Author’s response to reviews

Title: Secretion of fibronectin by human pancreatic stellate cells promotes chemoresistance to gemcitabine in pancreatic cancer cells

Authors:
MANOJ AMRUTKAR (manoj.amrutkar@medisin.uio.no)
Monica Aasrum (monica.aasrum@medisin.uio.no)
Caroline Verbeke (c.s.verbeke@medisin.uio.no)
Ivar P. Gladhaug (i.p.gladhaug@medisin.uio.no)

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Linda Gummlich
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Secretion of fibronectin by human pancreatic stellate cells promotes chemoresistance to gemcitabine in pancreatic cancer cells

Dear Editor,

On behalf of all authors, I would like to submit the revision of the manuscript “Secretion of fibronectin by human pancreatic stellate cells promotes chemoresistance to gemcitabine in pancreatic cancer cells”.

We are grateful to the reviewers for their valuable comments and suggestions that allowed us to substantially improve the manuscript. Please, find below our detailed point-by-point reply.

General comments about the revised manuscript:
Due to the inclusion of new data to comply with the requests of the reviewers figures Additional File 7_Figure S4 and Additional File 8_Figure S5 have been added to the revised manuscript to facilitate inclusion of the requested alterations. All changes are highlighted with Bright Green color in the revised manuscript.
Answers to reviewers’ comments – Reviewer #1

Comment 1. The study does not include proper controls. The pancreatic stellate cells cannot be compared to PC cell lines. They should compare them with PC cells taken from xenografts or PDX.

Response: We humbly disagree with the reviewer. We believe that this study includes relevant controls for each individual experiment. We would like to inform the reviewer that the present study aims at investigating the role of fibronectin, which is secreted by pancreatic stellate cells, in the development of chemoresistance to gemcitabine in pancreatic cancer cells. The study aim is not to compare pancreatic stellate cells with cancer cells, as suggested by the reviewer. Comparing PC cell lines with PC cells taken from xenografts or PDX is both beyond the scope of the original manuscript and of questionable information, given that the cancer cells would be of human origin and the stellate cells of murine derivation.

Comment 2. No study was shown on collagens?

Response: In the Discussion section, lines 323-329, page 13 we highlighted that the current knowledge regarding the role of different collagens in chemoresistance in pancreatic cancer is very limited. Furthermore, we pointed out that this study primarily focuses on the role of fibronectin in chemoresistance development in pancreatic cancer cells. However, following the reviewer’s advice, we performed additional experiments to investigate the effect of collagens on gemcitabine sensitivity. All PCC lines were seeded on plates that were/were not pre-coated with collagen type I solution from rat tail prior to incubation with gemcitabine (10 µM). Cell viability assessment using the MTT-based assay revealed no significant change in gemcitabine chemosensitivity in any of the PCC lines. In the revised manuscript, these results are shown in the figure Additional File 7_Figure S4 and the text is updated accordingly in Methods section, line 100, page 4 and Results section, lines 254-256, page 10. We believe that in order to understand the effect of collagens on chemosensitivity for gemcitabine, the specific role of each of the various collagens will have to be investigated, which is beyond the scope of the current manuscript.

Comment 3. The FN inhibit RGDS results are not impressive

Response: The commercially available fibronectin inhibitor RGDS, which was used in this study, has indeed limited effect, as pointed out by the reviewer, and we have now explicitly mentioned this in the Discussion section, lines 280-281, page 11 of the revised manuscript. In line with the reviewer’s comment, we also believe that there is a need for better fibronectin inhibitors, but these are not available as yet.

Comment 4. some language correction, Tumor vs tumours....

Response: We have now performed the language corrections. The changes are highlighted in Bright Green color in the revised manuscript.
Response: We kindly would like to inform the reviewer that the original manuscript Figure 6 includes experiments with a commercially available fibronectin inhibitor (RGDS), which is shown to inhibit the ERK phosphorylation that is induced by PSC-CM in the various PCCs used in this study. Following the reviewer’s suggestion, we have performed an experiment in which PCCs seeded on 96-well plates were incubated with SFM or PSC-CM for 24 hours and/or RGDS (20 µM) or PD98059 (a MEK/ERK inhibitor, 20 µM) for 4 hours prior to incubation with gemcitabine (10 µM) for 48 hours. Cell viability was determined using the MTT assay. Recent work by our group (Aasrum M., 2018, Journal of Cell Communication and Signaling) has shown that the MEK/ERK inhibitor PD98059 successfully inhibits ERK phosphorylation in AsPC-1 and PANC-1 cells. The results show that PD98059 blocks the chemoresistance to gemcitabine that is induced by PSC-CM. This concurs with the observation that RGDS-induced reduction of ERK phosphorylation similarly helps to overcome PSC-CM-induced chemoresistance. The results of this experiment are shown in the figure Additional File 8 Figure S5 of the revised manuscript and the text is updated accordingly in Methods section, line 104, page 4, Results section, lines 260-262, page 10 and Discussion section, lines 316-317, page 12.

Concluding remarks:

We believe that we have replied to all of the reviewers’ comments and have altered the manuscript accordingly.

As stated previously, we have no competing interests to declare.

I hope that our revised manuscript is now acceptable for publication in BMC Cancer and look forward to your reply.

Sincerely yours,

Manoj Amrutkar PhD,
Department of Hepato-Pancreato-Biliary Surgery,
Institute of Clinical Medicine, University of Oslo, Norway
Email: manoj.amrutkar@medisin.uio.no