Author's response to reviews

Title: SMAD4 Loss Triggers the Phenotypic Changes of Pancreatic Ductal Adenocarcinoma Cells

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Author's response to reviews: see over
Dear Editor,

Thank you very much for providing a comprehensive review of our manuscript. We are pleased to resubmit an extensively revised manuscript with additional supplementary data in relation to the existing data in the manuscript. Listed below, are the modifications that have been included in the revised manuscript to carefully address the reviewer’s comments (in chronological order)

Thank you for the consideration of the revised manuscript for publication. We are looking forward to hearing from you.

Best regards
Kuang

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Reviewer 1 major comments:
After the corrections the revised manuscript improved a lot. However, there are still a few minor changes necessary prior acceptance and publication.

Reply: Thank you very much for your carefully reading of the manuscript. We very much appreciate your valuable suggestions and kind advice.

- Figure 2 (D): Please show as a (supplementary) figure pictures of the invaded cells.

Reply: A supplementary figure has been added to present the images of invaded cells in our final manuscript.
- Figure 3 & 4: One beta-actin is not enough for all these different blots. At least another beta-actin blot should be added for each figure.

Reply: We have added another b-actin blot in Figure 3 & 4 of our revised manuscript.

- Figure 6: The authors did a calculation of the migration index and showed the results in the rebuttal letter, but did not integrate the new diagrams in the final manuscript.

Reply: Following your advice, the migration index has been presented in the supplementary information of our final manuscript.

For Reviewer 2

Answer:

Thank you very much for your critical comments. In our previous paper, through genetic engineered mouse models of PDAC, we demonstrated that SMAD4 deficiency altered PDAC tumor phenotype of KRAS\textsuperscript{G12D} INK4A/ARF heterozygous mice and; while tumors with intact SMAD4 frequently exhibited epithelial-to-mesenchymal transition (EMT), PDAC null for SMAD4 retained a differentiated histopathology with increased expression of epithelial markers such as E-cadherin and EGFR\textsuperscript{(1)}. SMAD4 deficiency influenced the differentiation state of Kras\textsuperscript{G12D} Ink4a/Arf mutant PDACs, producing tumors that retained epithelial differentiation in contrast to the frequent EMT observed in pancreatic cancers with intact Smad4. Our genetic mice demonstrated that Smad4 deficiency promoted progression to PDAC but produced tumors that retained differentiated ductal histopathology is consistent with a bimodal role of TGFb–Smad4 signaling in regulating the biology of PDAC.

In the present study, using human PDAC cell lines, in vitro wound healing, transwell migration assay and mouse xenograft models were assessed to confirm and determine the functional roles of SMAD4 in human PDAC.

Reference: