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TOPIC HIGHLIGHT

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Involvement of eicosanoids in the pathogenesis of pancreatic cancer: The roles of cyclooxygenase-2 and 5-lipoxygenase

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Abstract

The interplay between inflammation and cancer progression is a growing area of research. A combination of clinical, epidemiological, and basic science investigations indicate that there is a relationship between inflammatory changes in the pancreas and neoplastic progression. Diets high in ω-6 polyunsaturated fatty acids provide increased substrate for arachidonic acid metabolism by cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX) to form eicosanoids. These eicosanoids directly contribute to pancreatic cancer cell proliferation. Both COX-2 and 5-LOX are upregulated in multiple cancer types, including pancreatic cancer. *In vitro* studies using pancreatic cancer cell lines have demonstrated upregulation of COX-2 and 5-LOX at both the mRNA and protein levels. When COX-2 and 5-LOX are blocked *via* a variety of mechanisms, cancer cell proliferation is abrogated both *in vitro* and *in vivo*. The mechanism of COX-2 has been shown to include effects on apoptosis as well as angiogenesis. 5-LOX has been implicated in apoptosis. The use of COX-2 and 5-LOX inhibitors in clinical studies in patients with pancreatic cancer has been limited. Patient enrollment has been restricted to those with advanced disease which makes evaluation of these drugs as chemopreventive agents difficult. COX-2 and 5-LOX expression have been shown to be present during the early neoplastic changes of pancreatic cancer, well before progression to invasive disease. This indicates that the ideal role for these interventions is early in the disease process as preventive agents, perhaps in patients with chronic pancreatitis or hereditary pancreatitis.

Key words: Arachidonic acid; Eicosanoid; Cyclooxygenase-2; 5-lipoxygenase; Pancreatic cancer; Inflammation

Core tip: This review article highlights the relationship between inflammation and pancreatic cancer, specifically focusing on the enzymes cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LOX). The role of inflammation and tumor progression is a burgeoning area of research. This review delves into the research that has been conducted investigating COX-2 and 5-LOX and their relationship to pancreatic cancer both *in vivo* and *in vitro*. We discuss a variety of investigations including basic science, epidemiological, and clinical as they relate to pancreatic inflammation and eicosanoids.

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INTRODUCTION

The relationship between inflammation and cancer is well established. Rudolf Virchow noticed leukocytes in cancerous tissue as early as 1863 and conjectured that there was a link between chronic inflammation and neoplasia[1]. This theory has been validated by clinical examples such as Marjolin's ulcers which are squamous cell carcinomas that form in sites of chronic inflammation such as burn scars or chronic ulcers[2]. Other examples of inflammatory conditions with correlative cancers are inflammatory bowel disease and colorectal cancer, gastritis caused by *Helicobacter pylori* and gastric cancer, hepatitis and hepatocellular carcinoma, and chronic pancreatitis and pancreatic cancer. These examples highlight the impact of inflammation on the neoplastic process though the mechanism is unclear.

The inflammatory response is marked by cytokine release from epithelial cells which attract and activate inflammatory cells. When macrophages, neutrophils, fibroblasts, and mast cells are attracted to this inflammatory microenvironment, they produce reactive oxygen species (ROS) and stimulate epithelial cell proliferation[3]. The infiltration of these cells into the tumor microenvironment has been implicated in pancreatic tumor progression (Figure 1)[4-7]. ROS can directly cause DNA damage by increasing the probability that genetic mutation will occur. Combined with their effects on cellular proliferation, ROS increase the likelihood of neoplastic transformation[3,8]. A key step in the inflammatory process is the activation of the arachidonic acid pathway that produces eicosanoids. The purpose of this paper will be to review inflammatory mechanisms as they relate to pancreatic cancer, specifically the roles of cyclooxygenase (COX) and lipoxygenase (LOX), and how their metabolites contribute to carcinogenesis.

INFLAMMATION AND PANCREATIC CANCER

Pancreatic cancer is the fourth leading cause of cancer-related death in the United States, and the vast majority of those afflicted succumb to this disease. The 5-year survival rate is about 5%-6%[9]. Since the majority of pancreatic cancer is discovered late in the disease process, well after potentially curative surgery is an option, understanding the early oncogenic changes is necessary to aid in prevention. Since inflammation has been shown to be a key factor in the neoplastic process as it contributes to genetic changes and DNA damage, its role in pancreatic cancer is of particular interest.

Studying the mechanisms of pancreatitis in patients can be helpful for understanding inflammation as it relates to pancreatic cancer development. Patients with hereditary pancreatitis, a rare disease responsible for less than 1% of pancreatitis cases, have frequent episodes of acute inflammation[10]. Repeated episodes of pancreatitis result in fibrosis, chronic inflammation, and the eventual destruction of the gland[11]. This chronic inflammatory environment is thought to contribute to malignant transformation of pancreatic ductal cells. In patients with hereditary pancreatitis, the risk of developing pancreatic cancer is 53 times higher than unaffected individuals, and by 70 years of age, approximately 40% of these patients will develop pancreatic cancer[10]. Patients afflicted with non-hereditary chronic pancreatitis also have an increased risk of pancreatic cancer. Population studies suggest that patients with chronic pancreatitis are 17 times more likely to develop pancreatic cancer compared to age matched controls, and the risk is correlated with the duration of inflammation[12]. Therefore it will be important to understand the mechanisms that link pancreatitis to the development of pancreatic cancer.

The inflammatory process begins with the inappropriate release of proteolytic pancreatic enzymes that cause acinar cell injury[13]. This generates an immune response in which inflammatory cells are attracted to cytokines released from the cells at the site of injury. Our lab, as well as others, previously investigated the relationship between one of the major inflammatory cell types, mast cells, and pancreatic cancer[6,14]. We have shown that mast cell infiltration in pancreatic cancer specimens correlates with worse prognosis[6]. Ma demonstrated that pancreatic ductal adenocarcinoma (PDAC) cells promote mast cell migration and activation *in vitro*. The study also showed that blocking mast cell migration in an orthotopic PDAC mouse model decreased PDAC growth *in vivo*[15]. Similarly, Soucek demonstrated in an islet-cell tumor mouse model that mast cells mediate expansion of these tumors and are essential for tumor maintenance[5].

The generation of ROS and activation of the arachidonic acid pathway are also key steps in potentiating the inflammatory response[13]. The body mounts a natural response to chronic insults to the pancreas by releasing growth factors such as platelet-derived growth factor and transforming growth factor beta. This stimulates cell proliferation, which can potentially worsen DNA damage and increase genetic mutations[16].

EPIDEMIOLOGICAL STUDIES

Epidemiological studies have shown that high-fat diets, specifically with a high proportion of polyunsaturated omega-6 fatty acids, are associated with increased cancer rates, particularly in breast, pancreas, and prostate cancers[17-22]. Studies have shown that cancer incidence in an ethnic group often changes after migration and drastic dietary changes. An example is the migration of the Japanese to Western countries that have relatively higher fat diets compared to Japanese diets. Studies have reported increased colon, pancreas, breast, and prostate cancer incidence in individuals migrating to Western countries from Japan[21]. The relationship between a high-fat diet and pancreatic cancer was evaluated by a prospective study investigating obesity in various age groups including early adulthood, midlife, and older age. There were

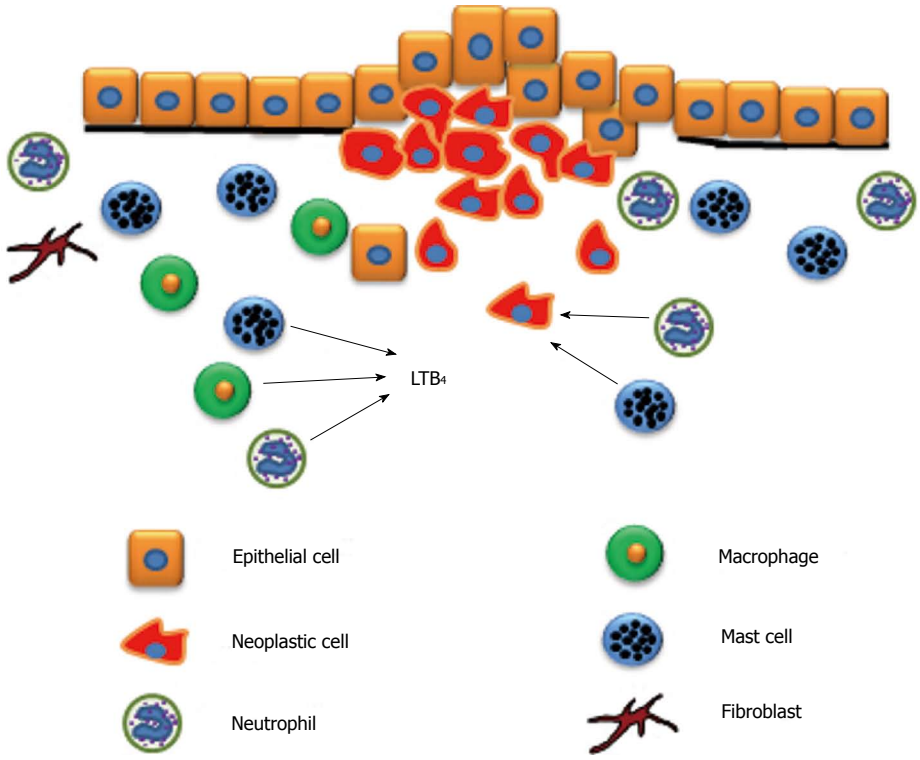

Figure 1 Inflammatory cell infiltration into the tumor microenvironment. As pancreatic adenocarcinoma progresses, inflammatory cells such as mast cells, neutrophils, and macrophages are attracted to the tumor microenvironment and enhance tumor growth. Leukotriene B_4 (LTB_4) is a chemotactic factor for macrophages, neutrophils, and mast cells. Fibroblasts are also activated and enhance collagen production.

significant positive associations between pancreatic cancer and obesity in all age groups studied[23]. Patients with the longest duration of obesity and diabetes were at the greatest risk for pancreatic cancer[23]. One of the mechanisms proposed for this association is the high content of arachidonic acid in animal fats. Arachidonic acid is metabolized to biologically active lipids by COX, LOX, and epoxygenase pathways to generate eicosanoids[24]. Eicosanoids have been implicated in various carcinogenic mechanisms including tumor progression and metastasis[25]. Studies conducted in EL-Kras transgenic mice fed a high ω-6 fatty acid diet demonstrated increased frequency and size of pancreatic neoplastic lesions as well as increased pancreatic mast cell densities[26]. In a related study, a high ω-3 fatty acid diet in EL-Kras transgenic mice was found to have a protective effect against the formation of pancreatic lesions. These mice had reduced incidence, frequency, and proliferative index of pancreatic precancer compared to those fed standard chow[27]. In unpublished findings by our lab, we demonstrate that EL-Kras transgenic mice fed high ω-6 fatty acid diets had increased PGE_2 and LTB_4 compared to their counterparts fed a high ω-3 fatty acid diet. Therefore, ω-3 and ω-6 fatty acids are involved in carcinogenic mechanisms and have opposing effects on pancreatic neoplasia, which is hypothesized to be mediated through the regulation of eicosanoid production.

Further evidence to support the role of eicosanoids in the carcinogenic process are epidemiological studies indicating that the use of non-steroidal anti-inflammatory drugs (NSAIDs) reduces the incidence of various solid tumors[24,28]. One study used a meta-analysis to examine the effect of regular NSAID use on colon, lung, breast, and prostate cancers. The results indicated that there is a risk reduction of 43% for colon cancer, 28% for lung cancer, 25% for breast cancer, and 27% for prostate cancer[28]. The role of NSAIDs and pancreatic cancer is not clear. Anderson conducted a prospective study with 28000 post-menopausal women and demonstrated a decreasing trend in pancreatic cancer incidence in women with more frequent aspirin use[29]. Alternatively, a study among United States adults followed for 18 years found no association between aspirin use and pancreatic cancer mortality[30]. A different prospective study in a large cohort of women with an 18 year follow-up showed an association with long-term aspirin use and pancreatic cancer although there was a higher prevalence of obesity and diabetes mellitus among patients who reported regular aspirin use[31]. A study conducted in the United Kingdom demonstrated that NSAID use for more than 773 d in the 5 years prior to diagnosis was associated with a 20% risk reduction of pancreatic cancer, although increasing doses did not have an impact on risk[32]. A meta-analysis involving 11 studies analyzing the association between pancreatic cancer and aspirin and other NSAIDS did not find a conclusive association[33]. The summary relative risk did not find an association between aspirin or other NSAIDS and pancreatic cancer, nor an association between regular use vs irregular use, nor frequency of aspirin or NSAID use[33].

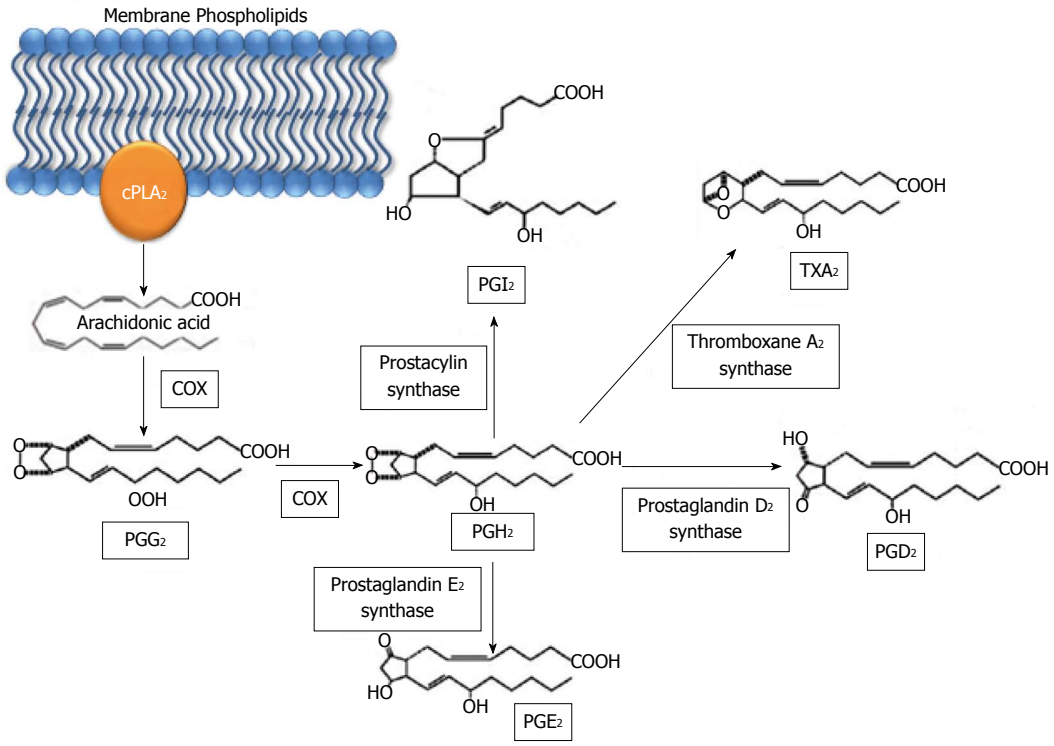

Figure 2 Metabolic pathway of prostaglandins *via* cyclooxygenase. Arachidonic acid is released from membrane phospholipids by phospholipase A_2 and converted to PGG_2 and subsequently PGH_2 by COX. PGH_2 is then converted to PGI_2, TXA_2, PGD_2, and PGE_2. cPLA2: Cytosolic phospholipase A_2; COX: Cyclooxygenase; PG: Prostaglandin; TX: Thromboxane.

BIOCHEMISTRY OF COX AND LOX

The ability of NSAIDs to exert their anti-inflammatory and anti-tumor effects by inhibiting the COX enzyme, which results in decreased prostanoid production, demonstrates the intimate relationship between inflammation and cancer[24]. There is evidence to suggest that 5-LOX, a close relative of COX-2, is essential for eicosanoid production and tumor pathogenesis. The precursors of eicosanoids are arachidonic acids. Both prostaglandins (PG) and leukotrienes (LT) are members of the eicosanoid family, which are lipid mediators made of a 20 carbon fatty acid derivative[34]. Eicosanoids are vital due to their distinct biological activity in the body and effectiveness in the nanomolar concentration range[34]. The two eicosanoid members that will be discussed in detail here are prostaglandins and leukotrienes.

Prostaglandins are made by most cells in the body, and they act as both paracrine and autocrine mediators[34]. Arachidonic acid is released from the membrane by the phospholipase $cPLA_2$ and acted on by prostaglandin G/H synthase (known as COX) to become an intermediate known as PGH_2[24] (Figure 2). There are two main forms of COX: COX-1 and COX-2. COX-1 is generally thought of as the constitutively expressed enzyme that is responsible for basal production of prostanoids for tissue homeostasis, and COX-2 is induced by cytokines and growth factors, particularly at sites of inflammation and neoplasia[13]. Therefore, COX-2 has a key role in the setting of inflammation and the tumor microenvironment[24].

Leukotrienes, while derived from the same precursor as prostaglandins, are functionally distinct. Leukotrienes are predominately produced by inflammatory cells, and once cellular activation occurs, $cPLA_2$ and 5-lipoxygenase (5-LOX) are translocated to the nuclear envelope[34]. LOX enzymes are a family of nonheme iron-containing dioxygenases with labeling based on the location of oxygen insertion at the carbon position of arachidonic acid[25]. The most common LOX enzymes are 5-, 8-, 12-, and 15-LOX[25]. These then form the corresponding hydroperoxyeicosatetraenoic acids (HPETE)[25]. Specifically, 5-LOX transforms arachidonic acid *via* a dehydration reaction to the unstable epoxide LTA_4[25]. LTA_4 is further oxidized to form either 5-HETE or the leukotrienes[25]. LTA_4 can be hydrolyzed by leukotriene A4 hydrolase in the cytoplasm or nucleus resulting in LTB_4 (Figure 3). LTB_4 is known as a potent chemoattractant, and its receptors are upregulated in pancreatic cancer[35]. LTA_4 can also be conjugated with glutathione to form LTC_4 by LTC_4 synthase. LTC_4 can then undergo extracellular metabolism resulting in LTD_4 and LTE_4[34]. The activation of 5-LOX is dependent upon the 5-LOX-activating protein (FLAP).

One of the ways in which LTB_4 directs chemotaxis and regulates neutrophil adhesion is by activating integrin receptors[34,36-39]. It has been demonstrated that local cell death causes "swarm-like" interstitial neutrophil clustering and LTB_4 plays an important role in intercellular communication between neutrophils and facilitates neu-

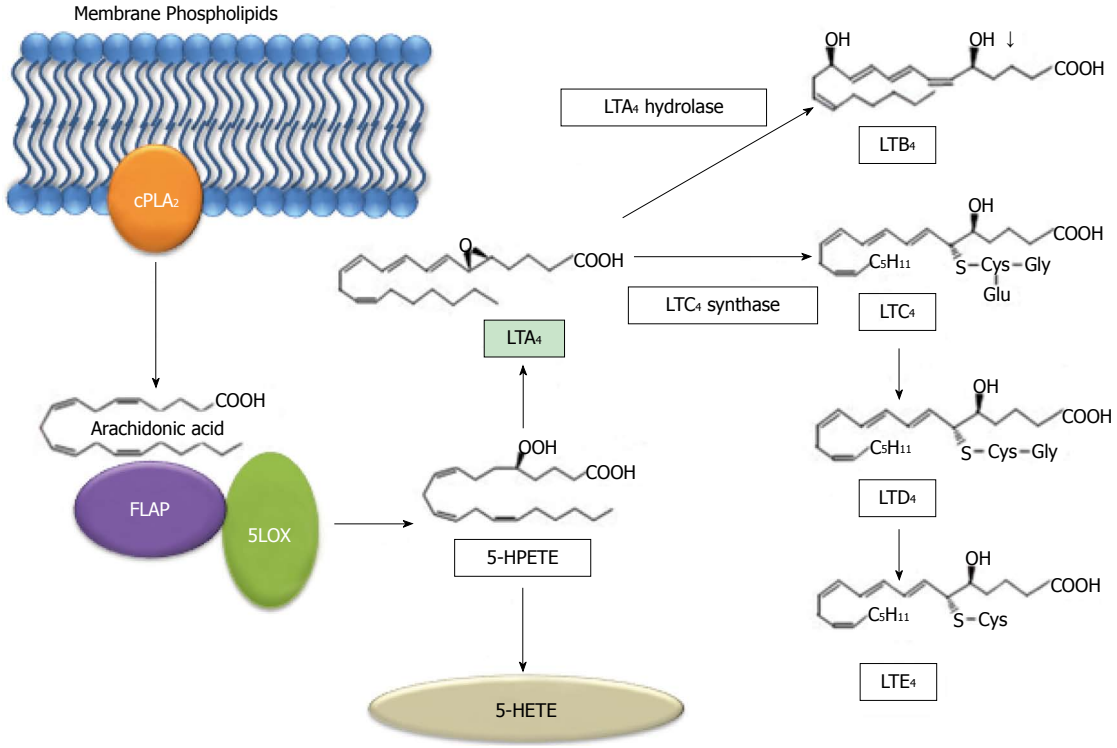

Figure 3 Metabolic pathway of arachidonic acid *via* 5- lipoxygenase. Arachidonic acid is released from membrane phospholipids by phospholipase A_2 and converted to 5-HPETE by 5-LOX and 5-LOX activating protein (FLAP). 5-HPETE can then form either 5-HETE or LTA_4. LTA_4 then becomes LTB_4 or LTC_4. LTC_4 can then form LTD_4 and subsequently LTE_4. cPLA2: Cytosolic phospholipase A_2; LOX: Lipoxygenase; FLAP: 5-LOX activating protein; HPETE: Hydroperoxyeicosatetraenoic acid; HETE: Hydroxyl 6 *trans* 8, 11, 14 *cis* eicosatetraenoic acid; LT: Leukotriene.

trophil movement through tissue[39]. In the tumor microenvironment, LTB_4 has been shown *in vivo* to enhance leukocyte recruitment into the tumor stroma[40].

ROLE OF COX IN PANCREATIC NEOPLASIA AND CANCER

COX-2 expression is upregulated in a variety of malignancies including colon, esophagus, breast, and pancreatic cancer[41-43]. Multiple studies have indicated that COX-2 is also important in carcinogenesis. One example in a murine model of familial adenomatous polyposis showed a marked reduction in the number and size of intestinal polyps in COX-2 null mice with an APC mutation[44].

The relationship between COX-2 and pancreatic cancer has been evaluated in multiple studies with the majority of the evidence demonstrating upregulated COX-2 expression in pancreatic cancer at both the mRNA and protein levels. One study showed that levels of COX-2 mRNA were increased 60-fold in pancreatic cancer compared to normal tissue. In addition, COX-2 protein was expressed in 9 out of 10 pancreatic cancer samples, while nontumor samples had no COX-2 expression[45]. Immunohistochemistry (IHC) confirmed COX-2 expression in malignant epithelial cells[45]. A different study demonstrated an increase in COX-2 expression using IHC when pancreatic carcinoma was compared to normal pancreas[43]. Five pancreatic cancer cell lines were studied, and COX-2 protein expression was detected in BxPC-3, Capan-1, and MDAPanc-3 cells, and increased levels of COX-2 mRNA were detected in 4 of the 5 cell lines[43]. When an NSAID was used, a dose-dependent inhibition of cellular proliferation was observed in all cell lines studied[43]. Kokawa *et al*[46] used different pancreatic cancer cell lines (KP-2, PNS-1, MiaPaca-2, and Panc-1) to show that COX-2 expression was upregulated in all 4 of them, and NSAID inhibition of cellular proliferation was correlated with the expression of COX-2. Maitra used automated cellular imaging to evaluate COX-2 expression not only in pancreatic adenocarcinoma but also its precursor, pancreatic intraepithelial neoplasia (PanIN). This showed an increase in the overall average number of positive cells from 19.2% in normal ducts to 36.3% in PanINs to 47.3% in adenocarcinomas[47]. This study suggests tumorigenic activity of COX-2 in preinvasive pancreatic lesions and a potential role for chemopreventive agents such as COX-2 inhibitors in pancreatic cancer.

While multiple studies have shown the association between pancreatic cancer and COX-2 expression, few have investigated the underlying mechanism of COX-2 and how it promotes neoplastic changes. Overexpression of COX-2 leads to increased tumor prostanoid levels, and PGE_2 is known to have several tumorigenic effects. PGE_2 has been implicated in the inhibition of apoptosis and the induction of proliferation and angiogenesis[48]. One group investigated the relationship between high-mobility group A1 (HMGA1) and COX-2 in pancreatic

cancer. The authors proposed that the HMGA1-COX-2 axis is a key molecular pathway in pancreatic cancer because the upregulation of COX-2 expression is HMGA1 dependent in various pancreatic cancer cell lines. It was first demonstrated that a positive correlation between HMGA1 and COX-2 expression in six pancreatic cancer cell lines (BxPC-3, HPAF-Ⅱ, MiaPaCa-2, Panc1, PL45, and XPA-3) existed[49]. COX-2 expression after knockdown of HMGA1 in two pancreatic cancer cell lines was evaluated and showed that HMGA1 binds to the COX-2 promoter to induce its expression[49]. A significant reduction in COX-2 expression after using an HMGA1 siRNA was observed, and COX-2 inhibitors blocked tumorigenesis in human pancreatic cancer xenografts that overexpressed HMGA1[49].

Another potential mechanism proposed for the involvement of COX-2 in tumorigenesis is its effect on angiogenesis. Chu compared the angiogenic effects of a COX-2 expressing pancreatic cancer cell line BxPC-3 with the COX-2 negative AsPC-1 cell line. The group found a significant increase in endothelial cell migration induced by BxPC-3 migration compared with AsPC-1. These findings were supported by data demonstrating that BxPC-3 treatment with a COX-2 inhibitor decreased the angiogenic responses of the endothelial cells[50]. Eibl *et al*[51] showed in a subset of pancreatic cancer cell lines that COX-2 increased PGE_2 which subsequently increased VEGF secretion. In a subsequent *in vivo* study, an orthotopic pancreatic cancer model in nude mice was used to demonstrate the effects of nimesulide, a selective COX-2 inhibitor, on angiogenesis. In mice with COX-2 positive tumors, nimesulide resulted in an increase in VEGF production by malignant cells but a compensatory decrease in production by nonmalignant cells, ultimately leading to reduced tumor angiogenesis and growth[52].

Ito's study on the effect of COX-2 on tumor invasion found that PGE_2 mediated pancreatic cancer cell invasion through induction of matrix metalloproteinase-2 expression. This induction was found to be dependent on an extracellular signal-regulated kinase (ERK)/Ets-1-dependent mechanism[53].

Another study investigated the expression of COX-2 on clinical outcomes and found no correlation between global COX-2 expression and clinical outcome. The clinical outcomes studied were survival, stage, tumor size, or vascular invasion[54]. The expression of COX-2 was related to an increase in perineural invasion[54].

Several preclinical mouse models evaluating pancreatic lesions have been reported. One particular transgenic model, LSL-KRASG12D; PDX-1-Cre, is a mouse with a KRAS mutation expressed in pancreatic progenitor cells. This model results in PanIN lesions which eventually develop through advanced PanIN lesions into adenocarcinoma[55]. The efficacy of a selective COX-2 inhibitor, nimesulide, was evaluated in this mouse model. Animals treated with nimesulide demonstrated significantly fewer PanIN lesions and decreased intrapancreatic prostaglandin E2 levels compared to mice on a control diet[55]. In two unpublished works from our group, another mouse model with mutant Kras expression targeted to acinar cells (EL-Kras)[56] have been crossed with COX-2 knock-out mice to generate cohorts of EL-Kras/COX-$2^{-/-}$ mice. These mice have a significantly reduced frequency of cystic papillary neoplasms compared with EL-Kras mice with wild-type COX-2. Also, mice that overexpress COX-2 in acinar cells develop hyperplastic, mildly dysplastic ducts with accompanying focal fibrosis and lymphocytic infiltration[57]. A different transgenic mouse model, BK5.COX2, results in COX-2 overexpression in the exocrine pancreas[58]. The resulting histology demonstrated pancreatitis-like changes with acinar-to-ductal metaplasia by 3 mo, and at 6-8 mo strongly dysplastic features. The described phenotype was completely prevented by maintaining the mice on a COX-2 inhibitor. Cell lines derived from lesions in these mice were tumorigenic when injected into nude mice. Both of these mouse models highlight the relationship between COX-2 and pancreatic cancer and will be important in future studies.

ROLE OF LOX IN PANCREATIC NEOPLASIA AND CANCER

Similar to COX-2, LOX has been implicated in several human cancers including lung, prostate, colon, breast, and pancreatic; however, relatively little research has been conducted to elucidate its role in cancer progression[59-61]. 5-LOX expression is upregulated in both pancreatic adenocarcinoma as well as in neoplastic lesions of the pancreas[25]. In a study by Hennig, three pancreatic cancer cell lines, AsPC-1, PANC-1, and MiaPaCa2, were found to have 5-LOX mRNA expression while normal human pancreatic cells did not express 5-LOX[35]. They also confirmed that 5-LOX protein was expressed in these cell lines and in two additional cell lines, Capan-1 and HPAF[35]. Moreover, the expression levels of both 5-LOX and its downstream metabolite LTB_4 were found to be significantly upregulated in pancreatic tumors compared with normal pancreatic tissue[35]. Interestingly, staining was evident in both the cancer cells as well as the ductal cells and adjacent islets. A follow-up study by Hennig *et al*[62] investigated 5-LOX expression in PanIN lesions. Greater than 90% of the ductal cells had strong positive 5-LOX staining in all grades of PanINs with no significant difference between grades of PanINs. This was compared to normal pancreatic specimens that had 0 to 7.5% of the ductal cells showing 5-LOX staining[62]. This study also reported that 5-LOX expression was present in pancreatic PanIN-like lesions in N-nitroso-bis (2-oxopropyl)-amine (BOP) treated hamsters as well as EL-Kras transgenic mice[62]. Ding reported similar results showing increased 5-LOX expression in MiaPaCa2, PANC-1, AsPC-1, and Capan2 pancreatic cancer cell lines at the mRNA level[63]. The general LOX inhibitor (NDGA), a 5-LOX inhibitor (Rev5901), and a FLAP inhibitor (MK-886), all inhibited thymidine incorpora-

tion in MiaPaCa2 cells indicating that these compounds induced growth inhibition in pancreatic cancer cells. Finally, it was demonstrated that arachidonic acid and linoleic acid induced pancreatic cancer cell proliferation[63].

While there have been no studies published to date examining mouse models deficient in 5-LOX, our lab is currently investigating this mouse model. We have developed a EL-Kras/5-LOX null mouse and preliminary results have indicated a decrease in pancreatic lesions in the 5-LOX null mice compared with their wildtype counterparts.

While it is well established that 5-LOX plays an important role in pancreatic tumor progression, fewer studies have investigated its underlying mechanism in this disease. Ding showed that the 5-LOX metabolite, 5(S)-hydroxyeicosatetraenoic acid [5(S)-HETE], stimulates pancreatic cancer cell proliferation in a time- and concentration-dependent manner[63]. In a subsequent study, Ding demonstrated that 5-(S)-HETE has mitogenic effects due to its role in the MEK/ERK and PI3 kinase/AKT pathways[64]. In an additional study, this group demonstrated that both the general LOX inhibitor (NDGA) and the 5-LOX inhibitor (Rev5901) induced apoptosis in four different pancreatic cancer cell lines[65]. Apoptosis was confirmed using three different methods including DNA propidium iodide staining, DNA fragmentation, and terminal deoxynucleotidyl transferase nick end labeling (TUNEL) assay in PANC-1, MiaPaCa2, Capan2, and HPAF cell lines[65]. A follow-up study performed by Tong further delineated the mechanism behind the LOX inhibitor-induced apoptosis showing that it is a mitochondria-mediated pathway[66]. Specifically, LOX inhibitors (NDGA and Rev5901) decreased Bcl-2 and Mcl-1 and increased Bax expression in human pancreatic cancer cells[66]. LOX inhibitors also induced cytochrome-c release and caspase-9 activation. The effect of the LOX inhibitors was also demonstrated *in vivo* where it blocked pancreatic cancer cell growth and induced apoptosis in athymic mice[66]. These studies suggest the relationship between 5-LOX and its role in apoptosis in the tumor microenvironment.

LTB_4 AND PANCREATIC CANCER

LTB_4 is a metabolite of 5-LOX and an important inflammatory mediator. LTB_4 is involved in recruiting inflammatory cells and is a potent chemokine for monocytes, neutrophils, and eosinophils. It also enhances adhesion and migration of neutrophils across the vascular endothelium[67]. BLT_1 and BLT_2 are two G-protein-coupled receptors that have a high and low affinity, respectively, for LTB_4[68]. LTB_4 is secreted from human pancreatic cancer cells and its receptors are upregulated in pancreatic cancer tissue as well as in multiple cell lines[35,69]. Similar to COX-2 and 5-LOX, BLT_1 and BLT_2 have also been found to be upregulated in PanIN lesions which suggests a potential role of LTB_4 and its receptors in chemoprevention[70].

Multiple LTB_4 receptor antagonists have been developed but earlier compounds had poor oral bioavailability[68]. A more stable and orally bioavailable compound was later developed, LY293111, which blocks LTB_4-mediated kinase phosphorylation[67]. LY293111 inhibits pancreatic cancer growth *in vivo* and *in vitro* through inhibition of proliferation and induction of apoptosis in a variety of pancreatic cancer cell lines (MiaPaCa-2, HPAC, Capan-1, Capan-2, PANC-1, and AsPC-1) in a time- and concentration-dependent manner[69,71]. When LTB_4 was added to the cancer cell lines, it stimulated proliferation and induced ERK1/2 phosphorylation in all six cell lines[69]. In a different study, LY293111 was found to cause cell cycle arrest in S phase and suppress cyclin A, cyclin E, and cdk2 expression[71]. When LY293111 was administered to athymic mice with human pancreatic cancer xenografts, the LTB_4 receptor antagonist suppressed growth of the subcutaneous xenografts[69].

CLINICAL CORRELATION

COX inhibitors

Multiple studies have been conducted evaluating the use of COX-2 inhibitors combined with different chemotherapy regimens. A phase Ⅱ trial of Uracil/Tegafur plus Leucovorin and Celecoxib combined with radiotherapy in patients with locally advanced pancreatic cancer did not show a significant response and resulted in substantial gastrointestinal toxicity[72]. A study of Celecoxib and 5-fluorouracil in patients with advanced pancreatic cancer who had progressed after gemcitabine-based chemotherapy showed promising results in that the Celecoxib was well tolerated and capable of inducing durable responses[73]. In a phase Ⅱ trial of gemcitabine, Irinotecan, and Celecoxib in patients with inoperable pancreatic cancer, the addition of Celecoxib was found to increase the percentage of patients achieving a one-year overall survival from about 3 mo to 9 mo and increased overall survival from about 6 mo to 18 mo[74]. Other studies in patients with advanced pancreatic cancer evaluated the combination of Celecoxib and gemcitabine or the combination of Gemcitabine, Celecoxib, and Cisplatin, but Celecoxib did not increase the efficacy of either chemotherapy regimen (Table 1)[75,76]. While the idea of using a COX-2 inhibitor is promising in patients with pancreatic cancer, it will likely be most effective as a preventive agent very early in the disease process as opposed to improving survival in those patients with advanced disease.

LOX inhibitors

Zileuton is a 5-LOX inhibitor of the N-hydroxyurea series, approved by the Food and Drug Administration in 1996 for the treatment of asthma[25]. It was shown in clinical trials to produce moderate airway improvement in asthmatics. While Zileuton has had promising effects for airway disease, this drug has not yet been tested in patients with cancer.

Several studies have investigated Zileuton in animal

Table 1 Clinical trials

Drug	Type	Trial	Type	Cancer	Outcome	Toxicity
Celecoxib	COX-2 inhibitor	Uracil/Tegafur, Leucovorin, Celecoxib + RT[72]	II	Pancreatic; locally advanced unresectable	No significant partial or complete response	Significant GI toxicity
		Celecoxib, 5-FU[73]	Pilot study	Pancreatic; advanced after Gemcitabine treatment	Durable response	Well tolerated
		Gemcitabine, irinotecan, celecoxib[74]	II	Pancreatic; unresectable	Increased OS from 6 m to 18 m	Well tolerated
		Gemcitabine, celecoxib[76]	II	Pancreatic; locally advanced or metastatic	No significant response	Well tolerated
		Gemcitabine, cisplatin, celecoxib[75]	II	Pancreatic; metastatic	No significant response	Well tolerated
LY293111	LTB_4 receptor antagonist	Irinotecan, LY293111[80]	I	Solid tumors (including pancreatic); locally advanced or metastatic	No significant response	Significant GI toxicity
		Gemcitabine, LY293111[81]	II	Pancreatic; locally advanced or metastatic	No significant response	Significant GI toxicity

Cox: Cyclooxygenase; LTB4: Leukotriene B4; RT: Radiation therapy; FU: Fluorouracil; OS: Overall survival; GI: Gastrointestinal.

studies and shown promising results for multiple cancers including carcinoma of the colon, lung, and pancreas. Zileuton was shown to reduce cell proliferation in murine colon adenocarcinoma cell lines[77]. In a xenograft model using human colon cancer cells, Zileuton inhibited tumor growth and reduced tumor mass[78]. In pancreatic cancer studies using the Syrian hamster model with BOP-induced pancreatic cancer, Zyflo (an extended release formulation of Zileuton) was found to reduce the incidence and size of the pancreatic cancer both alone and in combination with a COX-2 inhibitor[79].

LTB_4 RECEPTOR ANTAGONIST

A few clinical trials have been conducted using LY293111 in patients with pancreatic cancer. A phase I study demonstrated that LY29311 was well tolerated in combination with Irinotecan although no responses were seen[80]. A different study randomized patients with pancreatic cancer to gemcitabine and LY293111 *vs* gemcitabine and placebo. There was no significant difference in six-month survival or progression-free survival[81]. Finally, a study conducted in patients with non-small cell lung cancer receiving LY293111 and Cisplatin/Gemcitabine also did not show a survival benefit[82]. Similar to COX-2 inhibitors, an LTB_4 receptor antagonist would probably be most efficacious early in the disease process.

FLAP inhibitors

MK-886 is a FLAP inhibitor and inhibits leukotriene biosynthesis. It was first developed for use in asthma although clinical development was halted due to only a 50% inhibition of leukotriene production when used[83]. A second-generation FLAP inhibitor, MK-0591, had more potent inhibitory effects on leukotriene production, although it did not clinically perform as expected and was also discontinued[84].

Similar to Zileuton, MK-886 has shown promising results *in vitro* and *in vivo*. As mentioned above, MK-866 was shown to promote growth inhibition in a pancreatic cancer cell line. It was also shown *in vivo* to reduce pancreatic cancer development in a hamster model[85].

CONCLUSION

The inflammatory pathway is an important process in cancer progression. A combination of clinical studies, epidemiological studies, and basic science investigations indicate that there is a relationship between inflammatory changes in the pancreas and neoplastic progression. Intake of ω-6 polyunsaturated fatty acids provides increased substrate for COX and LOX mediated metabolism of arachidonic acid into eicosanoids. These eicosanoids directly contribute to pancreatic cancer cell proliferation. When COX-2 and 5-LOX are blocked *via* a variety of mechanisms, cancer cell proliferation is abrogated both *in vitro* and *in vivo*. The use of COX-2 and 5-LOX inhibitors in clinical studies in patients with pancreatic cancer has been limited. Patient enrollment has been restricted to patients with advanced disease which makes evaluation of these drugs as chemopreventive agents difficult. COX and LOX expression have been shown to be present during the early neoplastic changes of pancreatic cancer, well before progression to invasive disease. This indicates that the ideal role for these interventions is early in the disease process as preventive agents, perhaps in patients with chronic pancreatitis or hereditary pancreatitis. Further investigation is needed to broaden our understanding of the complex relationship between inflammation and pancreatic cancer and how these inflammatory pathways can be targeted to treat this deadly disease.

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