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5-Lipoxygenase and Leukotriene B_4 Receptor Are Expressed in Human Pancreatic Cancers But Not in Pancreatic Ducts in Normal Tissue

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The 5-lipoxygenase (5-LOX) pathway is critical for pancreatic cancer cell growth and escape from apoptosis. Inhibition of 5-LOX blocks proliferation and induces apoptosis in human pancreatic cancer cells. However, the expression of 5-LOX and its downstream signaling pathway have not been investigated in human pancreatic adenocarcinoma. Reverse transcriptase-polymerase chain reaction revealed expression of 5-LOX mRNA in all pancreatic cancer cell lines tested including, PANC-1, AsPC-1, and MiaPaCa2 cells, but not in normal pancreatic ductal cells. The expression of 5-LOX protein in pancreatic cancer cell lines was demonstrated by Western blotting. Finally, 5-LOX up-regulation in human pancreatic cancer tissues was verified by intense positive staining in cancer cells by immunohistochemistry. Staining for the 5-LOX protein was particularly evident in the ductal components of the more differentiated tumors but not in ductal cells in normal pancreatic tissues from cadaver donors. Immunohistochemistry also revealed strong staining of cancer tissues with an antibody to the receptor of the downstream 5-LOX metabolite, leukotriene B_4. The current study demonstrated marked expression of 5-LOX and the leukotriene B_4 receptor in human pancreatic cancer tissues. These findings provide further evidence of up-regulation of this pathway in pancreatic cancer and that LOX inhibitors are likely to be valuable in the treatment of this dreadful disease. ***(Am J Pathol 2002, 161:421–428)***

Pancreatic adenocarcinoma is characterized by a poor prognosis and lack of response to conventional therapy. This dreadful disease is now the fourth leading cause of cancer death in both men and women in the United States. The incidence has shown no significant sign of decline throughout the past 20 years and almost equals its mortality.[1–3] The 5-year survival rate for this disease is less than 4% and the median survival time after diagnosis is less than 6 months.[2,3] Surgical resection of the tumor is still the only effective treatment option, although only ~20% of carcinomas of the head of the pancreas are resectable.[4] Furthermore, the median survival even after apparent curative resection is only ~20 months, because of early tumor recurrence or rapid metastatic spread.[2,4] Other treatment options, such as chemotherapy or radiation therapy, provide limited palliation without significant improvement of survival in patients with nonresectable pancreatic cancer.[2] Therefore, new targets for chemopreventive and therapeutic agents need to be identified.

The results from epidemiological and animal studies suggest that a high-fat consumption is associated with an increased incidence and growth of tumors at several specific organ sites including pancreas, colon, breast, and prostate.[5,6] A recent review pointed out the important role of lipoxygenase (LOX) pathways in fat metabolism and in the regulation of pancreatic cancer cell proliferation and survival.[7] In summary, the mammalian 5-LOX pathway produces several potent biological mediators, including leukotriene B_4 (LTB_4) and the peptidoleukotrienes (LTC_4, LTD_4, or LTE_4) in addition to 5(S)-HETE.[8–10] The activity of 5-LOX is dependent on Ca^{++} and ATP, features that distinguish this enzyme from other LOXs.[11–14]

5-LOX is localized in the nucleus and cytosol but, on cellular activation the enzyme undergoes Ca^{++}-dependent translocation to the nuclear envelope.[12,15,16] The mechanism of translocation involves association of 5-LOX with a novel 18-kd membrane protein now known as 5-LOX-activating protein.[14–17] Transfection experiments have clearly shown that the presence of both 5-LOX and 5-LOX-activating protein are essential for leukotriene biosynthesis in intact cells.[14,17,18] 5-LOX is widely distributed in human tissues and is responsible for many physiological and pathophysiological events. In

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contrast to detailed investigation of the expression and functions of 5-LOX in human allergic and inflammatory diseases, information on the role of 5-LOX in cancer development and growth are limited. Certain prostate cancer cell lines produce the 5-LOX metabolite, 5(S)-HETE.[19–21] Hong and colleagues[22] showed that both 5-LOX and 5-LOX-activating protein mRNA are expressed in all of the lung, colon, and prostate cancer cell lines tested, whereas cyclooxygenase-2 (COX-2), 12-LOX, and 15-LOX were expressed in 60%, 35%, and 90% of these cell lines, respectively. Expression of 5-LOX protein has been confirmed in lung cancer by immunohistochemistry.[23] Expression of 5-LOX was also reported in human breast cancer, where it is related to cell proliferation.[24]

Immunohistochemistry has revealed that 5-LOX is expressed in porcine pancreatic acinar cells but not in porcine Langerhans islets or ductal cells.[25] Although weak 5-LOX immunoreactivity has been demonstrated in whole human pancreas extracts, to our knowledge the expression of 5-LOX in human pancreatic ductal cells and ductal adenocarcinoma cells has never been reported.[26]

In the present study, the expression of 5-LOX in normal pancreatic ductal cells from a human multiorgan donor and cancer cells was investigated by both nested reverse transcriptase-polymerase chain reaction (RT-PCR) and Western blotting. 5-LOX mRNA was not detected in normal human pancreatic ductal cells from a multiorgan donor but it was detected in all pancreatic cancer cell lines tested. Western blotting confirmed expression at the protein level. Furthermore, immunohistochemistry revealed that 5-LOX is up-regulated in surgical human pancreatic cancer tissues. These data support the concept that 5-LOX inhibitors are likely to be useful for prevention or treatment of pancreatic cancer.

Materials and Methods

Materials

RPMI 1640, Dulbecco's modified Eagle's medium, McCoy's 5A media, penicillin-streptomycin solution, and trypsin-ethylenediaminetetraacetic acid solution were purchased from Sigma Chemical Co. (St. Louis, MO). Fetal bovine serum was from Atlanta Biologicals (Norcross, GA). The monoclonal 5-LOX antibody was purchased from BD Pharmingen (San Diego, CA) and the polyclonal LTB_4 receptor antibody (lot 10925a) as well as the LTB_4 receptor (human)-blocking peptide from Cayman Chemicals (Ann Arbor, MI). RNAzol B reagent was purchased from TelTest (Austin, TX). RT-PCR reagents and the Prism ready reaction DyeDeoxy terminator cycle sequencing kit were from Perkin-Elmer (Foster City, CA). The horseradish peroxidase-conjugated secondary antibody, and Luminol reagents were from New England BioLabs (Beverly, MA). The TOPO PCR cloning vector and DNA ligation kit were obtained from Invitrogen (Carlsbad, CA). The normal goat serum, the streptavidin-peroxidase, the streptavidin-phosphatase, the diaminobenzidine reagent set, and the Histo-Mark-Red AP-system were purchased from Kirkegaard & Perry Laboratories (Gaithersburg, MD). The biotinylated secondary antibody (Multilink) was purchased from BioGenex (San Ramon, CA).

Cell Lines and Cell Cultures

The cell lines used, HPAF (well differentiated, heterogeneous), PANC-1 (poorly-differentiated), Capan2 (well differentiated), MiaPaCa-2, and AsPC-1 (poorly differentiated) were established from patients with pancreatic adenocarcinoma. All of the human pancreatic cancer cell lines were purchased from the American Type Culture Collection (Rockville, MD). PANC-1 and MiaPaCa2 were cultured in Dulbecco's modified Eagle's medium and Capan2 and AsPC1 were grown in McCoy's 5A media. HPAF was grown in minimal essential medium. Media were supplemented with 10% fetal bovine serum and cells grown as monolayers in a humidified atmosphere of 5% CO_2 at 37°C. Cells were routinely harvested by incubation of the cells in the trypsin-ethylenediaminetetraacetic acid solution for 10 minutes. The cells were pelleted by centrifugation (900 $\times$ *g*, 5 minutes) and suspended in fresh culture media before seeding into culture flasks. The human ductal cells (HUDs) were originally cultured from a human multiorgan donor.[27]

RT-PCR for 5-LOX

RNA from PANC-1, MiaPaCa2, AsPC-1, and Capan2 cells was isolated using RNAzol B, extracted with chloroform and precipitated with isopropanol. The RNA pellets were washed with 75% ethanol and the integrity confirmed on a 1% agarose gel. Then cellular RNA was reverse-transcribed into cDNA using each specific downstream primer and reverse transcriptase, according to the manufacturer's protocol (GeneAmp kit, Perkin Elmer). The total volume of the reaction was 20 μl containing the following: 2 μg of total RNA, 25 pmol of reverse primer with 25 mmol/L $MgCl_2$, 10$\times$ PCR buffer II, RNase/DNase-free water, 0.2 mmol/L of dNTPs, RNase inhibitor (20 U), and the reverse transcriptase (5 U). The thermocycler protocol for the RT phase is one hold at 42°C for 60 minutes, one hold at 99°C for 5 minutes, and one hold at 5°C for 5 minutes.

After the RT reaction, nested PCR was used to examine 5-LOX mRNA expression using human leukocyte RNA as positive control. PCR primers were designed based on the sequence of human 5-LOX cDNA. The first run PCR profile was 94°C for 15 seconds, 61°C for 30 seconds, and 72°C for 1 minute for 35 cycles with upstream 5′-CCCGGGGCATGGAGAGCAA-3′ and downstream 5′-CCAGGAACAGCTCGTTTTCCT-3′ primers. Five μl of first run PCR product was used for the nested PCR with the profile of 94°C for 15 seconds, 61°C for 30 seconds, and 72°C for 1 minute for 35 cycles with nested primers (upstream 5′-ATCAGGACGTTCACGGCCGA-3′; downstream 5′-GTCCACGATCTGCTCAATGGT-3′). The final PCR product was separated on 2% agarose gel and visualized with ethidium bromide.

Sequencing of 5-LOX RT-PCR Product

To confirm the 5-LOX RT-PCR product produced in our study is identical to that reported in the Gene Bank, the PCR product was sequenced using the TOPO TA Cloning kit for sequencing provided by Invitrogen following the manufacturer's instructions. One μl of our PCR product was used. This kit is designed for cloning and subsequent sequencing of *Taq*-amplified PCR products. The TOPO TA cloning kit for sequencing uses the TOPO cloning vector, pCR4-TOPO, with covalently bound topoisomerase I enzyme for 5 minute TOPO cloning and >95% recombinants. TOPO TA cloning is made possible by a unique enzyme, topoisomerase I. When combined with the pCR2.1-TOPO vector, the topoisomerase I activates the DNA (the PCR product), making it ready for rapid ligation with a PCR product with compatible overhangs.

Finally, samples were loaded on a 4.75, w/v, acrylamide gel containing 8.3 mol/L urea, 0.045 mol/L Tris-borate, and 0.001 mol/L ethylenediaminetetraacetic acid, and electrophoresed at 250 V (40 mA) for 14 hours at 4°C. DyeDeoxy-labeled DNA was detected using automated fluorescence spectrometry.

Western Blotting for 5-LOX

Thirty μg of pancreatic cancer cell lysates were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%). Proteins were transferred to nitrocellulose membranes by electroblotting using a mini semidry transfer-blotting apparatus (BioRad, Hercules, CA). The membranes were subsequently blocked in Tris-buffered saline (TBS) containing 0.1% Tween-20 and 5% nonfat milk, then treated with mouse monoclonal 5-LOX antibody at a 1:500 dilution in 5% nonfat milk and TBS containing 0.1% Tween-20 overnight at 4°C. The membrane-bound 5-LOX protein-antibody complex was reacted with horseradish peroxidase-conjugated goat anti-mouse antibody at 1:2000 dilution for 1 hour. 5-LOX protein was detected by chemiluminescence involving treatment of membrane with LumiGlo reagent and capture of light emissions to X-ray film.

Immunohistochemistry for 5-LOX and LTB_4 Receptor

Ten surgical pancreatic adenocarcinoma specimens and one liver metastasis from a patient with pancreatic adenocarcinoma were examined. Ten pancreas specimens from multiorgan donors were included as controls. Furthermore, we investigated nine tissue samples obtained from patients who underwent surgery because of chronic pancreatitis and seven specimens from the resection margins of patients with resected pancreatic adenocarcinoma.

All specimens were fixed in 10% buffered formalin, paraffin-embedded, and processed for histology by conventional methods. Sections 4-μm thick were prepared from paraffin blocks. After deparaffinization the slides were submerged in methanol containing 0.3% hydrogen peroxide for 30 minutes at room temperature to inhibit endogenous peroxidase activity (only for 5-LOX). Antigen retrieval was performed only for 5-LOX antigen by incubating the sections in 10 mmol/L citrate buffer (pH 6) in a microwave oven for 12 minutes (2 minutes high power, 10 minutes medium-low power). Thereafter, slides were cooled down to room temperature and then washed in TBS (0.1 mol/L, pH 7.4). The slides were incubated with normal goat serum for 30 minutes at room temperature. The slides were then incubated with the primary antibodies directed against 5-LOX (mouse monoclonal, 1:250) and LTB_4 receptor (rabbit polyclonal, 1:200) for 18 hours at 4°C overnight diluted in TBS containing 1% bovine serum albumin. The slides were then washed again in TBS and incubated with biotinylated secondary antibody (Multilink) against rabbit or mouse primary antibodies for 10 minutes at 37°C. Detection of the antibody complex was performed by the streptavidin-peroxidase reaction kit using diaminobenzidine as chromogen for 5-LOX and by streptavidin-phosphatase using the Histo-Mark-Red AP-system for the LTB_4 receptor. To ensure specificity of the primary antibodies, tissue sections were incubated in the absence of the primary antibodies and in addition the LTB_4 receptor antibody was quenched with the blocking peptide (50 μg/ml) for 1 hour at room temperature as controls. Counterstaining was performed with hematoxylin Gil No. 2. The stained tissue samples were verified by two pathologists.

Results

Expression of 5-LOX in Pancreatic Cancer Cell Lines

We used nested RT-PCR to detect the 5-LOX mRNA expression in pancreatic cancer cells. The PCR product is 336 bp, which is the expected size for the 5-LOX product. Because of the relatively lower abundance of 5-LOX mRNA in noninflammatory cells, the use of nested PCR increases the sensitivity. There are 14 exons and 13 introns in the genomic DNA of 5-LOX. The PCR primers used here are designed to stride two introns to exclude the genomic DNA-derived PCR product as the product. In parallel, human leukocyte RNA was used as a positive control in our study, because it has been reported that 5-LOX is expressed in leukocytes. Our results from nested RT-PCR show that 5-LOX mRNA is expressed in all of the pancreatic cancer cell lines tested, PANC-1, AsPC-1, and MiaPaCa2 (Figure 1).

It is widely believed that human pancreatic cancer cells are derived from the pancreatic ductal cells rather than acinar cells. RNA was extracted from the normal human pancreatic ductal cells and subjected to nested RT-PCR for 5-LOX mRNA expression. Compared with pancreatic cancer cells, 5-LOX was undetectable in the cultivated normal pancreatic ductal cells from a human multiorgan donor (Figure 2). To further confirm that the product from nested RT-PCR is derived from 5-LOX mRNA and identical to the 5-LOX cDNA sequence reported in the Gene

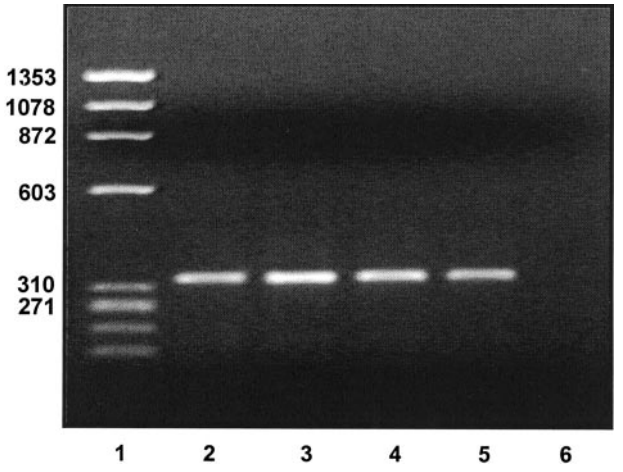

Figure 1. Expression of 5-LOX mRNA in pancreatic cancer cells. Total RNA was isolated from pancreatic cancer cells, reverse-transcribed, and then amplified by nested PCR. The PCR product was separated on 2% agarose gel and visualized by ethidium bromide staining. 5-LOX expression. **Lane 1**, DNA marker; **lane 2**, MiaPaCa2; **lane 3**, PANC-1; **lane 4**, AsPC-1; **lane 5**, leukocyte RNA; **lane 6**, non-RT control.

Bank, the PCR product was cloned into TOPO PCR sequencing plasmid and sequenced. The result showed that the sequence of our nested PCR product is identical to that reported in the Gene Bank (Table 1).

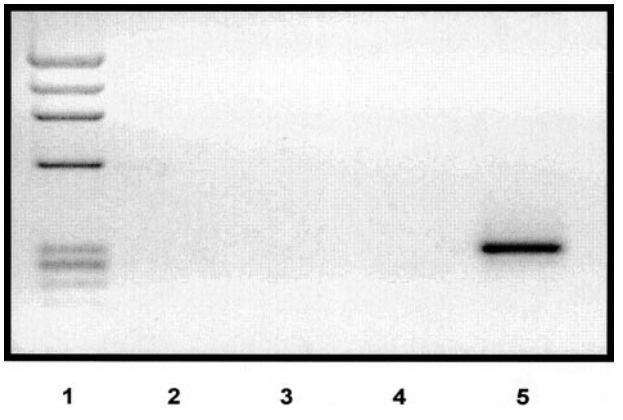

Figure 2. Expression of 5-LOX mRNA in pancreatic HUDs. Total RNA was isolated from pancreatic cancer cells, reverse-transcribed and then amplified by nested PCR. The PCR product was separated on 2% agarose gel and visualized by ethidium bromide staining. 5-LOX expression. **Lane 1**, DNA marker; **lane 2**, BOP-treated HUDs; **lane 3**, NNK-treated HUDs; **lane 4**, normal HUDs; **lane 5**, PANC-1.

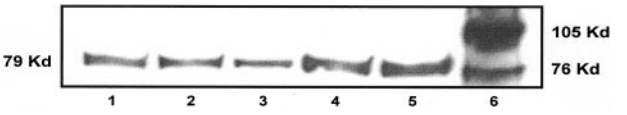

Figure 3. Expression of 5-LOX protein in pancreatic cancer cells. Western blotting. **Lane 1**, PANC-1; **lane 2**, AsPC-1; **lane 3**, HPAF; **lane 4**, MiaPaCa2; **lane 5**, Capan-l; **lane 6**, protein marker.

Furthermore, expression of 5-LOX protein in pancreatic cancer cell lines was confirmed with Western blotting using a monoclonal 5-LOX antibody. In accordance with mRNA expression, 5-LOX protein was also readily detected in the pancreatic cancer cell lines, including HPAF, PANC-1, AsPC-1, and CaPan-2 (Figure 3). These cell lines include poorly differentiated (PANC-1, AsPC-1, MiaPaCa2), well-differentiated (Capan2), and heterogeneous and well-differentiated (HPAF) pancreatic cancer cell lines. Apparently, this provides a good representation for different types of pancreatic cancer.

Expression of 5-LOX in Human Pancreatic Cancer Tissues

Performing immunohistochemistry with a monoclonal antibody against 5-LOX revealed that 5-LOX was expressed in 9 of 10 (90%) human pancreatic adenocarcinomas with an intense staining in ductal cells (9 of 10) and in adjacent islet cells (6 of 10). Moreover, the nucleus and nuclear envelopes of these ductal cells revealed the strongest positive staining. In the islet cells, intense staining was seen in the nucleus, nuclear envelope, and cytoplasm. A similar pattern of ductal and islet cell staining was seen in seven of nine chronic pancreatitis specimens as well as in six of seven pancreatic tissues obtained from the resection margins of pancreatic adenocarcinomas. However, the percentage of ductal cells showing a positive staining of the nuclear envelope was lower in some areas of these tissues compared with the cancers. In contrast, 5-LOX expression was only detected in a small proportion of normal ductal cells (0 to 7.5%) in 10 human pancreatic tissues from multiorgan donors. The staining in pancreatic islets showed a marked difference because of no (4 of 10) or very weak staining (6 of 10) in these tissues. Looking to one liver metastasis of a pancreatic adenocarcinoma we also found very intense

Table 1. RT-PCR Product of 5-LOX Identical to that Reported in the Gene Bank

RT-PCR product sequence of 5-LOX				
From 1478				
5′-				
1478 ATC				
1481 AGGACGTTCA	CGGCCGAGGT	GGTAGACATC	TACTACGAGG	GCGACCAGGT
1531 GGTGGAGGAG	GACCCGGAGC	TGCAGGACTT	CGTGAACGAT	GTCTACGTGT
1581 ACGGCATGCG	GGGCCGCAAG	TCCTCAGGCT	TCCCCAAGTC	GGTCAAGAGC
1631 CGGGAGCAGC	TGTCGGAGTA	CCTGACCGTG	GTGATCTTCA	CCGCCTCCGC
1681 CCAGCACGCC	GCGGTCAACT	TCGGCCAGTA	CGACTGGTGC	TCCTGGATCC
1731 CCAATGCGCC	CCCAACCATG	CGAGCCCCGC	CACCGACTGC	CAAGGGCGTG
1781 GTGACCATTG	AGCAGATCGT	GGAC		
-3′				

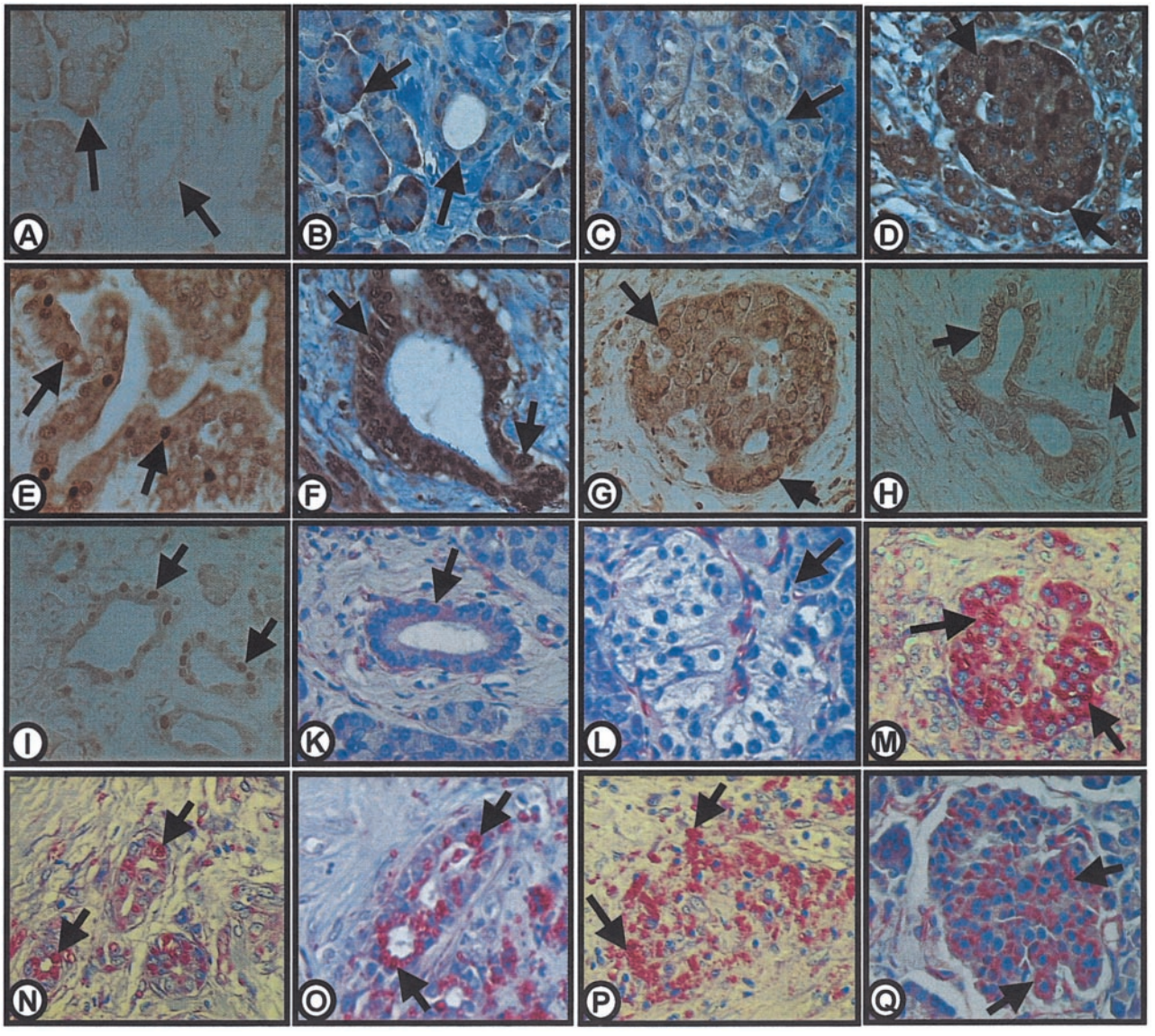

Figure 4. Expression of 5-LOX and LTB_4 receptor in human pancreatic tissues. Immunohistochemistry for 5-LOX is shown in panels **A–I**. **A** and **B:** Normal human pancreas with unstained ductal cells and light positive staining of the acinar cell cytoplasm. **C:** Unstained islet in normal human pancreas. **D** and **G:** Human pancreatic adenocarcinoma with intense positive staining in islet cells. **E** and **F:** Human pancreatic adenocarcinoma with intense positive staining in ductal cells. **H:** Positive stained duct in tissue adjacent to cancer. **I:** Positive stained duct in chronic pancreatitis. Immunohistochemistry for LTB_4 receptor is shown in panels **K–Q**. **K:** Pancreatic duct in normal human pancreas. **L:** Normal human pancreas with weak positive staining in an islet. **M:** Human pancreatic adenocarcinoma with strong positive staining in an adjacent islet. **N** and **O:** Human pancreatic adenocarcinoma with intense staining in ductal cells, and in the fibrotic area of the tissue (**P**). **Q:** Positive staining in an islet in chronic pancreatitis. Immunohistochemistry for 5-LOX used a mouse monoclonal antibody, 1:250, microwave pretreatment, incubation overnight at 4°C, and diaminobenzidine, and for the LTB_4 receptor used a rabbit polyclonal antibody, 1:200, incubation overnight at 4°C, and the HistoMark Red AP-System. Hematoxylin counterstain: **B–D**, **F**, and **K–Q**. Original magnifications, ×400.

staining in these ductal cells whereas the adjacent normal liver tissue was not stained. But as already described in porcine pancreas, we found a light positive staining of the acinar cell cytoplasm in all human pancreatic tissues (Figure 4).

Immunohistochemistry was also performed using an antibody to the receptor of the 5-LOX downstream metabolite, LTB_4. This revealed a strong intracytoplasmic and perinuclear staining in 10 of 10 (100%) human pancreatic adenocarcinoma tissues, particularly seen in surrounding islets, ductal cells, and also in the fibrotic parts of the tissues. The staining was similar in eight of nine chronic pancreatitis specimens and in seven of seven tissue samples from the resection margins of pancreatic adenocarcinomas, but the intensity appeared to be less than in cancer tissues. In comparison this staining was seen to a much lesser content or completely absent in all of the 10 normal pancreatic tissues from multiorgan donors. However, the stained structures in the cytoplasm have not been conclusively identified (Figure 4).

Negative controls for 5-LOX and LTB_4 receptor (first antibody omitted and quenching with the blocking peptide for the LTB_4 receptor) were obtained and showed no staining.

Discussion

Several studies have shown that essential polyunsaturated fatty acids, including arachidonic acid, play an important role in pancreatic cancer growth and development.[28–30] However, the underlying mechanisms are not fully understood. Several lines of evidence including our studies suggest that COX, especially COX-2 is important for pancreatic cancer growth.[31–36] COX-2 is overexpressed in both human pancreatic cancer cell lines and pancreatic cancer tissues compared to normal tissues. Blockade of COX enzymes significantly inhibits pancreatic cancer cell proliferation and induces apoptosis.

However, we have shown that the LOX pathways (both 5-LOX and 12-LOX) are even more critical for pancreatic cancer cell growth and survival than the COX pathway. Blockade of 5-LOX inhibits pancreatic cancer cell proliferation and induces apoptosis both *in vitro* and *in vivo*.[37,38] To our knowledge there is no information available for the expression of 5-LOX in human pancreatic cancer tissue at this time. Immunohistochemistry studies have only shown the 5-LOX expression in porcine pancreatic acinar cells but not in porcine Langerhans islets or ductal cells.[25] Staining was localized along the nuclear membranes of the acinar cells, demonstrated by immunoelectron microscopy.[25] 5-LOX expression and distribution was also investigated in rat pancreas, but no evidence of 5-LOX antigen or enzymatic activity was found in exocrine pancreas, purified β-cells, or other islet cells.[39] In contrast, 12-LOX is expressed in islet β-cells.[39] Quantifying 5-LOX activity in subcellular fractions of pancreas samples obtained from three human donors, 5-LOX was detectable in all samples, although enzyme activity was lower than in human leukocytes.[26] The highest specific activity of this enzyme displayed in membrane fractions of human pancreas samples and did not require arachidonic acid addition to be activated.[26] This is, at least in part, explained by the presence of unesterified arachidonic acid in pancreas samples.[26] Moreover, Western blotting has revealed that human pancreas contains low levels of 5-LOX-activating protein in addition to 5-LOX.[26]

Nevertheless, whether 5-LOX is expressed in human pancreatic ductal cells and ductal adenocarcinoma cells has not been reported before. Our results demonstrate that all pancreatic cancer cell lines tested in this study (including poorly differentiated and well-differentiated cell lines) express 5-LOX at both transcriptional and translational levels. In contrast to human pancreatic cancer cells, 5-LOX mRNA is undetectable in cultivated normal pancreatic ductal cells from a human multiorgan donor. Investigation of human pancreatic tissues using immunohistochemistry revealed that 5-LOX is expressed in the ductal cells of human pancreatic adenocarcinomas, as well as ducts and islets in chronic pancreatitis specimens and tissue adjacent to the tumor. In contrast, only sporadic expression was detected in pancreatic ductal cells in normal human pancreas. The staining in the cytosol of acinar cells reflects the constitutive expression of 5-LOX, whereas the staining of the nuclear envelope in ductal and islet cells is a result of Ca^{++}-dependent translocation and activation of this enzyme.[15,16] This supports our earlier findings of 5-LOX activation in pancreatic cancer cell lines.[7] Furthermore, to our knowledge, this is the first report of 5-LOX expression in pancreatic islet cells in response to pancreatic adenocarcinoma and chronic pancreatitis.[39] Strong staining for the LTB_4 receptor was seen in human pancreatic cancer tissues, tissues from the tumor resection margins, and in chronic pancreatitis specimens, particularly in islet and ductal cells. There was a marked difference in the number of stained cells in these tissues compared with normal pancreatic tissues from organ donors. The stained structures remain to be identified by further investigation. A connection between LTB_4 and insulin release was already investigated by two different groups in the past but with contrary results.[40–43] Therefore, further studies are necessary to find out why the LTB_4 receptor is up-regulated in pancreatic cancer, especially in tumor surrounding islets, and what is the underlying mechanism.

The immunohistochemical results show a consistent staining pattern for 5-LOX and the receptor for its downstream metabolite LTB_4, with strong staining for 5-LOX and the LTB_4 receptor in ductal and islet cells in the tissue from patients with pancreatic adenocarcinoma and chronic pancreatitis. The up-regulation of the 5-LOX pathway in ducts and islets of chronic pancreatitis tissues is intriguing and would suggest that the pancreas itself is producing immune modulators that may be involved in the inflammatory response in this disease. The positive staining for the LTB_4 receptor in stromal cells is more difficult to explain. The strong 5-LOX staining of the nuclear envelope provides evidence of the translocation and activation of this key enzyme and, therefore, an overproduction of its downstream metabolites, including LTB_4, which can lead to an overexpression of its receptor in different cells. The strongest expression of this receptor was revealed in pancreatic islet cells. These findings may be important in the understanding of the relationship between diabetes and pancreatic cancer as well as the importance of islets for the development of pancreatic adenocarcinoma, because of the known relationship between LOX-derived metabolites and insulin secretion.[40,41,43–56] However, further studies are necessary to confirm these suggestions.

The reason for investigating the expression of 5-LOX in human pancreatic ductal cells is the general belief that human pancreatic adenocarcinomas are derived from ductal epithelial cells rather than acinar cells.[57–59] The immunohistochemical results of the pancreatic tissue from the resection margins of surgically removed pancreatic adenocarcinomas were markedly different from those in normal pancreatic tissue from multiorgan donors. Positive staining for 5-LOX and LTB_4 receptor were seen in many ductal structures in the former specimens but not in the completely normal pancreas. The reason why this positive staining was seen in six of seven specimens of peritumoral pancreas is uncertain. However, the stained areas in these six specimens contained ductal changes reflecting secondary chronic pancreatitis. Similar immunohistochemical changes were seen in pancreatic tissues from patients with primary chronic pancreatitis. It is,

therefore, possible that chronic pancreatitis alone gives rise to up-regulation of the 5-LOX pathway. It is tempting to speculate that up-regulation of the 5-LOX pathway gives these ductal cells a growth advantage and thereby predisposes chronic pancreatitis patients to development of cancer. Reflecting these data we conclude that 5-LOX and the receptor for the downstream metabolite LTB_4 of this enzyme are markedly up-regulated in human pancreatic cancer supporting our findings in pancreatic cancer cell lines. This provides further evidence that LOX inhibitors are likely to be valuable in the treatment of this dreadful disease and therefore should find their way into clinical trials for chemoprevention as well as adjuvant therapies.

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