Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: ESPS Manuscript 7126 reivew.doc).

Title: Pancreatic Cancer and its Stroma: A Conspiracy Theory

Author: Zhihong Xu, Srinivasa P. Pothula, Jeremy S. Wilson, and Minoti V. Apte

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 7126

Thank you very much for the opportunity to submit a revised version of the above manuscript. We thank the reviewers for their insightful comments, which we feel have helped strengthen our review. Please find enclosed a 'point-by-point' response to each reviewer's comments and the revised manuscript with all modifications in the text highlighted (red fonts for addition and strikethrough for deletion).

We look forward to hearing from you.

With regards

Yours sincerely

Minoti Apte

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Point-by-point response to reviewers' comments

(1) Reviewer 00034174:

In their review article "Pancreatic Cancer and its Stroma: A Conspiracy Theory", Xu and collegues summarize the current literature on the contribution of pancreatic stellate cell's (PSC) to pancreatic carcinogenesis. The group has pioneered research into pancreatic stroma and their contribution to the field since 1998 is significant.

Please find my comments below.

i. Please avoid using secondary references (p3, ref 10. The original data on 100-fold increased cancer risk is from Dr. Whitcomb).

We thank the Reviewer for pointing this out. The reference has been updated as suggested. [The original

data reporting a 100 fold increase in risk of pancreatic cancer in patients of tropical pancreatitis comes from a paper by Chari et al, Pancreas 1994. This paper has subsequently been discussed by Dr. Whitcomb et al 2004.]

Revised manuscript, page 3, paragraph 2, Ref 15 – 16.

"…patients with tropical pancreatitis have been reported to have a 100-fold increased risk and an earlier onset of the disease compared to sporadic cases (Chari, Mohan et al. 1994; Whitcomb 2004)."

ii. p19. Angiogenesis: Could the authors explain why PDAC in humans and in various mouse models is a hypoxic tumor despite proangiogenic effects of PSC?

Angiogenesis is a complex process, and the response of endothelial cells in different parts of the tumour likely depends on the balance of the pro-angiogenic and anti-angiogenic factors within the surrounding microenvironment. It is possible that as activated PSCs lay down increasing fibrous stroma in central areas of the tumour, the blood vessels in that area are compressed, leading to insufficient perfusion and hypoxia. However, at the invading front of the tumour, where the collagenous stroma is significantly less dense, endothelial cell proliferation in response to activated PSC secretions can occur in a relatively unrestricted manner.

We have modified the text in the revised manuscript to reflect the above clarification. Revised manuscript, page 20, paragraph 2.

"Angiogenesis is a complex process, and the response of endothelial cells in different parts of the tumour likely depends on the balance of the pro-angiogenic and anti-angiogenic factors within the surrounding microenvironment. It is possible that as activated PSCs lay down increasing fibrous stroma in central areas of the tumour, the blood vessels in that area are compressed, leading to insufficient perfusion and hypoxia. However, at the invading front of the tumour, where the collagenous stroma is significantly less dense, endothelial cell proliferation in response to activated PSC secretions can occur in a relatively unrestricted manner."

iii. p20-21. In the figures shown, the number of Y chromosome positive cells in metastatic nodules looks pretty minor. Could the authors comment on the possible physiological role of PSC in tumor metastasis despite their insignificant number?

It is now accepted that cells can travel to metastatic sites through the circulation either as single cells, or more likely, as a cluster of cells. We believe that metastatic cell clusters in pancreatic cancer could comprise both cancer cells and PSCs from the primary tumour and propose that these PSCs perform a very important initial function at the metastatic sites, which is to facilitate seeding, survival and proliferation of the metastatic cancer cells at those sites. Also important is the likelihood that PSCs, via secretion of chemokines, subsequently recruit local stromal cells within the metastatic site, which further facilitates cancer cell growth. We would also note that although the number of PSCs identified by FISH in the metastatic nodule is relatively small, their presence indicates that the cells were not only able to travel from the primary tumour, but were able to survive in the metastatic nodule over the duration of the experiment. Given that the rate of proliferation of PSCs is significantly slower than that of cancer cells, it would be reasonable to expect that cancer cells comprise the bulk of the metastatic nodule.

We have modified the text in the revised manuscript to reflect the above clarification. Revised manuscript, page 21, paragraph 1.

"It is now accepted that cells can travel to metastatic sites through the circulation either as single cells, or

more likely, as a cluster of cells. We believe that metastatic cell clusters in pancreatic cancer could comprise both cancer cells and PSCs from the primary tumour."

"…we propose that PSCs that have travelled to the metastatic site perform a very important initial function at the metastatic sites, which is to facilitate seeding, survival and proliferation of the metastatic cancer cells at those sites. Also important is the likelihood that PSCs, via secretion of chemokines, subsequently recruit local stromal cells within the metastatic site, which further facilitates cancer cell growth."

iv. p24.

(i) It appears that the activation of PSC around precursor lesions is secondary to epithelial carcinogenesis. Here, it may be helpful to discuss the work of Collins et al. J Clin Invest, 2012.

As we have noted in our manuscript, and concurring with the reviewer's comment, it is likely that "… preneoplastic cells have the capacity to activate PSCs in the early stages of carcinogenesis." (revised manuscript, page 24, paragraph 3).

In order to retain the flow of text in our manuscript, we feel it may be better to discuss the work of Collins et al, J Clin Invest, 2012 in the section discussing the role of K-Ras in pancreatic cancer.

We have modified the text in the revised manuscript to reflect this change. Revised manuscript, page 4, paragraph 2.

"Collins et al (Collins, Bednar et al. 2012) have shown in mice bearing inducible K-Ras mutations, that oncogenic K-Ras initiates pancreatic carcinogenesis by hindering pancreatic repair after caerulein-induced pancreatitis. Importantly, inactivation of K-Ras mutation in these mice leads to tumour regression suggesting a role for oncogenic K-Ras in the maintenance of pancreatic cancer."

(ii) Is there any information on the genetic mutations of PSC.

To the best of our knowledge, there have been no reported studies on genetic mutations per se in PSCs, although as already discussed in our paper (revised manuscript, page 8, paragraph 4 and page 9 paragraph 1) differences in gene expression between normal versus disease-activated PSCs have been assessed.

(iii) Please comment on why normal cells should help tumor cells?

Using our orthotopic model, we have recently demonstrated that even when normal PSCs (isolated from normal human pancreas) are combined with cancer cells (AsPC-1) for a few weeks, the normal PSCs acquire an activated phenotype and are highly responsive to cytokines/growth factors secreted by cancer cells; in turn, these activated PSCs facilitate cancer cell growth. As we have discussed in the revised manuscript page 19, paragraph 2, cancer cells have the capacity to be highly efficient and effective at recruiting surrounding PSCs, so as to set up a conducive microenvironment (such as ECM) for their own growth.

v. p26. Although the authors mention the failed trial of Infinity using IPI-926, they refrain from speculation on this issue. It would be interesting to know the opinion of the authors why, despite optimistic preclinical results, there was an opposite effect in the clinic.

The reasons for the failure of this drug in the clinical setting are not entirely clear. The disappointing clinical outcome may reflect the fact that: i) results from a single preclinical model are not sufficient to account for the heterogeneity of human pancreatic cancer; 2) the effects described by Olive et al in the

preclinical model, particularly with regard to perfusion, were transient. Before taking treatments to the clinic, it would be prudent to ensure that robust, long lasting effects were demonstrable in the preclinical setting.

We have modified the text in the revised manuscript to address this question. Revised manuscript, page 27, paragraph 2.

"The reasons for the failure of this drug in the clinical setting are not entirely clear. The disappointing clinical outcome may reflect the fact that: i) results from a single preclinical model are not sufficient to account for the heterogeneity of human pancreatic cancer; 2) the effects described by Olive et al in the preclinical model, particularly with regard to perfusion, were transient. Before taking treatments to the clinic, it would be prudent to ensure that robust, long lasting effects were demonstrable in the preclinical setting."

(2) Reviewer 01437408:

Tumor-stroma interactions are increasingly acknowledged as a major determinant of pancreatic cancer (PC) progression. Xu et al. provide an excellent and up-to-date review of the recent literature. My only suggestions are:

i. With respect to the relevance of tumor-stroma interactions in PC, the authors have entirely focussed on experimental evidence. Although clinical data are scarce, it might be worth to discuss them as well (e.g., Erkan M et al., Clin Gastroenterol Hepatol 2008;6:1155-61; Watanabe I et al., Pancreas 2003; 26:326–33.)

We thank the reviewer for his/her comments. We have modified the text in the revised manuscript to discuss the above studies. Revised manuscript, page 18, paragraph 3.

"In this regard, two earlier clinical studies have reported findings to support a role for the stroma in cancer progression. Watanabe et al (Watanabe, Hasebe et al. 2003) have reported that the presence of fibrotic foci (which the authors postulated as representing intratumoural fibroblast proliferation) was associated with shorter survival in advanced pancreatic cancer, while Erkan et al (Erkan, Michalski et al. 2008) have reported that high α-SMA/collagen ratios in tumours correlated with poor prognosis. However, as detailed below, most of the in vivo evidence in support of tumour-stromal interactions in pancreatic cancer comes from experimental studies using tumour xenografts and genetically engineered mouse models."

ii. The authors might briefly review possible relationships between stellate cells and stem cells/progenitor cells (e.g., Mato E et al., Biochem J 2009;421:181-91.; Kordes C et al. PLoS One 2012;7:e51878.)

We have modified the text in the revised manuscript to discuss the above studies. Revised manuscript, page 9, paragraph 3 and page 10, paragraph 1.

"Recent evidence suggests that in addition to synthesising ECM proteins, PSCs may have other roles within the pancreas, for example, as progenitor cells. In this regard, Mato et al (Mato, Lucas et al. 2009) isolated and expanded pancreatic cells from lactating rats using mitoxantrone (a drug that acts through multidrug transporter systems) selection. They have reported that the surviving, mitoxantrone-resistant cells showed a PSC-like morphology (fibroblast-like with vitamin A lipid droplets), expressed the stem cell marker ABCG2 transporter (ATP binding cassette G2 transporter) and were able to secrete insulin after cell differentiation. More intriguingly, a recent study by Kordes et al (Kordes, Sawitza et al. 2012) has reported that clonally

expanded rat PSCs, when injected into hepatectomised recipient rats, are able to migrate to the liver and to reconstitute large parts of the liver by differentiating into hepatocytes and cholangiocytes, whereas muscle fibroblast do not show any such transformations."

(3) Reviewer 00039657:

This is review summarizing current knowledge about the role of pancreatic stellate cells and stroma in pancreatic cancer biology from the view of many aspects. This paper is well written and shows pivotal information about this area. Therefore, this review would be very useful for many researchers studying about pancreatic carcinoma.

We thank this reviewer for his/her encouraging comments.

3 References and typesetting were corrected

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

Sincerely yours,

Minoti Apte
Professor of Medicine
Director, Pancreatic Research Group
South Western Sydney Clinical School
Faculty of Medicine, University of New South Wales
Liverpool Hospital
Liverpool, NSW 2170
AUSTRALIA
Ph: 61-2-8738 9029
Fax: 61-2-9602 9441
E-mail: m.apte@unsw.edu.au