Cyclooxygenase-2 and Cytosolic Phospholipase A2 Are Overexpressed in Mucinous Pancreatic Cysts

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OBJECTIVES: **Expression of prostaglandin biosynthetic pathway enzymes in mucinous pancreatic cysts is unknown. Cyclooxygenase-2 (COX-2) inhibition is a potential cancer chemoprevention strategy for these lesions. We evaluated the expression of COX-2, cytosolic phospholipase A2 (cPLA2), and protein kinase B (AKT) in the epithelium of pancreatic cysts and correlated enzyme expression with aspirin (ASA) use and cyst fluid prostaglandin E_2 (PGE_2) concentration.**

METHODS: **Pathology of 80 resected pancreatic cysts was reviewed. Expression of COX-2, cPLA2, and AKT was quantified by tissue immunohistochemistry immunoreactivity scores (IRSs). IRS values were compared between cyst types and (in 30 cases) with matched cyst fluid PGE_2 concentrations.**

RESULTS: **The mean IRS was higher in the epithelium of mucinous vs nonmucinous cysts for COX-2 (6.1 ± 4.7 vs 3.2 ± 2.8, $P = 0.01$) and cPLA2 (6.9 ± 3.0 vs 2.9 ± 2.9, $P < 0.001$). Cyst epithelial COX-2 expression was higher in mucinous cysts with low-grade dysplasia vs those with high-grade dysplasia or invasive carcinoma (IRS 8.0 ± 3.9 vs 1.5 ± 2.9, $P < 0.001$), whereas the opposite was found for cPLA2 (6.2 ± 3.0 vs 8.6 ± 2.3, $P = 0.005$). Cyst fluid PGE_2 concentrations did not correlate with either the IRS or a history of low- to moderate-dose ASA use.**

CONCLUSIONS: **COX-2 and cPLA2 are overexpressed in the epithelium of mucinous pancreatic cysts. COX-2 and/or cPLA2 inhibition might prevent the emergence or progression of mucinous pancreatic cysts, but higher doses of ASA or nonsteroidal anti-inflammatory drugs may be necessary to substantially inhibit cyst epithelial COX-2 activity.**

Clinical and Translational Gastroenterology 2019;10:e-00028. https://doi.org/10.14309/ctg.0000000000000028

INTRODUCTION

Asymptomatic pancreatic cysts are common (1), and many are thought to be mucinous pancreatic cysts with some malignant potential, including intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs). Most pancreatic cysts are either observed or resected, depending on clinical and imaging features (2). There is currently no medical therapy for pancreatic cystic neoplasms, and chemoprevention strategies that prevent emergence and progression of pancreatic cysts are needed.

Eicosanoids, including prostaglandins and leukotrienes, are implicated in cancer pathogenesis (3). Enzymes of the prostaglandin biosynthetic pathway (including cyclooxygenase-2 (COX-2)) are overexpressed in colonic adenomas, and COX-2 inhibition prevents development of colon adenomas in patients with a history of colon polyps (4). It is unknown whether prostaglandin biosynthesis is upregulated in the epithelium of mucinous pancreatic cysts, whether epithelial COX-2 expression can be assessed by measurement of cyst fluid prostaglandin levels, and whether COX-2 inhibition is an effective chemoprevention strategy for pancreatic cysts. The aims of this study were to evaluate the expression of COX-2, cytosolic phospholipase A2 (cPLA2), and AKT (also known as protein kinase B) in the epithelium of mucinous vs nonmucinous pancreatic cysts, to compare expression of these prostaglandin biosynthetic pathway enzymes with cyst fluid prostaglandin E_2 (PGE_2) concentrations, and to assess the impact of aspirin (ASA) use on both cyst epithelial enzyme expression and cyst fluid PGE_2 levels.

METHODS

The Institutional Review Board (IRB) of the Mayo Clinic in Rochester, MN, approved this study. Pathology specimens of

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Received June 2, 2018; accepted January 16, 2019; published online April 15, 2019

resected pancreatic cystic lesions from 80 patients were reviewed retrospectively. Archived pathology specimens at Mayo Clinic Rochester, MN, obtained between 1988 and 2013, inclusive, were studied. Existing histology slides were reviewed to determine adequacy for inclusion, and additional sections of existing tissue blocks were obtained for immunohistochemistry (IHC). Cases were also preferentially included for which pancreatic cyst fluid specimens had been obtained preoperatively by endoscopic ultrasound (EUS) and were frozen at −80 °C and available for PGE_2 assay. Corresponding patient medical records were reviewed. Data regarding ASA or nonsteroidal anti-inflammatory drug (NSAID) use and dosage were abstracted from standard clinical questionnaires completed by Mayo Clinic patients at the time of their clinical evaluation and grouped into 3 hierarchical categories: daily ASA use (with or without NSAID use), daily NSAID use (with or without prn ASA), and neither (no daily ASA or NSAID use).

IHC

IHC was used to evaluate expression of COX-2, cPLA2, and AKT in MCN, IPMN, and nonmucinous cysts. All tissues were fixed in formalin and embedded in paraffin. Tissues were sectioned at 5 μm and placed on charged slides using a Leica RM2255 microtome (Leica, Buffalo, IL), and slides were stained using the Leica Bond III Stainer (Leica). Slides for phospho-cPLA2 and phospho-AKT were retrieved for 20 minutes using Epitope Retrieval 1 (Leica), and slides for COX-2 stain were retrieved for 20 minutes using Epitope Retrieval 2 (Leica). The COX-2 (Clone CX-294, Dako, Carpinteria, CA) and phospho-AKT (Clone LP18, Leica) antibodies were diluted in Bond Antibody Diluent (Leica), whereas the phospho-cPLA2 antibody (Sigma-Aldrich, St. Louis, MO) was diluted in Background Reducing Diluent (Dako). Slides stained with phospho-cPLA2 antibody were incubated in Protein Block (Dako) for 5 minutes. The primary antibody phospho-cPLA2 was used at 1:400; phospho-AKT was used at 1:500; and COX-2 was used at 1:75. All primary antibodies were incubated for 15 minutes. The detection system used was Polymer Refine Detection System (Leica). This system included the hydrogen peroxidase block, secondary antibody polymer, diaminobenzidine (DAB), and hematoxylin. Immunostaining visualization was achieved by incubating slides 10 minutes in DAB and DAB buffer (1:19 mixture) from the Bond Polymer Refine Detection System. To this point, slides were rinsed between steps with 1′ Bond wash buffer (Leica). Before and after DAB incubation, slides were rinsed in distilled water. Slides were counterstained for 5 minutes using Schmidt hematoxylin and molecular biology grade water (1:1 mixture), followed by several rinses in 1′ Bond wash buffer and distilled water. Once the IHC process was completed, slides were removed from the stainer and rinsed in tap water for 5 minutes. Slides were dehydrated in increasing concentrations of ethyl alcohol and cleared in 3 changes of xylene before permanent coverslipping in xylene-based medium.

All slides were reviewed by 1 pathologist (T.S.) using a well-established semiquantitative immunoreactivity scoring system (5). For each case, tissue staining was assessed in the cyst epithelium, pericystic stroma, pancreatic parenchymal endothelium, acini, islets, mucosa of large/interlobular ducts, and mucosa of small/interlobular ducts. The immunoreactivity score (IRS) was calculated by multiplying the intensity of staining (no staining, 0; mild, 1; moderate, 2; strong, 3) by the extent of staining (0% as 0; <10% as 1; 10%–50% as 2; 51%–80% as 3; 81%–100% as 4). This resulted in IRSs between 0 (no staining) and 12 (highest staining). Degree of dysplasia was also evaluated and categorized as none, low-grade, high-grade, or invasive carcinoma (6). IHC was performed on 60 mucinous lesions (23 MCN and 37 IPMN) and 20 nonmucinous lesions (13 serous cystadenomas, 1 dermoid cyst, 1 lymphangioma, 2 lymphoepithelial cysts, and 3 simple nonmucinous "retention" cysts). Representative images demonstrating histology scoring are shown in Figure 1. Clinical data were abstracted from patient records including ASA and NSAID use before surgery and cyst fluid aspiration.

To measure PGE_2 concentrations of pancreatic cyst fluid, samples were available from 30 patients who underwent preoperative EUS fine-needle aspiration, had provided written informed consent under an IRB-approved protocol, and had cyst fluid aliquots placed on ice and frozen at −80 °C within 90 minutes of collection. These cyst fluid specimens were thawed and centrifuged at 10,000*g* for 5 minutes at 4 °C. Supernatants (500 μL) were diluted with enzyme immunoassay buffer (EIA kit Cat No. 514010; Cayman Chemical, Ann Arbor, MI), and the samples were assayed at 1/1 (no dilution), 1/10, and 1/50 dilutions. The enzyme immunoassay is based on a competition between PGE_2 and PGE_2 acetyl cholinesterase conjugate (PGE_2Tracer) for a limited amount of PGE_2 monoclonal antibody after 18 hours of incubation at 4 °C. Two hundred microliters of Ellman reagent was used as the substrate for acetyl cholinesterase. The proportion of the bound tracer was determined spectroscopically, and the concentration of PGE_2 was determined using optimized standard curves from known concentrations. The product of this enzymatic reaction is determined using absorbance measured by a spectrophotometer. The least dilution yielding a result within the linear portion of the standard curves was considered most reliable and was multiplied by the appropriate dilution factor to yield a final result. Personnel blinded to histologic diagnoses conducted PGE_2 assays.

Statistical analysis

Continuous data were described as mean ± s.d. or median (minimum, maximum) and categorical data as count (percent). Characteristics of mucinous vs nonmucinous cysts and MCN vs IPMN mucinous cysts were compared using 2-sample *t* tests or Wilcoxon rank-sum tests, χ^2 tests, or Fisher exact tests.

Univariate linear regression of cyst and pancreatic tissue was used to predict the IRS and extent of staining measures, comparing mucinous and nonmucinous cysts, and R^2 was estimated. Using mucinous and nonmucinous cysts separately, the cyst epithelial IRS and extent of staining were compared between the cyst epithelium and various types of pancreatic tissues, with paired *t* tests. MCN vs IPMN mucinous cyst IRS and extent of staining were compared using 2-sample *t* tests.

Two-sample *t* tests were used to compare the IRS in low-grade dysplasia vs those with high-grade dysplasia or carcinoma. The relationship between the IRS, degree of dysplasia, and cyst type is displayed graphically as box plots overlaid with jittered points, whereas numerical comparisons of subtypes was performed with Kruskal-Wallis tests.

The association of PGE with epithelial COX-2 and cPLA2 IRS was visualized with jittered scatter plots color coded by

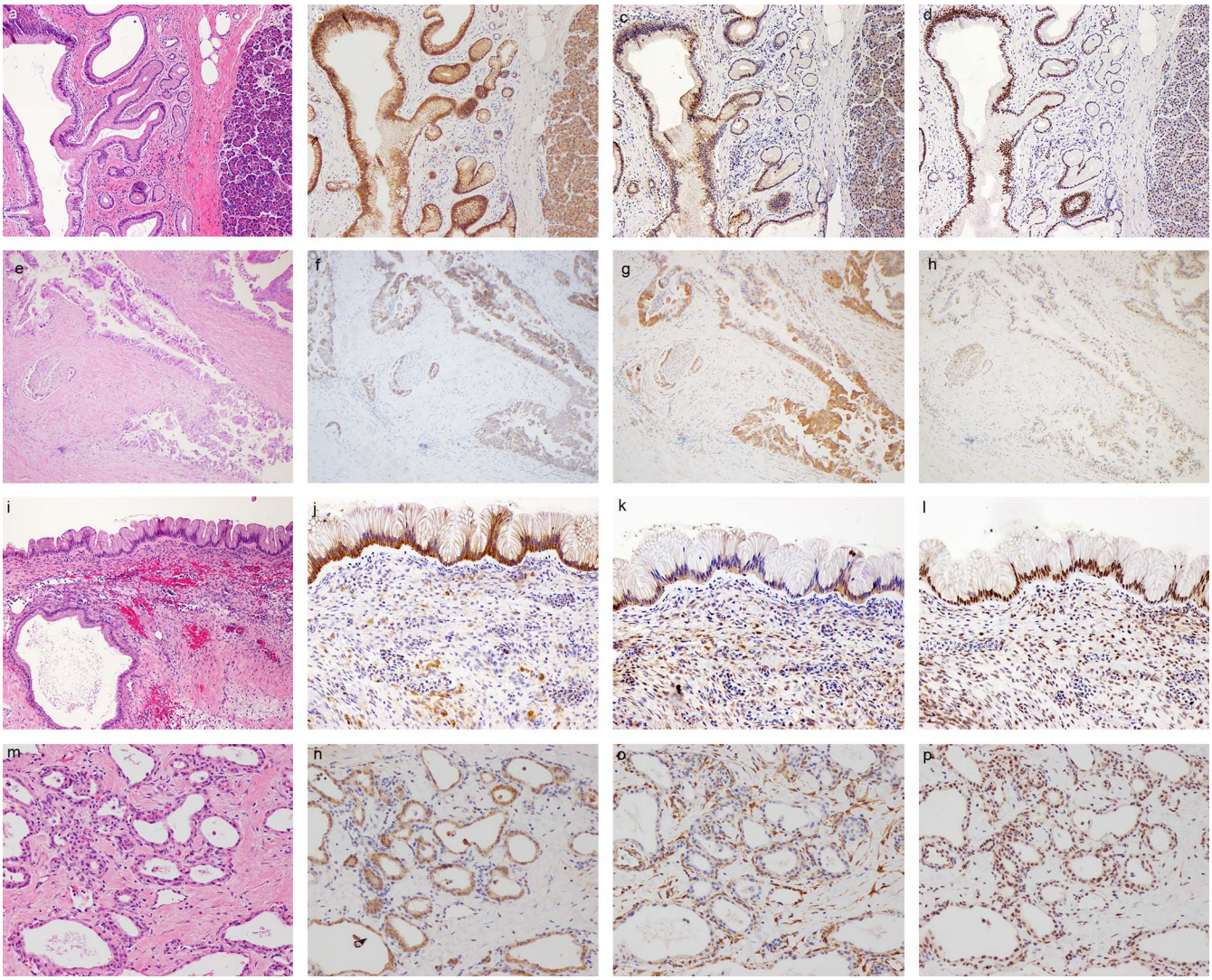

Figure 1. Cyclooxygenase-2 (COX-2), cytosolic phospholipase A2 (cPLA2), and AKT immunohistochemistry. (**a**–**d**) Intraductal papillary mucinous neoplasm (IPMN) with low-grade dysplasia, gastric epithelial subtype, and COX-2 epithelial immunoreactivity score (IRS) = 12. (**a**) Hematoxylin and eosin (H + E), (**b**) COX-2, (**c**) cPLA2, and (**d**) AKT. (**e**–**h**) IPMN with high-grade dysplasia, COX-2 epithelial IRS = 4. (**e**) H + E, (**f**) COX-2, (**g**) cPLA2, and (**h**) AKT. (**i**–**l**) MCN, COX-2 epithelial IRS = 12. (**i**) H + E, (**j**) COX-2, (**k**) cPLA2, and (**l**) AKT. (**m**–**p**) serous cystadenoma, COX-2 epithelial IRS = 4. (**m**) H + E, (**n**) COX-2, (**o**) cPLA2, and (**p**) AKT.

mucinous/nonmucinous status and overlaid with an overall univariate linear regression line per enzyme type. Univariate linear regression models were also fit within mucinous and nonmucinous cysts separately.

The effect of medication use was described with mean ± s.d. and investigated using 2-sample *t* tests and univariate linear regression to assess whether ASA use was associated with IRS values. Various subgroups were investigated, including mucinous cysts, nonmucinous cysts, and cysts for which PGE_2 data were available). When the NSAID use (with or without prn ASA) category was too small, as happened in several subset analyses, that category was excluded and 2-group comparisons were performed instead. A multiple variable linear regression model was constructed for the IRS, using 2 cyst types and 3 medication categories.

The data were analyzed using SAS v9.4 (SAS Institute, Cary, NC) and R v3.1 (R Development Core Team) software. All tests were 2 sided, and *P* values of less than 0.05 were considered statistically significant.

RESULTS

Among the 80 cases, there were 60 mucinous and 20 nonmucinous cysts. Fifty-three of the 80 subjects were women, and the median patient age was 64 years (range 29–87 years) for mucinous cysts and 61 years (range 23–81 years) for nonmucinous cysts. Demographic and clinical features are shown in Table 1. Among the 17 patients taking ASA daily, 12 were taking 81 mg/d, and 5 reported using doses between 162 and 325 mg/d. Six of these 17 subjects were also taking daily or prn NSAIDs at a variety of dosages. Among the 30 subjects with available cyst fluid PGE_2 values, 10 who were taking ASA daily and 18 who were taking neither ASA nor NSAIDs were included in relevant analyses; the 2 taking NSAIDs only were excluded because there were too few in this subgroup for analysis.

Table 1. Patient characteristics

	Mucinous cysts (n = 60)	Nonmucinous cysts (n = 20)	Total (N = 80)	*P* value
Age (median, range)	64 (29–87)	61 (23–81)	63 (23–87)	0.092[a]
Female	43 (72%)	10 (50%)	53 (66%)	0.076[b]
Tobacco use				0.003[c]
Current	3 (5%)	5 (25%)	8 (10%)	
Former	16 (27%)	3 (15%)	19 (24%)	
Never	26 (43%)	12 (60%)	38 (48%)	
Unknown	15 (25%)	0 (0%)	15 (19%)	
ASA and NSAID use				0.382[c]
Daily ASA (with or without NSAID)	13 (22%)	4 (20%)	17 (21%)	
NSAIDs with or without prn ASA	5 (8%)	4 (20%)	9 (11%)	
Neither	42 (70%)	12 (60%)	54 (68%)	
Cyst ≥3 cm in maximum diameter	18 (78%)	8 (80%)	26 (79%)	1.000[c]
Cyst fluid CEA				0.001[d]
N	20	10	30	
Median (range)	766 (0.5–6,852)	0.3 (0–6,218)	197 (0–6,852)	
Dysplasia grade				N/A
None	0	20 (100%)	20 (25%)	
Low	43 (72%)	—	43 (54%)	
High	1 (2%)	—	1 (1%)	
Invasive cancer	16 (27%)	—	16 (20%)	
IPMN subtype				
Gastric	13 (35%)	—		
Intestinal	13 (35%)	—		
Pancreaticobiliary	10 (27%)	—		
Mixed	1 (3%)	—		

P values compare mucinous and nonmucinous cysts.
ASA, aspirin; CEA, carcinoembryonic antigen; IPMN, intraductal papillary mucinous neoplasm; NSAID, nonsteroidal anti-inflammatory drug.
[a]Kruskal-Wallis test.
[b]Chi-square test.
[c]Fisher exact test.
[d]Wilcoxon test.

Enzyme expression

The mean IRSs for various cyst types are shown in Table 2. The IRS was higher in the epithelium of mucinous vs nonmucinous cysts for COX-2 (6.1 ± 4.7 vs 3.2 ± 2.8, $P = 0.01$) and cPLA2 (6.9 ± 3.0 vs 2.9 ± 2.9, $P < 0.001$) but not for AKT (7.7 ± 3.0 vs 6.7 ± 2.5, $P = 0.18$). In general, COX-2 and cPLA2 IRS in mucinous cyst epithelium were similar to duct mucosa, with lower scores in acini and nonmucinous cyst epithelium and very little immunoreactivity in stroma. When only mucinous cysts were considered, the MCN cyst epithelium had a higher IRS than IPMN for COX-2 (9.1 ± 3.1 vs 4.3 ± 4.6, $P < 0.001$) but not for cPLA2 (6.9 ± 3.3 vs 6.9 ± 2.9, $P = 0.95$) or AKT (8.1 ± 3.2 vs 7.5 ± 2.9, $P = 0.46$). Analyses were repeated using extent of IHC staining in place of IRSs, and similar results were obtained.

The mean COX-2 IRS in the epithelium of mucinous cysts with low-grade dysplasia was 5-fold higher than in those with high-grade dysplasia or carcinoma (8.0 ± 3.9 vs 1.5 ± 2.9, $P < 0.001$). The opposite was found for cPLA2 (6.2 ± 3.0 vs 8.6 ± 2.3, $P = 0.005$), and no significant difference was observed for AKT ($P = 0.58$). The relationship between COX-2 and cPLA2 IRS, degree of dysplasia, and cyst type is shown in Figure 2. The mean COX-2 IRS for cyst epithelium of IPMN gastric subtype was higher than for intestinal and pancreaticobiliary IPMN subtypes (7.9 ± 4.4 vs 1.7 ± 2.8 vs 3.1 ± 4.5, $P = 0.005$), whereas the mean cPLA2 IRS was lower in gastric than intestinal and pancreaticobiliary subtypes (5.3 ± 2.5 vs 7.2 ± 2.2 vs 8.7 ± 3.3, $P = 0.008$). No differences in the cyst epithelial IRS between IPMN subtypes were observed for AKT ($P = 0.82$).

ASA and NSAID use

Among the 80 cases studied, 71 reported either daily ASA use or no ASA/NSAID use, including 55 with mucinous cysts. Among these 71 cases, cyst epithelial IRS values for the daily ASA and no ASA/NSAID groups were 7.2 ± 4.4 and 4.9 ± 4.4, respectively, for COX-2 ($P = 0.06$), 6.2 ± 3.8 and 6.1 ± 3.3, respectively, for

Table 2. **Mean IRS of the pancreatic cyst epithelium and parenchyma**

Cyst type	Cyst Epithelial IRS (mean ± s.d.)	Pancreatic tissue				*P* value[a] (cyst epithelium vs pancreatic tissue)	*P* value[b] (mucinous vs nonmucinous)
		Stromal IRS (mean ± s.d.)	Acini IRS (mean ± s.d.)	Small duct IRS (mean ± s.d.)	Large duct IRS (mean ± s.d.)		
COX-2							
Mucinous (IPMN + MCN)	6.1 ± 4.7	0.1 ± 0.8	4.9 ± 3.6	6.2 ± 2.8	5.4 ± 3.1	Large ducts: $P = 0.01$ Small ducts: $P = 0.15$ Acini: $P = 0.10$ Stroma: $P < 0.001$	Epithelium: $P = 0.01$, $R^2 = 0.08$ Stroma: $P = 0.47$, $R^2 = 0.007$ Acini: $P = 0.74$, $R^2 = 0.002$ Small ducts: $P = 0.64$, $R^2 = 0.004$ Large ducts: $P = 0.86$, $R^2 = 0.001$
Nonmucinous (other)	3.2 ± 2.8	0.0 ± 0.0	5.3 ± 4.0	6.7 ± 3.6	5.1 ± 3.4	Large ducts: $P = 0.06$ Small ducts: $P = 0.02$ Acini: $P = 0.09$ Stroma: $P < 0.001$	
cPLA2							
Mucinous (IPMN + MCN)	6.9 ± 3.0	5.5 ± 3.2	1.0 ± 1.2	5.4 ± 2.2	5.6 ± 2.3	Large ducts: $P = 0.03$ Small ducts: $P = 0.06$ Acini: $P < 0.001$ Stroma: $P = 0.008$	Epithelium: $P < 0.001$, $R^2 = 0.26$ Stroma: $P = 0.43$, $R^2 = 0.008$ Acini: $P = 0.55$, $R^2 = 0.005$ Small ducts: $P = 0.08$, $R^2 = 0.06$ Large ducts: $P = 0.51$, $R^2 = 0.01$
Nonmucinous (other)	2.9 ± 2.9	4.9 ± 3.3	1.2 ± 1.0	4.1 ± 2.4	5.0 ± 2.6	Large ducts: $P = 0.27$ Small ducts: $P = 0.13$ Acini: $P = 0.11$ Stroma: $P = 0.07$	
AKT							
Mucinous (IPMN + MCN)	7.7 ± 3.0	4.6 ± 2.7	3.0 ± 3.0	6.7 ± 2.5	6.7 ± 3.0	Large ducts: $P = 0.009$ Small ducts: $P = 0.01$ Acini: $P < 0.001$ Stroma: $P < 0.001$	Epithelium: $P = 0.18$, $R^2 = 0.02$ Stroma: $P = 0.85$, $R^2 = 0.0005$ Acini: $P = 0.50$, $R^2 = 0.007$ Small ducts: $P = 0.36$, $R^2 = 0.02$ Large ducts: $P = 0.73$, $R^2 = 0.003$
Nonmucinous (other)	6.7 ± 2.5	4.8 ± 2.7	3.7 ± 4.0	7.5 ± 3.4	7.1 ± 4.1	Large ducts: $P = 1$ Small ducts: $P = 0.57$ Acini: $P = 0.01$ Stroma: $P = 0.008$	

COX-2, cyclooxygenase-2; cPLA2, cytosolic phospholipase A2; IPMN, intraductal papillary mucinous neoplasm; IRS, immunoreactivity score; MCN, mucinous cystic neoplasm.
[a]Paired *t* tests.
[b]Two-sample *t* tests.

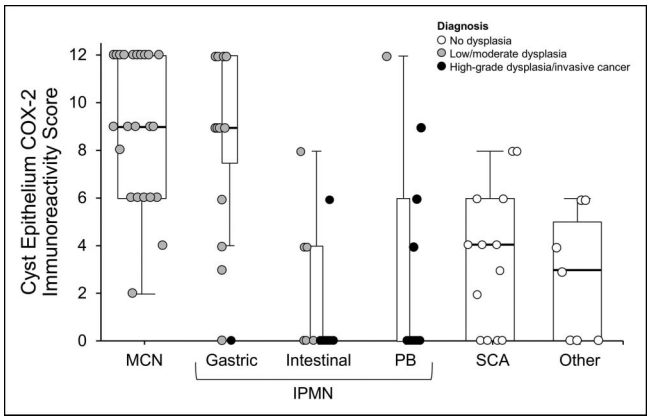

Figure 2. Epithelial COX-2 and cytosolic phospholipase A2 immunoreactivity scores of various pancreatic cyst types. COX-2, cyclooxygenase-2; IPMN, intraductal papillary mucinous neoplasm; MCN, mucinous cystic neoplasm; PB, pancreaticobiliary; SCA, serous cystadenoma.

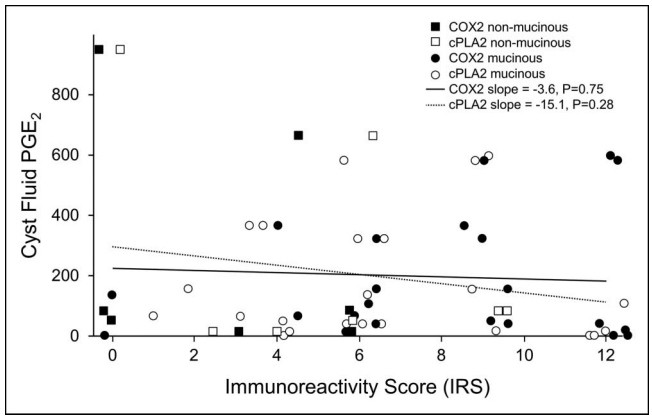

Figure 3. Association between pancreatic cyst fluid PGE_2 concentration and cyst epithelial COX-2 expression (IRS). COX-2, cyclooxygenase-2; cPLA2, cytosolic phospholipase A2; IRS, immunoreactivity score; PGE_2, prostaglandin E_2.

cPLA2 ($P = 0.88$), and 7.6 ± 3.4 and 7.5 ± 2.9, respectively, for AKT ($P = 0.90$). In multivariable analysis of the 71 cases, there was a statistically significant association between the cyst epithelial COX-2 IRS and both cyst type (mucinous vs nonmucinous) and ASA use ($P = 0.007$ and 0.046, respectively). Subjects with mucinous cysts had a 3.4-point higher cyst epithelial COX-2 IRS, and those taking daily ASA had a 2.4-point higher IRS.

When considering only the 55 mucinous cyst cases with either daily ASA use or no ASA/NSAID use, epithelial IRS values for the daily ASA and no ASA/NSAID groups were 9.0 ± 3.1 and 5.3 ± 4.8, respectively, for COX-2 ($P = 0.01$), 7.2 ± 3.3 and 6.9 ± 3.0, respectively, for cPLA2 ($P = 0.74$), and 7.4 ± 3.1 and 7.9 ± 3.0, respectively, for AKT ($P = 0.61$). Among these 55 cases, the mean cyst epithelial COX-2 IRS was higher in those with low-grade dysplasia than those with high-grade dysplasia or carcinoma (7.9 ± 4.0 vs 1.7 ± 3.0, $P < 0.001$), and the opposite was found for cPLA2 (6.4 ± 3.0 vs 8.5 ± 2.5, $P = 0.02$), with no significant difference for AKT ($P = 0.40$).

Cyst fluid PGE_2 concentrations

Among the 80 cysts, 30 had matched pancreatic cyst fluid PGE_2 concentrations available, including 23 mucinous cysts (22 with low-grade dysplasia and 1 with invasive cancer) and 7 nonmucinous cysts (all with no dysplasia). In all cases, cyst fluid was obtained during preoperative EUS-guided fine-needle aspiration. As shown in Figure 3, there was no significant correlation between cyst fluid PGE_2 concentrations and cyst epithelial COX-2, cPLA2, or AKT IRS ($P = 0.75$, 0.28, and 0.40). When nonmucinous and mucinous cysts were analyzed separately, results were similar, with no statistically significant association observed (nonmucinous $P = 0.54$, 0.28, and 0.36; mucinous $P = 0.49$, 0.86, and 0.64). There was no statistically significant correlation between ASA use and cyst fluid PGE_2 concentrations either for all cyst types (n = 28, $P = 0.66$) or for mucinous cysts (n = 21, $P = 0.22$).

DISCUSSION

Asymptomatic pancreatic cysts are common (1), and most are thought to be mucinous pancreatic cystic neoplasms such as IPMN. Because these lesions have a low but real risk of malignant transformation, they are either resected or observed over time, depending on the presence of worrisome clinical or imaging features (2). Colonic adenomas share some histologic similarities with IPMN, and inhibition of the COX-2 enzyme is an effective chemoprevention strategy for colon adenomas (4). We studied the expression of prostaglandin biosynthetic pathway enzymes in the pancreatic cyst epithelium to assess whether a similar strategy might be appropriate for pancreatic cysts. We found that COX-2 and cPLA2 are upregulated in the epithelium of mucinous pancreatic cysts compared with the epithelium of nonmucinous cysts (which have negligible malignant potential). Enzyme expression in surrounding pancreatic tissues was assessed as an internal control and was similar between groups. In addition, COX-2 was markedly overexpressed in mucinous cysts with low-grade dysplasia and appeared downregulated in cysts with high-grade dysplasia or invasive cancer, whereas mean cPLA2 IRSs were higher in cysts with high-grade dysplasia or invasive cancer. These findings support the concept that COX-2 and/or cPLA2 inhibition might prevent pancreatic cyst emergence or progression of cysts not already harboring invasive malignancy.

COX-2 plays an important role in chronic pancreatic inflammation and pancreatic cancer pathogenesis. COX-2 is overexpressed in the ducts and acini of patients with chronic pancreatitis (7,8) and regulates both chronic pancreatic inflammation and pancreatic stellate cell activity (9). Induction of the COX-2 enzyme increases production of PGE_2, and elevated levels of PGE_2 are found in the pancreatic juice of patients with chronic pancreatitis (10). COX-2 expression is increased in pancreatic intraepithelial neoplasia (PanIN) lesions, with expression peaking in PanIN2 and diminishing in PanIN3 (11). In mouse models, coactivation of K-Ras and COX-2 augments formation of intraductal papillary mucinous neoplasm (12). COX-2 expression is upregulated in human pancreatic ductal adenocarcinoma (13), and tumors that are deficient in COX-2 may use exogenous local sources of prostaglandins (14).

Because pancreatic cyst fluid can be sampled during EUS, we sought to determine whether pancreatic cyst fluid PGE_2 concentrations correlate with COX-2 expression in the underlying cyst epithelium and are a biomarker of COX-2 activity. We were not able to demonstrate an association between cyst fluid PGE_2 and epithelial COX-2 expression. Several considerations may

explain this result. Because many mucinous pancreatic cysts communicate with the pancreatic duct, cyst fluid aspirates may contain variable amounts of cyst epithelial secretions and pancreatic juice, which would be expected to have low PGE_2 levels in the absence of chronic pancreatitis (10). In addition, our findings might be due to inactivity of expressed COX-2 or rapid metabolism of PGE_2 in the cyst epithelium or the cyst lumen.

Our findings suggest that low doses of ASA may not be sufficient to significantly inhibit COX-2 activity in mucinous pancreatic cysts. We found that daily low- to moderate-dose ASA use was associated with overexpression of COX-2 in the mucinous cyst epithelium; however, cyst fluid PGE_2 levels did not correlate with ASA use. This implies that oral ASA use at low to moderate doses induces compensatory upregulation of COX-2 in the cyst epithelium but does not significantly suppress overall enzyme activity. This is an important issue for design of chemoprevention trials. Ideally, a drug and dose that substantially inhibits human pancreatic COX-2 activity (as determined by measurement of PGE_2 concentrations in cyst fluid, pancreatic tissue, or pancreatic juice) would be used in chemoprevention studies.

Available data provide some indirect evidence for effects of ASA and NSAIDs in the human pancreas. Rectally administered indomethacin effectively prevents post-endoscopic retrograde cholangiopancreatography pancreatitis (15), suggesting significant acute anti-inflammatory activity in the gland. A recent large study of participants enrolled in the Health Professionals Follow-up Study or the Nurses' Health Study found no association between regular ASA use and future risk of pancreatic cancer, even among persons taking 16 or more 325 mg ASA tablets per week (16). It is unclear whether these findings are applicable to pancreatic cysts because most pancreatic adenocarcinomas are thought to arise from PanIN lesions, not MCN or IPMN. In a retrospective Japanese study, low-dose ASA use appeared to prevent further pancreatic duct dilation in patients with main duct IPMN (17). Studies demonstrating inhibition of pancreatic PGE_2 production by higher oral ASA doses, or an oral NSAID, would inform design of chemoprevention trials in patients with pancreatic cysts.

We found that cPLA2 enzyme expression is also increased in mucinous pancreatic cysts. In contrast to COX-2, however, cPLA2 is more highly expressed in mucinous cysts with high-grade dysplasia than those with low-grade dysplasia. cPLA2 is the rate-limiting enzyme in the prostaglandin biosynthetic pathway and appears to protect against gastrointestinal mucosal injury induced by COX-2 inhibitors via a mitochondrial effect (18). Inhibition of cPLA2, perhaps by statins (19), might also be an effective pancreatic cyst chemoprevention strategy.

Limitations of our study include its retrospective nature. The sample size of lesions with matched pancreatic cyst fluid specimens was small. ASA and NSAID use was determined from standard questionnaires completed by patients at the time of their clinical care at our institution, rather than prospective patient interviews. Strengths of the study include histological assessment of a large number of well-annotated pancreatic cystic lesions of diverse types and the ability to correlate histologic findings with medication use and cyst fluid PGE_2 concentrations.

In summary, COX-2 and cPLA2 are overexpressed in the epithelium of mucinous pancreatic cysts. These findings support the concept that inhibition of these prostaglandin pathway biosynthetic enzymes might prevent the emergence or progression of MCN and IPMN. ASA use at low doses upregulates mucinous cyst epithelial COX-2 expression but does not decrease cyst fluid PGE_2 concentration, raising the question of whether higher ASA doses or NSAIDs would more effectively inhibit pancreatic COX-2 activity. Further study is required to identify a drug regimen that substantially inhibits pancreatic COX-2 and/or cPLA2 activity and would be appropriate for chemoprevention trials.

CONFLICTS OF INTEREST

Guarantor of the article: Mark Topazian, MD.

Specific author contributions: Planning and conducting the study: E.M., T.S., B.B., P.L., and M.T. Collecting and interpreting data: E.M., T.S., B.B., C.W.W., F.E., and M.T. Drafting and reviewing the manuscript, and approved the final version of the manuscript: All authors.

Financial support: None.

Potential competing interests: None.

Study Highlights

WHAT IS KNOWN

- ✓ Pancreatic cysts are common, and mucinous cysts (such as MCN and branch duct IPMN) have malignant potential.
- ✓ Medical treatments for pancreatic cysts are needed.

WHAT IS NEW HERE

- ✓ The cyclooxygenase-2 (COX-2) and cPLA2 enzymes are overexpressed in the epithelium of mucinous pancreatic cysts compared with nonmucinous cysts.
- ✓ Cyst fluid PGE_2 concentrations did not correlate with a history of low- to moderate-dose ASA use.

TRANSLATIONAL IMPACT

- ✓ COX-2 and cPLA2 may be targets for chemoprevention of pancreatic cyst emergence and progression.
- ✓ Higher doses of ASA or NSAIDs may be necessary to substantially inhibit cyst epithelial COX-2 activity.

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