January 16, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format: **6661_review_wjg**.

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

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Name of Journal: *World Journal of Gastroenterology*

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I appreciate the constructive criticism from the reviewers and am grateful for their time. The manuscript has been improved according to the suggestions of reviewers:

Reviewer #1

1. *"The references should be labeled in table 1"*

Response:
References were added to Table 1.

2. *"The contribution of figure 1 to the manuscript could be greatly improved by insertion of arrows indicating mechanisms and or any proposed intercellular interactions between the various cell-types."*

Response:
Arrows were added to Figure 1 to indicate intercellular interactions.

3. *"A corresponding figure should be provided for the Cox-2 pathway (see p 8)."*

Response:
A new figure was added demonstrating the COX-2 pathway. This is now Figure 2 and the LOX pathway figure is now Figure 3 (it was previously Figure 2).

Reviewer #2

1. "…it is necessary to include the update information on the studies using animal model, such as genetically engineered models"

Response:
The reviewer's point about including animal models is well-taken as this is an important aspect of investigation. A paragraph dedicated to transgenic animal models was added on page 12 and is copied below.
"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. 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. In two unpublished works from our group, another mouse model with mutant Kras expression targeted to acinar cells (EL-Kras) have been crossed with Cox-2 knock-out mice to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. A different transgenic mouse model, BK5.COX2, results in COX-2 overexpression in the exocrine pancreas. The resulting histology demonstrated pancreatitis-like changes with acinar-to-ductal metaplasia by 3 months, and at 6-8 months 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."

An additional study involving an orthotopic mouse model in pancreatic cancer was also added on page 11 and is copied below.
"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."

2. "…it is necessary to include the update information on the summary review on different NSAIDs such as Aspirin"

Response:
The relationship between aspirin/NSAIDs and pancreatic cancer is very important and there is a paragraph devoted to the literature studying this association (page 7, paragraph 2). We have also added more sources to strengthen the section (page 8). The added paragraph is below.

"A study conducted in the United Kingdom demonstrated that NSAID use for more than 773 days 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. A meta-analysis involving 11 studies analyzing the association between pancreatic cancer and aspirin and other NSAIDS did not find a conclusive association. 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."

3. "…it is necessary to include the update information on interaction of unsaturated fatty acid and COX2/LOX, etc."

Response:
The reviewer points out an important relationship between unsaturated fatty acids, eicosanoids, and their relationship to pancreatic cancer. The actual pathways from unsaturated fatty acids to the eicosanoids via the COX-2 and 5-LOX enzymes are illustrated in figures 2 and 3. In addition, a paragraph on page 7 specifically discusses the relationship between the specific fatty acids (ω-3 and ω-6) and their impact in mouse models on pancreatic lesions. We have added a sentence on page 7 including additional unpublished data specifically addressing PGE_2 and LTB_4 in relation to the fatty acids, included below.

"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."

Reviewer #3

1. *"I would recommend to discuss in more details the mechanism for eicosanoid effect on cancer development through cell adhesion regulation."*

Response:
Thank you for this suggestion as cell adhesion is a very important role of eicosanoids. A paragraph was added detailing the role of LTB_4 in neutrophil chemotaxis, adhesion, and influence in the pancreatic tumor microenvironment on page 9, included below.
"One of the ways in which LTB_4 directs chemotaxis and regulates neutrophil adhesion is by activating integrin receptors. 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 neutrophil movement through tissue. In the tumor microenvironment, LTB_4 has been shown in vivo to enhance leukocyte recruitment into the tumor stroma."

2. *"COX-2 is a constitutive enzyme expressed in brain tissue in neurons, I would recommend to avoid statements like "COX-2 is absent in most cells until it is induced by cytokines and growth factors". In addition, COX-2 is induced through cytokine-independent mechanism as well."*

Response:
The sentence mentioned above starting on page 8, was changed to, **"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"**

3. *"The subtitle "Mechanism of COX-2" (p. 10) is not informative; I would recommend to correct it. The same for "Mechanism of 5-LOX" and "LTB4.""*

Response:
The subtitles have been changed. They are now, **"The Role of COX in Pancreatic Neoplasia and Cancer," "The Role of LOX in Pancreatic Neoplasia and Cancer,"** and "**LTB_4 and Pancreatic Cancer**."

The formatting for the title was changed, the author information was changed, and the "author contribution" section was added.

Thank you for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely,

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