Cyclooxygenase-2 Expression Is Up-Regulated in Human Pancreatic Cancer


Abstract

A large body of evidence suggests that cyclooxygenase-2 (COX-2) is important in gastrointestinal cancer. The purpose of this study was to determine whether COX-2 was expressed in adenocarcinoma of the human pancreas. Quantitative reverse transcription-PCR, immunoblotting, and immunohistochemistry were used to assess the expression of COX-2 in pancreatic tissue. Levels of COX-2 mRNA were increased by >60-fold in pancreatic cancer compared to adjacent nontumorous tissue. COX-2 protein was present in 9 of 10 cases of adenocarcinoma of the pancreas but was undetectable in nontumorous pancreatic tissue. Immunohistochemical analysis showed that COX-2 was expressed in malignant epithelial cells. In cultured human pancreatic cancer cells, levels of COX-2 mRNA and protein were induced by treatment with tumor-promoting phorbol esters. Taken together, these results suggest that COX-2 may be a target for the prevention or treatment of pancreatic cancer.

Introduction

Adenocarcinoma of the pancreas is one of the most lethal malignancies. Its mortality rate is in excess of 95% of its incidence rate. In the United States, ~25,000 new cases of pancreatic cancer are diagnosed annually (1). Pancreatic cancer now ranks fourth and fifth as a cause of cancer death in men and women, respectively, in the United States (1). Unfortunately, >90% of pancreatic cancer patients present with metastatic disease or advanced local disease, precluding a curative surgical resection. Chemotherapy has not resulted in a significant survival benefit, and the 5-year survival rate is <1.3% in the United States (1), with a median survival of 4.1 months. On the basis of these observations, it is clear that new molecular targets are needed for the prevention and treatment of pancreatic cancer.

Results from recent studies have established the presence of two distinct COX enzymes, a constitutive enzyme (COX-1) and an inducible form (COX-2). COXs catalyze the formation of prostaglandins from arachidonic acid. COX-1 is thought to be a housekeeping gene with essentially constant levels of expression, whereas COX-2 is an early response gene that, like c-jun and c-fos, is induced rapidly by growth factors, tumor promoters, oncogenes, and carcinogens (2).

Multiple lines of evidence suggest that COX-2 is important in carcinogenesis. For example, COX-2 is up-regulated in transformed cells (3) and in various forms of cancer (4–7), whereas levels of COX-1 are relatively constant. Moreover, a null mutation for COX-2 is found in APC-mutant mice, a murine model of familial adenomatous polyposis (8). COX-2 knockout mice also developed ~75% fewer chemically induced skin papillomas than control mice (9). In addition to the genetic evidence implicating COX-2 in carcinogenesis, newly developed selective inhibitors of COX-2 protect against gastrointestinal tumor formation (8, 10). Here, we investigated whether COX-2 was up-regulated in pancreatic cancer. Our data show that levels of COX-2 are increased in adenocarcinoma of the pancreas and raise the possibility that selective inhibitors of COX-2 may be useful in the prevention or treatment of this disease.

Materials and Methods

Materials. RPMI 1640, DMEM, fetal bovine-serum, penicillin, streptomycin, COX-2, and β-2-microglobulin primers were from Life Technologies, Inc. (Grand Island, NY). RNAeasy Mini kits were from Qiagen (Santa Clarita, CA). GeneAmp RNA PCR kits were from Perkin Elmer Corp. (Norwalk, CT). GenElute Agarose Spin Columns were from Supelco (Bellevfonte, PA). Lowry protein assay kits, PMA, and secondary antibody to IgG conjugated to horseradish peroxidase were from Sigma Chemical Co. (St. Louis, MO). The COX-2 standard for immunoblotting was from Cayman Chemical Co. (Ann Arbor, MI). The COX-2 polyclonal antibody, PG-27, was from Oxford Bio-medical Research, Inc. (Oxford, MI). Nitrocellulose membranes were from Schleicher & Schuell (Keene, NH). Western blotting detection reagents (ECL) were from Amersham Pharmacia Biotech. The tyramide signal and amplification kit was from NEN Life Science (Boston, MA). The Vector Blocking Kit was from Vector Laboratories (Burlingame, CA).

Patient Samples. Biopsy specimens were obtained at the time of surgery from 10 patients with adenocarcinoma of the exocrine pancreas. Tissue samples were taken from a nonnecrotic area of the tumor and from adjacent nontumorous tissue; samples were immediately frozen in liquid nitrogen and subsequently stored at −80°C. Informed consent was obtained from each patient. The study was approved by the Committee on Human Rights in Research at Weill Medical College of Cornell University.

Tissue Culture. Three human pancreatic adenocarcinoma cell lines (Su 86.86, BxPC-3, and Panc-1) were obtained from American Type Culture Collection (Manassas, VA). The Su 86.86 and BxPC-3 cell lines were maintained in RPMI 1640; the Panc-1 cell line was maintained in DMEM supplemented with 10% FCS, 100 units/ml penicillin, and 100 μg/ml streptomycin. Cells were plated for experimental use in complete medium and allowed to attach and grow for 48 h in a 5% CO2/water-saturated incubator at 37°C. The medium was then replaced with serum-free medium. Twenty-four h later, cells were treated with vehicle or PMA under serum-free conditions.

Western Blotting. Frozen tissue was thawed in ice-cold homogenization buffer containing 150 mM NaCl, 100 mM Tris-buffered saline (pH 8), 1% Tween 20, 50 mM diethyldithiocarbamate, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml trypsin-chymotrypsin inhibitor, and 10 μg/ml pepstatin. Tissues were homogenized using a glass-on-glass tissue homogenizer. Homogenates were centrifuged at 11,750 × g for 10 min at 4°C to remove the particulate material.

Cellular lysates were prepared by traveling cells with the same lysis buffer that was used for the tissue samples. Lysates were sonicated for 20 s on ice and centrifuged at 11,750 × g for 10 min to sediment the particulate material. The protein concentration of the supernatant was measured using the Lowry protein assay kit. Immunoblot analysis for COX-2 was performed as in previous studies (11).
Results

Levels of COX-2 mRNA and Protein Are Increased in Pancreatic Cancer. To analyze the expression of COX-2, we developed a sensitive competitive RT-PCR assay in which the amount of COX-2 mRNA could be measured from minute quantities of RNA (0.5 μg total RNA). This method relies on the coamplification in the same tube of known amounts of competitor DNA (Fig. 1A) with COX-2 cDNA, obtained after reverse transcription from total tissue RNA. The competitor and target use the same PCR primers but yield amplicons with a different size (Fig. 1A), allowing their separation on a gel at the end of the reaction. Increased amounts of COX-2 mRNA were detected in 10 of 10 pancreatic cancers compared with nontumorous tissue (Fig. 1B). Overall, there was a >60-fold increase in amounts of COX-2 mRNA in tumors (mean, 135 fg/μg total RNA) versus nontumorous (mean, 2 fg/μg total RNA) tissue. To determine whether levels of COX-2 protein were also increased in pancreatic cancer,
Western blot analysis of paired tumorous and nontumorous tissue was performed (Fig. 1C). COX-2 protein was detected in tumor tissue from 9 of 10 patients but was undetectable in nontumorous tissue in the same patients.

Immunohistochemical analysis of 10 cases of pancreatic cancer revealed that COX-2 expression was multifocal and moderate to strong in the majority of cases. Expression of COX-2 was localized to tumor cells, not to surrounding stromal cells or infiltrating inflammatory cells (Fig. 2A). The staining pattern was predominantly granular, perinuclear cytoplasmic. This staining was specific for COX-2 because immunoreactivity was lost when the antiserum to COX-2 was incubated with human recombinant COX-2. Staining for COX-2 was negligible in nontumorous tissue (Fig. 2B) with the exception of pancreatic endocrine cells. Interestingly, moderate COX-2 immunoreactivity was detected in metastatic pancreatic adenocarcinoma in regional lymph nodes from two patients.

**COX-2 Is Induced by Tumor-promoting Phorbol Esters in Human Pancreatic Carcinoma Cell Lines.** To determine whether the up-regulation of COX-2 mRNA and protein seen in pancreatic tumors was reproducible in vitro, we analyzed three human pancreatic cancer cell lines: Su 86.86, Panc 1, and BxPC-3. As shown in Fig. 3, COX-2 mRNA and protein were detected under basal conditions in the Su 86.86 cell line. Treatment with PMA, a tumor promoter, induced COX-2 mRNA and protein in as little as 3 h. Similar results were obtained in the Panc 1 and BxPC-3 cell lines (data not shown).

**Discussion**

In this study, we discovered that amounts of COX-2 mRNA and protein are increased in pancreatic cancer. Immunohistochemistry revealed that COX-2 was expressed in tumor cells. COX-2 was also induced by PMA in cultured human pancreatic cancer cell lines. To our knowledge, this is the first evidence that COX-2 is up-regulated in pancreatic cancer.

COX-2 can potentially predispose to carcinogenesis via multiple mechanisms. In extrahepatic tissues in which cytochrome P450 content is low, COX may be important for metabolism of carcinogens. For example, several classes of chemical carcinogens, e.g., dihydrodiol derivatives of polycyclic aromatic hydrocarbons, aromatic amines, and heterocyclic amines, are activated to mutagenic derivatives by COX (12). The metabolism of carcinogens by COX-2 may be important, therefore, for understanding the link between cigarette smoking (1) or consumption of grilled or fried meat (1) and pancreatic cancer. Additionally, enhanced synthesis of prostaglandins, a consequence of up-regulation of COX-2, favors the growth of malignant cells by increasing cell proliferation (13), promoting angiogenesis.

![Fig. 2. COX-2 is expressed in malignant epithelial cells in pancreatic adenocarcinoma. A, pancreatic adenocarcinoma shows diffuse, strong, granular, perinuclear cytoplasmic immunoreactivity with anti-COX-2 antibody (×200). B, benign ductule surrounded by histologically unremarkable pancreatic acini showing no COX-2 immunoreactivity (×200).](image)
therapy or radiotherapy as a novel treatment for pancreatic cancer. Inhibiting COX-2 will be useful alone or in combination with chemotherapy. It will be important to establish whether celecoxib, a specific cyclooxygenase-2 inhibitor, is safer than traditional nonsteroidal anti-inflammatory drugs. On the basis of results of this study, it will be important to determine which of these mechanisms are important in adenocarcinoma of the pancreas.

It also is interesting to consider the possible link between the known genetic alterations in pancreatic cancer and COX-2. Mutations in the Ki-ras oncogene (18) are common in pancreatic cancer. Levels of COX-2 are increased in Ras-transformed epithelial cells (3, 19). It is reasonable to postulate, therefore, that activation of the Ras pathway contributes to the up-regulation of COX-2 in pancreatic cancer. Mutations of the Apc gene also occur in pancreatic cancer (20). The potential significance of the link between COX-2 and Apc was highlighted by the finding that COX-2 deficiency protects against tumor formation in mice carrying a defective Apc gene (8).

Recently, selective inhibitors of COX-2 have been developed. These compounds possess anticancer properties (8, 10) and appear to be safer than traditional nonsteroidal anti-inflammatory drugs. On the basis of results of this study, it will be important to establish whether inhibiting COX-2 will be useful alone or in combination with chemotherapy or radiotherapy as a novel treatment for pancreatic cancer.
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