Enhanced erbB-3 Expression in Human Pancreatic Cancer Correlates with Tumor Progression

Helmut Friess, Yoichiro Yamanaka, Michael S. Kobrin, David A. Do, Markus W. Büchler, and Murray Korc

Division of Endocrinology, Diabetes and Metabolism, Departments of Medicine and Biological Chemistry, University of California, Irvine, California 92717 [Y. Y., M. S. K., D. A. D., M. K.], and Department of Visceral and Transplantation Surgery, University of Berne, CH-3010 Berne, Switzerland [H. F., M. W. B.]

ABSTRACT

The erbB-3 gene encodes a transmembrane protein that is related to the epidermal growth factor (EGF) receptor and erbB-2. We compared erbB-3 expression in the normal human pancreas, human pancreatic carcinomas, and cultured human pancreatic cancer cell lines. Northern blot analysis of total RNA revealed the anticipated 6.2-kb mRNA transcript in all 19 normal pancreatic samples. In 17 of 27 pancreatic cancers, there was a 6.7-fold increase (P < 0.001) in erbB-3 mRNA levels. Southern blot analysis did not reveal erbB-3 gene amplification. Four of six pancreatic cancer cell lines exhibited the 6.2-kb erbB-3 mRNA transcript, and all four cell lines coexpressed the epidermal growth factor receptor and erbB-2. Using a highly specific antibody, we determined that faint to moderate erbB-3 immunoreactivity was present in the ductal cells in the normal pancreas. In 47% (27/58) of the pancreatic cancers, there were many cancer cells with intense erbB-3 immunostaining. The presence of erbB-3 in the cancer cells was associated with advanced tumor stage and shorter survival postoperatively. These data indicate that a significant proportion of human pancreatic cancers overexpress erbB-3, and that erbB-3 may contribute to disease progression in this disorder.

INTRODUCTION

Expression of growth factors and growth factor receptors may play an important role in tumor pathogenesis and may influence the clinical course and therapeutic responsiveness of human malignancies (1). A considerable body of evidence indicates that overexpression of certain transmembrane receptors that possess intrinsic tyrosine kinase activity is associated with malignant transformation (1–3). For example, overexpression of the EGF3 receptor in transfection experiments may lead to malignant transformation (2). Furthermore, enhanced levels of expression of the EGF receptor have been correlated with increased metastatic potential and poor prognosis in brain, breast, lung, and bladder carcinomas (4–7). This receptor belongs to a family of four transmembrane receptors (8–10). In addition to the human EGF receptor (HER-1), the members of this family are erbB-2 or human EGF receptor 2 (HER-2), erbB-3 or human EGF receptor 3 (HER-3), and erbB-4 or human EGF receptor 4 (HER-4). Each of these receptors is characterized by an extracellular ligand-binding domain that contains a high proportion of cysteine residues clustered in two distinct regions and an intracellular region containing a conserved tyrosine kinase domain (8–11). erbB-2 overexpression also leads to malignant transformation and has been implicated in the aggressiveness of certain malignancies (2, 3, 12, 13). The potential role of erbB-3 and erbB-4 in the neoplastic process has not yet been fully established.

Pancreatic cancer is a devastating disease with an overall 5-year survival rate of <0.4% (14, 15). The concomitant overexpression of the EGF receptor with either EGF and/or TGF-α is associated with enhanced pancreatic tumor aggressiveness and significantly shorter postoperative survival periods (16). In contrast, enhanced erbB-2 expression in pancreatic cancer correlates with a more differentiated phenotype and does not appear to be associated with tumor progression (17). Although erbB-3 immunostaining has been reported in human pancreatic tumors (18), the clinical relevance of this observation and its relationship to expression at the mRNA level are not known. Therefore, in the present study, we carried out Northern and Southern blot analyses of human pancreatic tumors, and investigated the relationship between the immunohistochemical presence of erbB-3 in these cancers and tumor grading, staging, and postoperative survival. We now report that there is an increase in the expression of the 6.2-kb erbB-3 transcript in pancreatic cancers in association with increased erbB-3 immunoreactivity and decreased patient survival.

PATIENTS AND METHODS

Pancreatic Tissues. Normal human pancreatic tissue samples were obtained from 19 previously healthy individuals (7 females and 12 males; median age, 39 years; range, 14–57 years) through an organ donor program. Pancreatic cancer tissue samples were obtained from 29 female and 29 male patients undergoing surgery for pancreatic cancer. The median age of the pancreatic cancer patients was 63 years, with a range of 31–77 years. A partial duodenopancreatectomy (Whipple resection) or a left resection of the pancreas (distal resection) was carried out...
Fig. 1  Northern blot analysis of pancreatic tissue RNA. Total RNA (20 μg/lane) from seven normal and eight pancreatic cancer samples was size fractionated, transferred onto nylon membranes, and hybridized as described in “Patients and Methods” using a 32P-labeled erbB-3 cRNA probe (1 × 106 cpm/ml; 5-day exposure) and a 32P-labeled 7S cDNA probe (5 × 105 cpm/ml; 24-h exposure). All normal samples exhibited the 6.2-kb erbB-3 mRNA transcript, but in two samples the band was only visible on the original autoradiograph. Left, migration positions of 28S and 18S ribosomal subunits.

in 41 and 17 patients, respectively. According to the TNM classification of the International Union Against Cancer (19), there were four grade 1, 33 grade 2, 20 grade 3, and 1 grade 4 duct cell adenocarcinomas. There were 18 patients with stage I, 12 patients with stage II, 24 patients with stage III, and 4 patients with stage IV disease.

Freshly removed tissue samples were immediately fixed in Bouin or 10% formaldehyde solution for 12 to 24 h and paraffin-embedded for histological analysis. In addition, tissue samples from 27 randomly selected patients with pancreatic cancer and from all normal organ donors were frozen in liquid nitrogen immediately upon surgical removal and maintained at −80°C until use for RNA and DNA extraction. All studies were approved by the Ethics Committee of the University of Berne (Berne, Switzerland) and by the Human Subjects Committee at the University of California (Irvine, CA).

Cell Culture. PANC-1, ASPC-1, MIA PaCa-2, and CAPAN-1 human pancreatic cancer cells and A431 human vulvar carcinoma cells were obtained from the American Type Culture Collection (Rockville, MD). T3M4 and COLO-357 human pancreatic cancer cells were a gift from R. S. Metzgar at Duke University. Cells were grown in monolayer culture at 37°C in a humidified air atmosphere. PANC-i, MIA PaCa-2, and COLO-357 cells were grown in DMEM. T3M4, ASPC-i, and CAPAN-i cells were grown in RPMI 1640 medium. Media contained antibiotics and 10% fetal bovine serum.

Northern Blot Analysis. Total RNA was extracted using the guanidine isothiocyanate method and size fractionated on a 1.2% agarose/1.8 m formaldehyde gel (20). Poly(A⁺) RNA was prepared from total RNA in a limited number of cases, using oligo(dT)-cellulose column chromatography, as previously reported (21). Total RNA (20 μg) or poly(A⁺) RNA samples were electrotransferred onto nylon membranes (Genescreen; DuPont, Boston, MA) and cross-linked by UV irradiation (20, 21). 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 (20, 21). In the case of antisense riboprobe, the blots were prehybridized overnight at 65°C and then hybridized for 18 h at 65°C in the presence of 1 × 106 cpm/ml of the labeled antisense.

Fig. 2  Northern blot analysis of total and poly(A⁺) RNA. Total RNA (20 μg/lane) from two different normal pancreatic tissues and the corresponding poly(A⁺) RNA (10 μg/lane) were size fractionated, transferred onto nylon membranes, and hybridized as described in “Patients and Methods” using the 32P-labeled erbB-3 cRNA probe (1 × 106 cpm/ml). A major 6.2-kb mRNA band and a minor 1.6-kb mRNA band are clearly visible when total RNA was used. Both mRNA moieties are markedly enhanced when poly(A⁺) RNA was used. Exposure times were 4 days for total RNA and 6 h for poly(A⁺) RNA. Left, migration positions of 28S and 18S ribosomal subunits.
Southern blot analysis. Following EcoRI digestion, genomic DNA (10 μg/ lane) was size fractionated and transferred onto nylon membranes as described in “Patients and Methods.” The filters were hybridized with 32P-labeled erbB-3 cDNA (1 × 10^6 cpm/ml; 4-day exposure) and 32P-labeled β-actin cDNA (1 × 10^6 cpm/ml; 24-h exposure) to verify equivalent DNA loading. Two bands corresponding to the erbB-3 gene were detectable. There was no evidence for gene amplification in any of the tissue samples. Left, positions of the molecular weight markers (kb).

Fig. 3 Southern blot analysis. Following EcoRI digestion, genomic DNA (10 μg/ lane) was size fractionated and transferred onto nylon membranes as described in “Patients and Methods.” The filters were hybridized with 32P-labeled erbB-3 cDNA (1 × 10^6 cpm/ml; 4-day exposure) and 32P-labeled β-actin cDNA (1 × 10^6 cpm/ml; 24-h exposure) to verify equivalent DNA loading. Two bands corresponding to the erbB-3 gene were detectable. There was no evidence for gene amplification in any of the tissue samples. Left, positions of the molecular weight markers (kb).

Southern Blot Analysis. Pancreatic tissues were pulverized in liquid nitrogen and incubated for 16 h at 50°C in a buffer containing 100 mM NaCl, 10 mM Tris-HCl (pH 8.0), 25 mM EDTA, 0.5% SDS, and 0.1 mg/ml proteinase K (17). DNA was purified by phenol/chloroform extraction, ethanol precipitated, and digested with the restriction endonuclease EcoRI and BgII (17). DNA samples were electrophoresed on 0.7% agarose gels (10 μg/lane) and transferred onto Hybond N Plus nylon membranes (Amersham Corp., Arlington Heights, IL) by alkaline capillary transfer. The membranes were prehybridized and hybridized for 16 h at 42°C, as previously reported (17). To confirm equivalent DNA loading of all lanes, the blots were rehybridized with a cDNA probe for human β-actin (American Type Culture Collection, Rockville, MD). The blots were exposed at −80°C to Kodak XAR-5 film with Kodak intensifying screens.

Probe Synthesis. A cDNA encoding sequences corresponding to the erbB-3 gene was produced by amplification with the PCR of single-stranded cDNA that was reverse transcribed from human placenta RNA. The cDNA, consisting of a 454-bp fragment corresponding to a portion of the intracellular domain (nucleotides 3507–3961) of human erbB-3 cDNA (9), was subcloned into a pGEM3zf vector (Promega Biotechnology, Madison, WI). Authenticity of the reverse transcriptase PCR product was confirmed by sequencing. [α-32P]dCTP (Amersham) were used for cRNA and cDNA probe synthesis, respectively (20, 21).

Immunohistochemistry. A highly specific anti-erbB-3 mAb (RTJ1; Novocastra Laboratories Ltd., Newcastle, England) that was raised against a synthetic peptide corresponding to a region in the cytoplasmic domain of the human erbB-3 protein was utilized for immunohistochemistry (22). It has been previously shown that human carcinoma cell lines exhibit immunostaining with this antibody only if they overexpress c-erbB-3 mRNA (22). In the present study, 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 sections for 60 min (23°C) with 0.6% hydrogen peroxide in methanol. The sections were then incubated for 30 min (23°C) with 10% normal goat serum prior to overnight incubation (4°C) with the anti-c-erbB-3 antibody at a 1:20 dilution using 0.01 M PBS and 1% BSA. Bound antibody was detected with a biotinylated goat antimouse IgG secondary antibody and streptavidin-peroxidase complex, and incubated with diaminobenzidine tetrahydrochloride (0.03%) as the substrate. Counterstaining was done with Mayer’s hematoxylin.

Statistical Analysis. Results are expressed as median and range or as mean ± SE. For statistical analysis, the Student’s t test, rank sum Wilcoxon test, and χ² test were used.
Postoperative survival periods were computed according to the method of Kaplan and Meier and compared by both the generalized Wilcoxon and the log rank test (25). Survival data, age, gender, tumor stage, and tumor grade were also analyzed by multivariate analysis using Cox proportional hazards regression (25). Significance was defined as \( P < 0.05 \).

RESULTS

**Molecular Analysis.** Northern blot analysis of total RNA demonstrated the presence of low levels of erbB-3 mRNA in all normal pancreatic tissue samples (Fig. 1). Although all of these samples exhibited a 6.2-kb erbB-3 mRNA transcript, in two of the samples the 6.2-kb band was only visible on the original autoradiographs (Fig. 1). In some samples, there was a smaller erbB-3 transcript with an approximate size of 1.6 kb (Fig. 1). This smaller transcript was also visible on Northern blots using poly(A\(^+\)) RNA from the normal human pancreas (Fig. 2), indicating that the 1.6-kb transcript was not due to cross-hybridization with rRNA moieties.

The 6.2-kb erbB-3 transcript was expressed at high levels in 17 of 27 (63%) carcinoma samples. Densitometric analysis indicated that, by comparison with the normal pancreas, these 17 samples exhibited a 6.7-fold increase (\( P < 0.001 \)) in the levels of the 6.2-kb transcript (range, 1.8–9.1-fold increase). Analysis of the data for all 27 cancers revealed a 3.6-fold increase in this mRNA moiety. Some of the cancer samples exhibited the 1.6-kb transcript, but its levels were not increased by comparison with the normal tissues.

Southern blot analysis was next carried out to determine whether the increase in erbB-3 mRNA levels in pancreatic cancer was associated with amplification of the erbB-3 gene. Following EcoRI digestion of 10 \( \mu \)g of genomic DNA and hybridization with the 454-bp erbB-3 cDNA probe, two fragments of the erbB-3 gene were detectable (Fig. 3). There were no aberrant bands in any of the cancer tissues. A similar analysis following HindIII digestion also did not reveal any aberrant DNA fragments (data not shown). Densitometric analysis and calculation of the absorbance ratio of the erbB-3 fragments with the corresponding \( \beta \)-actin signals did not reveal erbB-3 gene amplification in any of the cancer tissue samples.

We next sought to determine whether cultured human pancreatic cancer cell lines express the 1.6-kb transcript, and whether these cells coexpress all of the members of the EGF receptor family (Fig. 4). Northern blot analysis of RNA isolated from six pancreatic cancer cell lines did not reveal the 1.6-kb transcript in any of the cell lines. In contrast, T3M4, COLO-357, and CAPAN-1 cells expressed high levels of the 6.2-kb erbB-3 transcript. ASPC-1 cells expressed intermediate levels of this transcript, which was not detected in PANC-1 and MIA PaCa-2 cells. All of the cell lines exhibited the 4.8-kb erbB-2 (HER-2) transcript, which in the case of PANC-1 cells was only faintly visible on the original autoradiograph, as well as the 10.5-kb EGF receptor (HER-1) transcript, which in the case of CAPAN-1 cells was only faintly visible on the original autoradiograph. Only T3M4, COLO-357, and PANC-1 cells readily exhibited the 5.5-kb EGF receptor transcript, and only A431 human vulvar carcinoma cells appeared to express the 2.8-kb EGF receptor transcript (Fig. 4). However, in contrast to many of the pancreatic cancer cells, A431 cells appeared to express very low levels of the erbB-2 and erbB-3 transcripts. Northern blot analysis using 5 \( \mu \)g poly(A\(^+\)) RNA indicated that all six pancreatic cancer cell lines expressed both the 10.5- and 5.5-kb EGF receptor transcripts, whereas the erbB-4 transcript was not detected (data not shown).

**Immunocytochemical Studies.** In the normal human pancreas faint to moderate erbB-3 immunoreactivity was present in the cytoplasm of most ductal cells in both the intralobular (Fig. 5A) and interlobular (Fig. 5B) ducts. In contrast, erbB-3 immunostaining was not visible in the acinar cells (Fig. 5, A and B). In many of the pancreatic cancer samples, the cancer cells exhibited moderate to intense cytoplasmic as well as membranous erbB-3 immunoreactivity (Fig. 5, C and D). In some areas adjacent to the cancer cells, mild erbB-3 immunoreactivity was evident in the acinar cells (Fig. 5E), whereas in
Fig. 5 Immunohistochemical staining of erbB-3 in the human pancreas. Faint to moderate erbB-3 immunoreactivity was present in intralobular ducts (A, arrowhead), ductules (A, arrows), and interlobular ducts (B) in the normal human pancreas. Pancreatic cancer cells in poorly differentiated (C) and well-differentiated (D) carcinomas exhibited moderate to intense erbB-3 immunoreactivity. In the exocrine pancreas adjacent to the cancer cells, there was mild erbB-3 immunoreactivity in the acinar cells (E), which was greatly enhanced in regions exhibiting pseudoductular metaplasia (F). ×100.

Other areas there were regions of pseudoductular metaplasia with strong erbB-3 immunoreactivity (Fig. 5F).

To determine whether there was a correlation between the immunohistochemical data and clinicopathological findings, immunohistochemical analysis was performed in 58 tumor samples using established grading criteria (23, 24). Positive erbB-3 immunostaining was seen in 47% (27/58) of the tumor samples. $\chi^2$ analysis of these data indicated that the presence of erbB-3 in
Table 1  Relationship between the presence of erbB-3 and tumor stage and grade in 58 patients with pancreatic cancer*

<table>
<thead>
<tr>
<th>erbB-3 Immunoreactivity</th>
<th>Tumor stage</th>
<th>Tumor grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n = 31)</td>
<td>1 II-IVb</td>
<td>1 I II III + IV</td>
</tr>
<tr>
<td>Positive (n = 27)</td>
<td>4 23 12 15 1</td>
<td>1 14 11 1</td>
</tr>
</tbody>
</table>

* Positive immunostaining was defined as strong immunoreactivity in more than 10% of the cancer cells.

b P < 0.05 by x² analysis, by comparison to stage I.

Fig. 6  Survival curves. Kaplan-Meier plots of the postoperative survival data in patients whose tumors were negative for erbB-3 immunostaining versus patients whose tumors were positive for erbB-3 immunostaining. Patients whose tumors were negative for erbB-3 lived longer than patients whose tumors were positive, as determined by either the generalized Wilcoxon test (P < 0.02) or the log rank test (P < 0.04).

The c-erbB-3 gene encodes a M, 180,000 transmembrane polypeptide that shares close structural homology with the EGF receptor and c-erbB-2 (9). The gene was detected by low stringency hybridization of v-erbB to normal genomic human DNA and was mapped to human chromosome 12q13 (9). It is expressed in a variety of normal tissues and in breast and ovarian cancers (9, 26–28). Furthermore, its overexpression has been correlated with the presence of lymph node metastases in breast cancer, but not with tumor size, histological grade, or patient survival (27). The catalytic region of the kinase-like domain of erbB-3 lacks four amino acid residues that are conserved in tyrosine as well as serine/threonine kinases and is devoid of intrinsic tyrosine kinase activity (29, 30). Nonetheless, there are at least two mechanisms that lead to tyrosine phosphorylation of erbB-3. First, phosphorylation may occur as a result of binding to a family of closely related growth factors called neu differentiation factors (or heregulins) and heterodimerization with erbB-2 (30, 31). Second, erbB-3 may undergo tyrosine phosphorylation following heterodimerization with activated EGF receptor (32, 33).

Previous work from our laboratory has demonstrated that EGF receptor and erbB-2 mRNA levels are each 3.0-fold elevated in pancreatic cancers by comparison to the normal pancreas (17, 20). In the present study, Northern blot analysis revealed a 6.7-fold increase in erbB-3 mRNA levels in 17 of 27 pancreatic cancer samples, and a 3.6-fold overall increase in the levels of this transcript. Inasmuch as there was no evidence for erbB-3 gene amplification, erbB-3 overexpression was most likely due to increased transcription rates and/or enhanced message stability. In contrast, we did not detect erbB-4 mRNA transcripts by Northern blot analysis in either the normal or cancerous pancreas.4 Thus, our data indicate that erbB3 is frequently coexpressed at high levels with the EGF receptor and erbB-2 in human pancreatic cancers. This conclusion is supported by our observation that three of six pancreatic cancer cell lines coexpress all three receptors, and that all of the pancreatic cancer cell lines coexpressed at least two of these receptors.

A small 1.6-kb erbB-3 mRNA transcript was observed in some of the normal and cancer samples. The biological significance of this smaller erbB-3 transcript is not clear. A 1.4-kb transcript that may result from alternative splicing and that has been shown to encode a secreted extracellular form of erbB-3 has been reported in gastric cancer cell lines (34). Inasmuch as the erbB-3 probe used in the present study corresponds to a region encoding a portion of the intracellular domain of the receptor, the 1.6-kb transcript does not represent a secreted form of erbB-3. Furthermore, the failure of cultured pancreatic cancer cell lines to exhibit this transcript, and the fact that its levels were not increased in the cancers suggest that it is only expressed in the normal pancreas. Thus, its presence in some of the pancreatic cancers may derive from normal cells around the tumor mass.

We found faint to moderate erbB-3 immunoreactivity in the ductal cells of the normal human pancreas, in agreement

* H. Friess and M. Korc, unpublished observations.

the cancer cells was associated with a more advanced tumor stage (P < 0.05), but not with the histological grade of the tumor (Table 1). One patient died at the 10th postoperative day from a massive pulmonary embolism and was therefore excluded from the survival analysis. Survival data were not available on four additional patients. Analysis of the data on the remaining 53 patients indicated that the presence of erbB-3 was associated with a shorter postoperative survival (8.7 ± 4.5 months, mean ± SD) by comparison with patients whose tumors did not exhibit erbB-3 immunoreactivity (13.4 ± 7.3, mean ± SD). This difference was statistically significant when analyzed by either the generalized Wilcoxon test (P < 0.02) or by the log rank test (P < 0.04; Fig. 6). Survival data were also analyzed by multivariate analysis in relation to gender, tumor stage, and grade and erbB-3 immunoreactivity. Advanced tumor stage (III-IV) was an independent prognostic marker (P < 0.01), whereas erbB-3 immunoreactivity was not an independent marker (P < 0.09). When patient age was included in the analysis, advanced tumor stage and younger age were independent markers correlating with poor prognosis. Furthermore, patients whose cancers were erbB-3 positive were younger than erbB-3-negative patients (P < 0.05).

**DISCUSSION**

The c-erbB-3 gene encodes a M, 180,000 transmembrane polypeptide that shares close structural homology with the EGF receptor and c-erbB-2 (9). The gene was detected by low stringency hybridization of v-erbB to normal genomic human DNA and was mapped to human chromosome 12q13 (9). It is expressed in a variety of normal tissues and in breast and ovarian cancers (9, 26–28). Furthermore, its overexpression has been correlated with the presence of lymph node metastases in breast cancer, but not with tumor size, histological grade, or patient survival (27). The catalytic region of the kinase-like domain of erbB-3 lacks four amino acid residues that are conserved in tyrosine as well as serine/threonine kinases and is devoid of intrinsic tyrosine kinase activity (29, 30). Nonetheless, there are at least two mechanisms that lead to tyrosine phosphorylation of erbB-3. First, phosphorylation may occur as a result of binding to a family of closely related growth factors called neu differentiation factors (or heregulins) and heterodimerization with erbB-2 (30, 31). Second, erbB-3 may undergo tyrosine phosphorylation following heterodimerization with activated EGF receptor (32, 33).

Previous work from our laboratory has demonstrated that EGF receptor and erbB-2 mRNA levels are each 3.0-fold elevated in pancreatic cancers by comparison to the normal pancreas (17, 20). In the present study, Northern blot analysis revealed a 6.7-fold increase in erbB-3 mRNA levels in 17 of 27 pancreatic cancer samples, and a 3.6-fold overall increase in the levels of this transcript. Inasmuch as there was no evidence for erbB-3 gene amplification, erbB-3 overexpression was most likely due to increased transcription rates and/or enhanced message stability. In contrast, we did not detect erbB-4 mRNA transcripts by Northern blot analysis in either the normal or cancerous pancreas.4 Thus, our data indicate that erbB3 is frequently coexpressed at high levels with the EGF receptor and erbB-2 in human pancreatic cancers. This conclusion is supported by our observation that three of six pancreatic cancer cell lines coexpress all three receptors, and that all of the pancreatic cancer cell lines coexpressed at least two of these receptors.

A small 1.6-kb erbB-3 mRNA transcript was observed in some of the normal and cancer samples. The biological significance of this smaller erbB-3 transcript is not clear. A 1.4-kb transcript that may result from alternative splicing and that has been shown to encode a secreted extracellular form of erbB-3 has been reported in gastric cancer cell lines (34). Inasmuch as the erbB-3 probe used in the present study corresponds to a region encoding a portion of the intracellular domain of the receptor, the 1.6-kb transcript does not represent a secreted form of erbB-3. Furthermore, the failure of cultured pancreatic cancer cell lines to exhibit this transcript, and the fact that its levels were not increased in the cancers suggest that it is only expressed in the normal pancreas. Thus, its presence in some of the pancreatic cancers may derive from normal cells around the tumor mass.

We found faint to moderate erbB-3 immunoreactivity in the ductal cells of the normal human pancreas, in agreement...
with the low levels of erbB-3 mRNA in these samples. In the pancreatic tumors, many cancer cells exhibited strong erbB-3 immunostaining, which, with one exception, was increased only modestly in the exocrine parenchyma adjacent to the cancer cells. Of note, erbB-3 immunoreactivity in the cancers was associated with a more advanced tumor stage and shorter postoperative survival. In contrast, using the same immunohistochemical criteria that were used in the present study, we did not find a correlation between erbB-2 immunostaining in pancreatic cancer and postoperative patient survival (17). Furthermore, the presence of EGF receptor correlated with decreased survival only when the cancer cells also exhibited EGF and/or TGF-α immunostaining (16).

It is not clear why among the members of the EGF receptor family erbB-3 should have an especially important role in pancreatic cancer. Similarly, it is not readily evident why younger patients with pancreatic cancer have more aggressive disease and why their cancers exhibit erbB-3 immunoreactivity more frequently than pancreatic cancers from older patients. However, it is known that tyrosine phosphorylation of erbB-3 is dependent on its ability to heterodimerize with other members of the EGFR receptor family (30–33). Once phosphorylated, erbB-3 efficiently recruits the p85 regulatory subunit of phosphatidylinositol 3 kinase, a signaling molecule that does not bind directly to other members of the EGFR receptor family (32, 33). It is conceivable, therefore, that co-expression of high levels of the EGF receptor, erbB-2, and erbB-3 in human pancreatic cancer may allow for excessive activation of signaling pathways that would not be activated in the absence of erbB-3. This excessive activation may be further enhanced by the overexpression of multiple ligands that bind to the EGF receptor, including EGF (20), TGF-α (20), heparin-binding EGF-like growth factor (35), and amphiregulin (36), and by the frequent presence of K-ras mutations in pancreatic cancers (37–39). Together, these perturbations may combine to contribute to disease progression and enhanced biological aggressiveness in pancreatic cancer. Our findings thus raise the possibility that therapeutic interventions aimed at abrogating erbB-3-mediated signaling may have a potentially important role in this disorder.

ACKNOWLEDGMENTS
We thank Dr. Michael J. Schell for assisting with statistical analysis of survival data.

REFERENCES
23. Yamanaka, Y., Friess, H., Büchler, M., Beger, H. G., Uchida, E., Onda, M., Kobrin, M. S., and Korc, M. Overexpression of acidic and
31. Tzahar, E., Levkowitz, G., Karunagaran, D., Yi, L., Peles, E., Lavi, S., Chang, D., Liu, N., Yayon, A., Wen, D., and Yarden, Y. ErbB-3 and erbB-4 function as the respective low and high affinity receptors of all
Enhanced erbB-3 expression in human pancreatic cancer correlates with tumor progression.

H Friess, Y Yamanaka, M S Kobrin, et al.


Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/1/11/1413

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.