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13 January 2014

To,

Jin-Lei Wang, Director, Editorial Office
Baishideng Publishing Group Co., Limited

Title: Pancreatic cancer organotypics: high throughput, preclinical models for pharmacological agent evaluation.

Author: Stacey J Coleman, Jennifer Watt, Prabhu Arumugam, Leonardo Solaini, Elisabeta Carapuca, Mohammed Ghallab, Richard P Grose, Hemant M Kocher.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6460

Dear Editor/Director,

Thank you very much for the opportunity to re-present our manuscript for publication in the World Journal of Gastroenterology. Please find enclosed the edited manuscript in Word format (file name: 6460-review.doc).

The manuscript has been improved according to the suggestions of reviewers and editorial office. Point by point response is appended.

Sincerely yours,

Hemant Kocher

Digitally signed by Hemant Kocher
DN: cn=Hemant Kocher, o=Barts and the London, ou=Tumour Biology, Institute of Cancer, email=h.kocher@qmul.ac.uk, c=GB
Date: 2014.01.13 18:22:38 Z

Hemant Kocher on behalf of all co-authors

1 Format has been updated according to ‘BPGs Revision Policies for Topic Highlight’.

2. As requested figures have been modified such that text and lines can be moved.

3. Revision has been made according to the suggestions of the reviewer

(1) Page 4, paragraph 1 : The sentence “Furthermore, matrix metalloproteinases ……..invasion” appears to be incomplete.

This sentence has now been amended and is highlighted in the new version of the submitted review

(2) Page 4, paragraph 1, last sentence: The authors state that “The net result is a unique tumour microenvironment inaccessible to chemotherapy….”. I do not think that the microenvironment itself is inaccessible to chemotherapy. It is the cancer cells within the microenvironment that are inaccessible to chemotherapy. Please rewrite the sentence appropriately.

This text has now been amended as follows to reflect this comment and is highlighted in the new version of the review submitted:

The net result is a unique tumour micro-environment, where tumour cells become inaccessible to chemotherapy and metastasise readily, leading to poor chemotherapy response rate.

(3) Page 5 : The authors mention the use of immortalised pancreatic stellate cell lines. There is some controversy in the literature about whether the available immortalised PSC lines closely represent the primary cells. A cautionary note should be inserted here to indicate that while immortalised PSC lines can certainly be used to provide proof of concept, it would be wise to ensure that the findings are subsequently confirmed using primary PSCs.

We have now amended and inserted the following text to incorporate the reviewer’s suggestions

The isolation and immortalisation of PSCs from human and rat pancreas has provided an additional tool for studying PSC activation and can overcome the limitations of culturing primary stellate cells. While immortalised stellate cells have provided a valuable tool in the study of PSC function, it is important to validate findings using primary PSCs

(4) Page 6, paragraph 2 : In terms of hypoxic conditions in pancreatic cancer, it should be noted that this can differ significantly in different regions of the same tumour. Thus, while the central areas of the tumour with dense stroma can be hypoxic, the same may not be the case at the invading front of the tumour

We have now changed the text to the following

Furthermore, in areas of the tumour that are hypoxic as a result of hypovascularity and profuse stroma this provides an environment in which pancreatic cancer cells thrive.

(5) Page 10, paragraph 2 : Please clarify the sentence “Importantly, these results were….. resembling human PDAC”. How would ATRA lead to increased “histology resembling human PDAC”?

We have now amended the text accordingly.

(6) . As the authors have noted, no single in vitro model can entirely replicate the in vivo system. In the Conclusion section, it may be useful to include the point that while 3D organotypic models are an important tool in elucidating molecular mechanisms of stroma-tumour interactions and are also useful for testing new treatment regimens, it remains critical that therapeutic approaches are subsequently tested in orthotopic or transgenic models of the disease, particularly because these animal models lend themselves to assessment of the effects of interventions on metastasis (a factor which the authors have acknowledged is the prime driver of the poor prognosis in pancreatic cancer).

We have now added the following text to reflect the reviewers comments

Although the organotypic model provides a physiologically relevant means to study the tumour stroma interactions and the use of new therapies to target the cross talk, it remains a simplified representation of the complex in vivo situation and it still remains critical to test new therapies in orthotopic or transgenic models of the disease. However, the use of the organotypic model as a preclinical tool is becoming increasingly important and our group, as well as others, are modulating the 3D cultures to recapture other important aspects of the tumour microenvironment that can influence cancer cell behaviour. Thus, 3D organotypic models have potential for bridging the gap between cell based discovery and complex animal models.

……

4 References and typesetting were corrected

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