Author’s response to reviews

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

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Linda Gummlich
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Re-submission – Manuscript # BCAN-D-18-03347 R1
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
To comply with the requests of the reviewer, a new Figure 7 and Table 1 have been added to the revised manuscript to facilitate inclusion of the requested alterations. Figure 1 has been altered accordingly. All text related changes in the revised manuscript are as indicated below.
Answers to the reviewers’ comments – Reviewer #1

Comment 1. In my opinion, the last paragraph of Introduction reveals a lot about the results which is not needed. Introduction needs to just lay the foundation for the study and perhaps provide a logic to the necessity and importance of study. I suggest that the authors do not describe major results rights here (i.e. modify the last sentence to tone down on results)
Response: We have now modified the last paragraph of the Introduction as suggested by the reviewer. In the revised manuscript, Introduction section, Lines 84-86, Page 4 following text has been added: “….for its secretome content….. pancreatic cancer chemoresistance.”

Comment 2. Why do I see 'tumor' and several other words/phrases highlighted in green in the manuscript?
Response: These highlights were made in response to the changes requested by one of the reviewers. These have now been removed from the revised manuscript.

Comment 3. Why did the authors evaluate the effect of a single dose of gemcitabine against PSC cultures in Fig 1A? Why was this dose chosen? Is it possible to use increasing doses of gemcitabine and report IC-50 values for PSCs, similar to IC-50s for cell cultures in Fig 1C to get a general idea of the cytotoxicity of gemcitabine against PSCs.
Response: We believe that the reviewer may possibly have misunderstood Figure 1 in the original manuscript. In Figure 1A, PSC cultures were evaluated for cytotoxicity using a varying concentration range (0.01 – 100 µM) of gemcitabine, Figure 1B shows a similar dose-response curve for cancer cells. Thus, no single dose was used in either of the experiments. No significant change in the cell viability of PSCs was detected across all the gemcitabine doses that were tested, hence calculations of IC50 on PSCs were meaningless. In the context of the present study, further work on determining IC50 for gemcitabine in PSCs is considered therapeutically irrelevant, since the highest dosage used for PSCs (100 µM) is well above the dose that resulted in maximum effects on the cancer cells.

Comment 4. I suggest that Fig 1C be presented as a stand-alone Table.
Response: We have now created a separate Table as suggested by the reviewer. The results can be seen in Table 1 in the revised manuscript.

Comment 5. Are FN effects and MEK/ERK pathways mutually exclusive? Their inter-dependence / hierarchy has not been evaluated?
Response: A previous study by Sawai et al (2008) showed that the basement membrane proteins fibronectin and collagen IV induced phosphorylation of ERK1/2 and thereby invasion and metastasis of pancreatic cancer cells BxPC-3, Panc-1 and SW-1990. In the same study, the use of RGDS peptide (FN inhibitor), Integrin antibody and PD98059 (MEK/ERK inhibitor) resulted in inhibition of ERK1/2 phosphorylation through the blocking of integrin signal-mediated effects, thereby inhibiting invasion of pancreatic cancer cells. In the present study, our data show that the PSC-CM induced ERK1/2 phosphorylation was inhibited both by RGDS (via blocking of integrin 1-signal) and PD98059 (intracellular ERK inhibition in the cancer cells), which in turn counteracted gemcitabine chemoresistance induced by fibronectin. This confirmed that inhibition of ERK1/2 phosphorylation led to the blocking of cell proliferation and survival pathways. Also, we found no effect of fibronectin on the AKT pathway. Whether there are other ERK- and AKT-independent effects of fibronectin, is considered beyond the scope of this study. In the revised manuscript, we have expanded the discussion regarding the involvement of the ERK pathway. In the revised manuscript Discussion section, Lines 317-322, Page 13 following text has been added: “A previous study by Sawai et al….. pancreatic cancer cell proliferation and survival.”
Comment 6. Looks like FN inhibition has profound effects on ERK signaling but not to the same extent on gemcitabine sensitivity?
Response: We agree with the reviewer that the proportional effects on ERK signaling and gemcitabine sensitivity are not similar, i.e. the effect of PSC-CM on the development of chemoresistance was significantly higher compared to the effect of FN alone. This suggests that secretome components other than FN may also contribute to chemoresistance to gemcitabine. In addition, it is conceivable that the fibronectin inhibitor RGDS may not be sufficient to block down-stream integrin-mediated effects on ERK signaling affecting gemcitabine sensitivity. In the revised manuscript Discussion section, Lines 311-313, Page 12 the following text has been added: “Notably, FN displayed…… pathways also might be involved.”

Comment 7. Considering the effects in BxPC3 cells, is there a possibility of cell line-specific effects?
Response: We agree with the reviewer and have therefore amended the text accordingly in Discussion section, Lines 282-284, Page 11. “…indicating possible cell-line…… with wild type KRAS (44).”

Comment 8. Finally, I highly recommend providing a cartoon figure summarizing the explored mechanism.
Response: We have now created a cartoon figure summarizing the findings of the study as shown in Figure 7 in the revised manuscript.

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,

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