials and Methods

Materials

The following were purchased: pGEM3Zf and pGEM7Zf vectors from Promega Biotechnology (Madison, WI); Genius 3 (nonradioactive nucleic acid detection kit), Genius 4 (nonradioactive RNA labeling kit), and proteinase K from Boehringer Mannheim (Indianapolis, IN); yeast tRNA from GIBCO BRL (Gaithersburg, MD); RPA II kit for ribonuclease protection assays from Ambion (Austin, TX); aqueous mounting medium from Dako Corp. (Carpinteria, CA); Tween-20 from Bio-Rad Laboratories (Hercules, CA); glycine and formamide from Fisher Scientific (Fair Lawn, NJ); goat anti-human KGF (FGF-7, N-14) and rabbit anti-human FGFR-2 (Bek, C-17) polyclonal antibodies from Santa Cruz Biotechnology (Santa Cruz, CA); Immobilon-P nitrocellulose membranes from Millipore Corp. (Bedford, MA); enhanced chemiluminescence (ECL) substrate from Pierce (Rockford, IL); HistoMark Biotin/Streptavidin-peroxidase kit and biotinylated goat anti-guinea pig IgG secondary antibodies from Kirkegaard & Perry Laboratories (Gaithersburg, MD); Vectastain Universal ABC Elite Peroxidase kit from Vector Laboratories (Burlingame, CA); RPMI medium, fetal bovine serum (FBS), penicillin G, and streptomycin from Irvine Scientific (Irvine, CA). All other chemicals and reagents were purchased from Sigma Chemical Corp. (St. Louis, MO). In addition, a highly specific anti-human FGFR-2 monoclonal antibody was a gift from Prizm Pharmaceuticals (San Diego, CA), and T3M4 human pancreatic cancer cells were a gift from Dr. R. S. Metzgar, Duke University (Durham, NC).

Tissue Samples

Pancreatic carcinoma samples (four male, six female; mean age, 55.7 years; range, 32 to 68 years) were obtained from patients undergoing surgery for pancreatic cancer. Normal pancreatic tissues (four male, one female; mean age, 41.8 years; range, 18 to 55 years) were obtained from organ donors through an organ donor program. Tissues were fixed in Bouin's solution or 10% paraformaldehyde solution (PFA) for 18 to 20 hours and embedded in paraffin. All studies were approved by the Human Ethics Committees of the University of California, Irvine, and the University of Bern, Switzerland.

Probe Preparation

A 297-bp *Bam*HI-*Hind*III cDNA fragment, corresponding to nucleotides 461 to 764 of the human KGF cDNA sequence¹¹ was generated by polymerase chain reaction

(PCR) amplification of single-stranded cDNA that was reverse transcribed (RT) from human placental RNA, as previously described.¹⁸ The primers used for KGF cDNA preparation corresponded to nucleotides 461 to 481 (5'-CTGACATGGTCCTGCCAAC-3') and 745 to 764 (5'-GAGAAGCTTCCAAGTCCACTGCTCCTG-3') of the human KGF cDNA. A 168-bp *Bam*HI-*Hind*III cDNA fragment, corresponding to nucleotides 1349 to 1516 of the human KGFR cDNA sequence¹⁶ was similarly generated by RT-PCR. The primer pair used for KGFR cDNA preparation was derived from sequences located on either side of exon IIIb of the human KGFR cDNA, corresponding to nucleotides 1349 to 1367 (5'-GCGGATCCTTCTCAAGCACTCGGGGA-3') and 1498 to 1516 (5'-GCAAGCTTCCAGG-CGCTTGCTGT-3'). A cDNA encoding sequences corresponding to the human FGFR-2 receptor cDNA¹⁹ was generated by RT-PCR from PANC-1 human pancreatic cancer cells. The primers used for FGFR-2 were derived from sequences located on either side of the human FGFR-2 cDNA that are specific for exon IIIc, corresponding to nucleotides 1103 to 1122 (5'-GCGGATCCTCAAGGTTCTCAAGGCCG-3') and 1254 to 1273 (5'-GTAAGCTTCCAG-GCGCTGGCA GAAC-3'). The 297-bp KGF cDNA fragment and the 171-bp FGFR-2 cDNA fragment were subcloned separately into the pGEM3Zf vector, and the 168-bp KGFR cDNA was subcloned into pGEM7Zf. Authenticity of the three fragments was confirmed by sequencing. The probes were labeled with digoxigenin-UTP by SP6 or T7 RNA polymerase using the Genius 4 RNA labeling kit.

In Situ Hybridization

In situ hybridization was performed as previously reported^{20,21} with minor modifications. Briefly, tissue sections (4 μ m thick) were placed on 3-aminopropyl-methoxysilane-coated slides, deparaffinized, and incubated at 23°C for 20 minutes with 0.2 N HCl and at 37°C for 15 minutes with 20 μ g/ml proteinase K. The sections were then post-fixed for 5 minutes in phosphate-buffered saline (PBS) containing 4% paraformaldehyde, incubated briefly twice with PBS containing 2 mg/ml glycine and once in 50% (v/v) formamide/2X SSC for 1 hour before initiation of the hybridization reaction by the addition of 100 μ l of hybridization buffer. The hybridization buffer contained 0.6 mol/L NaCl, 1 mmol/L EDTA, 10 mmol/L Tris/HCl (pH 7.6), 0.25% SDS, 200 μ g/ml yeast tRNA, 1X Denhardt's solution, 10% dextran sulfate, 40% formamide, and 100 ng/ml of the indicated digoxigenin-labeled riboprobe. Hybridization was performed in a moist chamber for 16 hours at 42°C. The sections were then washed sequentially with 50% formamide/2X SSC for 30 minutes at 50°C, 2X SSC for 20 minutes at 50°C, and 0.2X SSC for 20 minutes at 50°C. For immunological detection, the Genius 3 nonradioactive nucleic acid detection kit was used. The sections were washed briefly with buffer 1 solution (100 mmol/L Tris/HCl and 150 mmol/L NaCl, pH 7.5) and incubated with 1% (w/v) blocking reagents in buffer 1 solution for 60 minutes at 23°C. The sections were then incubated for 30 minutes at 23°C with a 1:2000 dilution of alkaline-phosphatase-conjugated polyclonal

Figure 1. Immunohistochemistry of KGF and KGFR/FGFR-2 in the normal pancreas. **A:** KGF was present in some of the endocrine islet cells (outlined by arrowheads) and ductal cells (arrows). **B:** Abundant KGFR/FGFR-2 immunoreactivity was present in the islet cells (outlined by arrowheads). **C:** Localization of endocrine β -cells in serial sections using an anti-porcine insulin antibody, cross-reactive with human insulin (arrowheads). Magnification, $\times 450$.

sheep anti-digoxigenin Fab fragment containing 0.2% Tween 20. The sections were then washed twice for 15 minutes at 23°C with buffer 1 solution containing 0.2% Tween 20 and equilibrated with buffer 3 solution (100 mmol/L Tris/HCl, 100 mmol/L NaCl, 50 mmol/L MgCl₂, pH 9.5) for 2 minutes. The sections were then incubated with color solution containing nitroblue tetrazolium and X-phosphate in a dark box for 2 to 3 hours. After the reaction was stopped with TE buffer (10 mmol/L Tris/HCl,

1 mmol/L EDTA, pH 8.0), the sections were mounted in aqueous mounting medium.

Immunohistochemistry

A highly specific goat anti-human KGF and two different anti-human FGFR-2 antibodies were used for immunohistochemistry. The anti-KGF antibody was an affinity-purified goat polyclonal antibody raised against a peptide corresponding to amino acids 33 to 46 mapping at the amino terminus of the KGF precursor of human origin. This antibody reacts with KGF of human origin by immunoblotting and ELISA but does not react with any other member of the FGF family (Santa Cruz Biotechnology). The C-17 anti-FGFR-2 antibody from Santa Cruz was an affinity-purified rabbit polyclonal antibody raised against a peptide corresponding to amino acids 789 to 802 mapping at the carboxy terminus of the FGFR-2 precursor of human origin. This antibody reacts principally with FGFR-2 and KGFR and may cross-react to a limited extent with FGFR-1, -3, or -4 (Santa Cruz Biotechnology). Therefore, a second anti-FGFR-2 antibody from Prizm Pharmaceuticals was also used. This mouse monoclonal antibody is directed against the acid box region (TDGAEDFVSEN) located in the extracellular domain of FGFR-2 and shared by both FGFR-2 and KGFR but not by other FGF receptors. Therefore, it is highly specific for FGFR-2 and KGFR and does not cross-react with other FGF receptors.²² Its specificity has been previously demonstrated in immunoblotting studies and ELISAs.²² Because both the polyclonal and monoclonal anti-FGFR-2 antibodies recognize KGFR in addition to FGFR-2, positive immunostaining obtained with either antibody was reported as reflecting KGFR/FGFR-2 immunoreactivity.

Paraffin-embedded sections (4 μ m) were subjected to immunostaining using the streptavidin-peroxidase technique.^{23,24} Endogenous peroxidase activity was blocked by incubation for 30 minutes with 0.3% hydrogen peroxide in methanol. Tissue sections were incubated for 15 minutes (23°C) with 10% normal rabbit serum for the KGF antibody and 10% normal goat serum for the polyclonal FGFR-2 antibody and then incubated for 16 hours at 4°C with the KGF (1:500 dilution) and polyclonal FGFR-2 antibody (1:500 dilution) in PBS containing 1% bovine serum albumin (BSA). To perform immunostaining with the monoclonal anti-FGFR-2 antibody, sections were incubated for 20 minutes (23°C) with 5% normal horse serum and then incubated for 30 minutes at 23°C with the antibody (1:500 dilution) in PBS containing 1% bovine serum albumin. Bound antibodies were detected with biotinylated rabbit anti-goat IgG secondary antibodies for KGF staining, goat anti-rabbit IgG secondary antibodies for staining with the polyclonal anti-FGFR-2 antibody, and biotinylated universal antibody for staining with the monoclonal anti-FGFR-2 antibody. For insulin staining, guinea pig polyclonal anti-porcine insulin antibodies (1:3000 dilution in PBS containing 1% bovine serum albumin), cross-reactive with human insulin, and biotinylated goat anti-guinea pig IgG secondary antibodies were used after incubation with 10% normal goat serum. Sections were then treated with streptavidin-peroxidase complex,

Figure 2. Immunohistochemistry of KGFR/FGFR-2 using a monoclonal antibody. **A:** In the normal pancreas, mild to moderate KGFR/FGFR-2 was present in the endocrine islet cells (outlined by **arrowheads**) and ductal cells (**arrow**). **B:** In the pancreatic cancers, abundant KGFR/FGFR-2 immunoreactivity was present in the ductal-like cancer cells. **C:** Moderate to strong KGFR/FGFR-2 immunoreactivity was present in the small ductal cells and endocrine islets adjacent to the cancer cells (outlined by **open arrowheads**). **D:** Moderate to strong KGFR/FGFR-2 immunoreactivity was present in the ductal cells of the large ducts adjacent to the cancer cells. Magnification, $\times 600$ (A), $\times 400$ (B and D), and $\times 200$ (C).

using diaminobenzidine tetrahydrochloride as the substrate, and counterstained with Mayer's hematoxylin. However, in the case of the monoclonal anti-FGFR-2 antibody, the avidin-peroxidase complex was used. Some sections were incubated with nonimmunized goat anti-IgG for KGF and rabbit anti-IgG for FGFR-2 or without primary antibodies, which did not yield positive immunoreactivity.

Cell Culture

T3M4 human pancreatic cancer cells were grown in RPMI medium supplemented with 10% fetal bovine serum, penicillin G (100 U/ml), and streptomycin (100 μ g/ml) and maintained in monolayer culture at 37°C in humidified air with 5% CO₂.

Immunoblotting and Ribonuclease Protection Assay

We previously reported that T3M4 human pancreatic cancer cells express exceedingly low levels of KGFR that are detectable only after amplification by PCR.¹⁷ To de-

termine whether these cells express FGFR-2, immunoblotting¹⁰ was carried out using the monoclonal anti-FGFR-2 antibody that does not cross-react with other FGF receptors.²² In addition, a ribonuclease protection assay was carried out using total RNA isolated from these cells. For this purpose, RNA (10 μ g/sample) was hybridized overnight (42°C) with a [α -³²P]CTP-labeled FGFR-2 riboprobe (100,000 cpm/sample). Single-stranded RNA was then digested with RNase A/T1, size-fractionated on a 6% polyacrylamide/8 mol/L urea gel, and subjected to autoradiography.¹⁰

Results

In the normal pancreas, KGF immunoreactivity was present in a focal but intense pattern in a few islet cells (Figure 1A, arrowheads). Many ductal cells in the small ducts exhibited faint KGF immunoreactivity (Figure 1A, arrows). Occasional acinar cells and vascular smooth muscle cells (VSMCs) also exhibited faint KGF immunoreactivity (not shown). Using the polyclonal anti-FGFR-2 antibody, intense and abundant KGFR/FGFR-2 immunoreactivity was present in many islet cells (Figure 1B,

Figure 3. Expression of KGF, KGFR, and KGFR/FGFR-2 in human pancreatic cancer tissues. Immunostaining revealed moderate to strong KGF (A) immunoreactivity in the cytoplasm of the cancer cells and faint to moderate KGFR/FGFR-2 immunoreactivity (B) in these cells. *In situ* hybridization analysis of serial sections revealed moderate KGF (C), FGFR-2 (D), and KGFR (E) mRNA signals in the cancer cells. Hybridization with the sense KGFR probe (F) did not yield any specific signals. Magnification, $\times 400$.

arrowheads) but was faint in the ductal cells (arrows) and completely absent in the acinar cells and VSMCs. With the monoclonal anti-FGFR-2 antibody, KGFR/FGFR-2 immunoreactivity was diffuse but faint in the islet cells (Figure 2A, arrowheads), of moderate intensity in the ductal cells (Figure 2A, arrow), and absent in the acinar cells (Figure 2A). As expected, many islet cells were strongly positive for insulin (Figure 1C, arrowheads).

In 6 of 10 pancreatic cancer samples, KGF immunoreactivity was present in many of the cancer cells in a diffuse cytoplasmic pattern that was of moderate intensity

(Figure 3A; Table 1). Moderate to strong KGF immunoreactivity was also present in the endocrine islets and in some fibroblasts and VSMCs (not shown). Using the polyclonal anti-FGFR-2 antibody, faint to moderate KGFR/FGFR-2 immunoreactivity was present in the cancer cells in 7 of the same 10 cancer samples (Figure 3B). Using the monoclonal anti-FGFR-2 antibody, strong KGFR/FGFR-2 immunoreactivity was present in the cancer cells (Figure 2B). Most KGF-positive cancers were also positive for KGFR/FGFR-2 (Table 1). Thus, all six cancer samples that were strongly positive for KGF exhibited

Table 1. Summary of KGF and KGFR/FGFR-2 Immunohistochemistry

Number of cases	KGF	KGFR/FGFR-2
4	++	++
2	+	+
1	—	+
3	—	—

In 6 of 10 cases, the cancer cells were positive for both KGF and KGFR/FGFR-2 immunoreactivity. In 4 of 10 cases, the cancer cells were negative for KGF immunoreactivity. In 3 of 10 cases, the cancer cells were negative for KGFR/FGFR-2 immunoreactivity. Immunoreactivity was scored as follows: —, <10% of the cancer cells exhibited positive immunoreactivity and were defined as negative; +, 11 to 80% of cancer cells were positive; ++, >80% of the cancer cells were positive.

KGFR/FGFR-2 immunoreactivity, whereas three of the four cancers that were negative for KGF were also negative for KGFR/FGFR-2.

Previously, we reported that cultured pancreatic cancer cell lines express variable levels of KGF.¹⁷ To determine whether pancreatic cancer cell lines express FGFR-2, we next characterized FGFR-2 expression in T3M4 cells by immunoblotting and ribonuclease protection. Immunoblotting with the monoclonal anti-FGFR-2 antibody revealed a distinct band of approximately 140 kd (Figure 4A). Because this cell line expresses a negligible level of KGFR that is detectable only by PCR,¹⁷ the 140-kd band most likely represents FGFR-2. Expression of FGFR-2 at the RNA level was confirmed with a highly specific ribonuclease protection assay, which revealed the presence of a 278-bp protected band in T3M4 cells (Figure 4B).

In all 10 cancer samples, the ductal and acinar cells that were close to the cancer cells exhibited chronic

pancreatitis-like changes consisting of foci of acinar cell degeneration, ductal cell proliferation, ductal dilatation, and increased stromal elements. In these regions, the ductal cells exhibited moderate to strong KGF immunoreactivity (Figure 5, A and B) whereas the degenerating acinar cells (Figure 5A) exhibited moderate KGF immunoreactivity. Using the polyclonal anti-FGFR-2 antibody, the same ductal and acinar cells exhibited faint to moderate KGFR/FGFR-2 immunoreactivity (Figure 5, C and D). Using the monoclonal anti-FGFR-2 antibody, moderate to strong KGFR/FGFR-2 immunoreactivity was present in the same cells (Figure 2, C and D). Diffuse and strong KGF immunostaining was evident in the islet cells (Figure 5, A and B). Using the polyclonal anti-FGFR-2 antibody, the same islet regions exhibited focal and intense KGFR/FGFR-2 immunoreactivity (Figure 5, C and D), whereas with the monoclonal anti-FGFR-2 antibody the KGFR/FGFR-2 signal in the islet cells was less intense and more diffuse (Figure 2C). Staining of serial sections with anti-insulin antibodies revealed the insulin-secreting islet cells (Figure 5, E and F), which consistently exhibited strong KGF and KGFR/FGFR-2 immunoreactivity. Some of the ductal cells forming the dilated ducts adjacent to the islets also exhibited strong insulin immunoreactivity (Figure 5, E–G). The same ductal cells also exhibited strong immunoreactivity for KGF (Figure 5B) and KGFR/FGFR-2 (Figures 2C and 5D).

In situ hybridization analysis using highly specific riboprobes was used next to delineate the exact sites of synthesis of KGF, KGFR, and FGFR-2 and to more clearly differentiate between the sites of expression of KGFR and FGFR-2. This analysis revealed low levels of KGF, KGFR, and FGFR-2 mRNA in the ductal cells within the normal human pancreas and moderate levels of these mRNA species in many of the islet cells (data not shown). In the cancer tissues, moderate KGF (Figure 3C), FGFR-2 (Figure 3D), and KGFR (Figure 3E) *in situ* hybridization signals were observed in the cancer cells. The *in situ* hybridization signals for all three mRNA moieties were strong in the islet cells (Figure 6) and adjacent ductal cells, including the ductal cells forming the dilated structures next to the islets (Figure 6). All three *in situ* hybridization signals were of faint to moderate intensity in the adjacent degenerating acinar cells (Figure 6). The corresponding sense probes did not yield any positive signals (Figures 3F and 6D).

Discussion

KGF is generally synthesized in stromal fibroblasts and other types of mesenchymal cells.²⁵ Several lines of evidence suggest that KGF acts in a paracrine manner to promote epithelial cell growth and wound healing. Thus, after its release from mesenchymal cells, KGF binds to KGFR, which is expressed in a variety of epithelial cells, including keratinocytes²⁶ and mammary^{27,28} and corneal epithelial cells.²⁹ KGF is believed to be an important mitogen for these cells. It has also been shown to enhance the proliferation of hepatocytes,³⁰ mucous-secreting cells in the gastrointestinal tract,³⁰ and pulmonary

Figure 4. FGFR-2 expression in T3M4 pancreatic cancer cells. **A:** Immunoblotting. Cell lysate from T3M4 cells (30 μ g) was transferred to an Immobilon P membrane and subjected to immunoblotting using a 1:500 dilution of the monoclonal anti-FGFR-2 antibody (2 μ g/ml). A single band (approximately 140 kd) was visible after visualization by ECL. **B:** Ribonuclease protection assay. Total RNA (10 μ g/sample) was hybridized overnight (42°C) with a [α -³²P]CTP-labeled FGFR-2 antisense riboprobe (326 nt, 100,000 cpm). Single-stranded RNA was then digested with RNase A/T1, size-fractionated on a 6% polyacrylamide/8 mol/L urea gel, and subjected to autoradiography. A single 278-bp protected RNA fragment was visible, confirming that the cells express FGFR-2.

Figure 5. Analysis of KGF and KGFR/FGFR-2 immunoreactivity in the chronic pancreatitis-like lesions. **A and B:** Moderate to strong KGF immunoreactivity was present in the ductal cells and endocrine islets (outlined by **open arrowheads**) and, to a lesser extent, in the degenerating acinar cells (**a**). **C and D:** Analysis of serial sections revealed faint to moderate KGFR/FGFR-2 immunoreactivity in the ductal cells and acinar cells (**a**) and focal but intense KGFR/FGFR-2 immunoreactivity in the endocrine islets (outlined by **open arrowheads**). **E-G:** Insulin immunoreactivity was evident in the β -cells within the endocrine islets (outlined by **open arrowheads**) and in a few ductal cells (**solid arrowheads**) adjacent to the islets and forming dilated ductal structures. Magnification, $\times 100$ (**A**, **C**, and **E**), $\times 200$ (**B**, **D**, and **F**), and $\times 400$ (**G**).

type II pneumocytes.³¹ KGF may have an especially important role in epidermal wound healing, as its expression increases 160-fold in rat skin after injury.³²

Previously, we reported that the normal human pancreas expresses low levels of KGF mRNA.¹⁷ In the present study we determined that KGF is expressed principally in the endocrine islet cells and, to a lesser extent,

in the ductal cells in the normal human pancreas. By *in situ* hybridization, KGFR exhibited a similar pattern of distribution. Furthermore, using a polyclonal antibody, strong immunostaining for KGFR/FGFR-2 was observed in the islet cells and weak staining was observed in the ductal cells. In contrast, using a monoclonal antibody, KGFR/FGFR-2 immunoreactivity was of moderate inten-

Figure 6. *In situ* hybridization analysis of KGF, KGFR, and FGFR-2 in chronic pancreatitis-like regions. *In situ* hybridization of serial sections revealed moderate to strong KGF (A), KGFR (B), and FGFR-2 (C) mRNA signals in the ductal and islet cells (outlined by open arrowheads) and faint to moderate mRNA signals in the degenerating acinar cells (a). The ductal cells (solid arrowheads) that formed dilated ductal structures adjacent to the islets exhibited a strong signal for all three mRNA species. Hybridization with sense probes did not yield any specific signals (D). Magnification, $\times 200$.

sity in the ductal cells and relatively faint in the islet cells. Despite these slight differences between the two antibodies, the immunostaining and *in situ* hybridization data raise the possibility that KGF and KGFR participate in the regulation of pancreatic functions in the islet and ductal cells. Two observations support this hypothesis. First, KGF-expressing transgenes exhibit pancreatic ductal hyperplasia and a marked increase in insulin-containing ductular epithelial cells.³³ The abundance of KGF in the endocrine islets within the chronic pancreatitis-like lesions may thus explain the presence of insulin-containing ductal cells next to the islet. Second, the *in vivo* injection of KGF leads to enhanced pancreatic ductal cell proliferation.³⁴ Inasmuch as the *in situ* hybridization data confirmed that FGFR-2 was also present in the islets, it is possible that other FGFs acting via FGFR-2 may also contribute to regulation of islet cell function.

In the present study we also determined that KGF is expressed in some of the cancer cells within the pancreatic tumor mass and that these cells often also express KGFR and FGFR-2. Although the anti-receptor antibodies used in the present study cannot distinguish between KGFR and FGFR-2, the *in situ* hybridization data confirmed that the cancer cells often co-expressed these receptors. The co-localization of KGF and KGFR in these

cells indicates that there is a potential for a KGF-dependent autocrine loop in pancreatic cancer. In support of this hypothesis, a number of cultured human pancreatic cancer cell lines are known to co-express KGF and KGFR, and KGF is a mitogen in one of these cells.¹⁷ Furthermore, as shown in the present study, pancreatic cancer cell lines are capable of expressing FGFR-2. Together with the *in vivo* data demonstrating that cancer cells within the pancreatic tumor mass express KGFR and FGFR-2, these observations suggest that FGFR-2 also has a role in pancreatic cancer cell growth.

By immunohistochemistry, KGF and KGF/FGFR-2 were abundant in the chronic pancreatitis-like lesions adjacent to the cancer cells. These regions harbor proliferating ductal cells, degenerating acinar cells, occasional endocrine islets and an abundant stroma. By *in situ* hybridization, the ductal and acinar cells within these regions uniformly expressed high levels of KGF, KGFR, and FGFR-2 mRNA. This parenchymal overexpression suggests a potential for paracrine interactions between the exocrine cells and the cancer cells and is in agreement with our previous finding that aFGF and bFGF are overexpressed in both the cancer cells and the adjoining pancreatic parenchyma.⁷ The abundance of KGF and KGFR may also contribute to the proliferation of normal

ductal cells that is frequently observed in the chronic pancreatitis-like regions. A similar mechanism has been proposed to contribute to epidermal hyperplasia in psoriasis.³⁵ Furthermore, the co-localization of FGFR-2 with KGFR is consistent with the fact that KGFR and FGFR-2 derive from the same gene and raises the possibility that FGFR-2 also contributes to aberrant epithelial-mesenchymal interactions in human pancreatic cancer.

The mechanisms that lead to overexpression of KGF and its receptor are not known. It is established, however, that KGF mRNA expression is induced by certain cytokines, such as interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , IL-6, and TGF- α as well as by platelet-derived growth factor (PDGF).^{36,37} Furthermore, the promoter region of KGF is known to be activated by IL-1 and IL-6.³⁸ PDGF and TGF- α are overexpressed in pancreatic cancer.^{5,39} It is also possible that some of the KGF-stimulating cytokines are expressed in the chronic pancreatitis-like lesions close to the cancer cells. Together, these growth factors and cytokines may induce the overexpression of KGF mRNA in pancreatic cancer. Increased levels of KGF, acting via the overexpressed KGFR, may then contribute to the acinar cell regeneration and ductal cell proliferation that occurs in the chronic pancreatitis-like lesions.

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