Peer Review File

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Reviewer A

The paper titled "Construction of a prognostic model with exosome biogenesis- and release-related genes and identification of RAB27B in immune infiltration of pancreatic cancer" is interesting. The predictive signature can predict overall survival, help elucidate the mechanism of exosome biogenesis and release, and provide immunotherapy guidance for PDAC patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The abstract is not adequate and needs further revisions. The research background does not indicate the clinical needs of this research focus.

Author reply: Thanks for your suggestion. We have modified our text as advised (see Page 1, line 19-23).

Changes in the text: Background: Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive and fatal disease. Exosomes are extracellular vesicles that plays a vital rule in the progression and metastasis of PDAC. However, the specific mechanism of exosome biogenesis and release in the tumorigenesis and development of pancreatic cancer remains elusive. Novel biomarkers and a reliable prognostic signature and urgently required to accurately stratify patients and optimize clinical decision-making.

2) What is the greatest advantage of the prognostic model in this study? What is the biggest problem we are facing? It is suggested to add relevant content to the discussion.

Author reply: Thanks for your suggestion. We have modified our text as advised (see Page19, line17-26).

Changes in the text: To the best of our knowledge, this study is the first attempt to developed and validated a risk prediction model associated with the process of exosome biogenesis and release in PDAC patients, which showed a great efficacy on survival prediction. We believe these could serve as a preliminary exploration and lay foundation for subsequent high-quality research. Furthermore, we identified RAB27B as the core gene in our risk signature and demonstrated its

inhibitory effect on the release of exosomes from PDAC cells. Also, we found RAB27B could inhibit PDAC cellular apoptosis while facilitate proliferation and migration by in vitro experiments. However, there are still several limitations in our study. First and foremost, this study mainly collected data from retrospective public databases, and thus further prospective research is necessary to validate our results. Also, an independent external validation cohort with greater sample size and long-term follow-up is needed in the future.

3) In this study, bioinformatics approaches were employed to develop the model. It is suggested to add further functional experiments to study its role in vivo and potential molecular mechanisms.
Author reply: We feel great thanks for your professional review work on our article. We understand that in vivo experiments and exploration of potential molecular mechanisms may better reveal RAB27B in exosome biogenesis and release process and immune infiltration of pancreatic cancer. However, in the present study, we mainly focused on the construction and validation of a exosome biogenesis- and release-related risk model, and we think that the PCR, WB, BCA, IHC and in vitro experiments may not be optimal, but should be sufficient to validate our results. The in vivo experiments are under investigation in our laboratory. Unfortunately, results are unavailable at this point. We will report on this in detail in our future study. Again,thank you for giving us the opportunity to strengthen our manuscript with your valuable comments and queries.

4) What key changes may RAB27B cause in immune infiltration and biological pathways of pancreatic cancer? Please discuss based on the author's own understanding and literature.
Author reply: We sincerely appreciate the valuable comments. Given that RAB27B is a key protein participating exosome biogenesis, it may recruit some regulators into the exosome, which could regulate some biological processes in the immune cells within tumor microenvironment. An recent paper showed that RAB27B could link NRAS protein in the inner side of the cell membrane by palmitoylation, which could stably stimulate c-RAF/MEK/ERK signaling (PMID: 37317963). Therefore, in pancreatic cancer cells, RAB27B might recruit some key proteins into exosomes by palmitoylation as well, which affect the immune infiltration and biological pathways of pancreatic cancer. We will explore the fine mechanism in our further study.

5) What might be the main active components in exosomes? What are the key regulatory genes? It is suggested to increase the research of related content, which may be more meaningful.

Author reply: We sincerely appreciate the valuable comments. We have modified our text as advised (see Page17, line23-33).

Changes in the text: Exosome biogenesis consists of cargo sorting, MVB formation, MVB transportation, and MVB-plasma membrane fusion, which are regulated by some key molecules. RNA binding proteins, such as hnRNPA2B1, hnRNPK, major vault protein (MVP), and FMR1 are considered to participate in the exosome sorting process in different cancer models(46-48). MVB formation is the center to exosome biogenesis, and ESCRT-dependent/independent pathways mediated membrane budding and intraluminal vesicles generation are key components in this process(49, 50). In the regulation of exosome transportation, many Rabs are proposed to participate in this process, including Rab2b, Rab5a, Rab9a, Rab27a and Rab27b(15). Also, Hsu et al. proposed that docking of multivesicular bodies to the plasma membrane is mediated by the Rab35 protein, which promotes exosome secretion(51). Besides, Rab7 is found to be a critical gene in determining multivesicular body degradation(52, 53).

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Exosomal miR-125b-5p derived from cancer-associated fibroblasts promotes the growth, migration, and invasion of pancreatic cancer cells by decreasing adenomatous polyposis coli (APC) expression, PMID: 37201069”. It is recommended to quote the article.

Author reply: We sincerely appreciate the valuable comments. We have modified our text as advised (see Page17, line15-16).

Changes in the text: Recently, many studies have revealed the crucial role of exosomes in pancreatic cancer development, metastasis and drug resistance(40-45).

7) What are the relevant characteristics of the tumor microenvironment of pancreatic cancer? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Author reply: We think this is an excellent suggestion.We have explain “the relevant characteristics of the tumor microenvironment of pancreatic cancer”, where the change can be

found in the revised manuscript (see Page18, line25-32). As for “the possible goals of future drug development”, we believe this is a profound question worth exploring, but it has been covered in detail in several papers published this year (PMID: 38167055, 38193476, 38672552), so we will not repeat it in our paper.

Changes in the text: The PDAC tumour microenvironment (TME) contains tumour cells, stromal cells, immune cells, extracellular matrix and a variety of soluble molecules. Among them, the extracellular components play an essential role in the process of tumour development, invasion and metastasis. In PDAC TME, tumour cells only account for a small part, and most of the rest consists of stromal components, which contain a variety of immunosuppressive cell infiltration, such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and cancer-associated fribroblasts (CAFs), with immunosuppression as a prominent feature(66, 67). In contrast, anti-tumor immune cells such as CD8+ T cells and NK cells are infiltrated to a lesser extent.

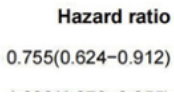
Thank you for giving us the opportunity to strengthen our manuscript with your valuable comments and queries.We have worked hard to incorporate your feedback and hope that these revisions persuade you to accept our submission.

Reviewer B

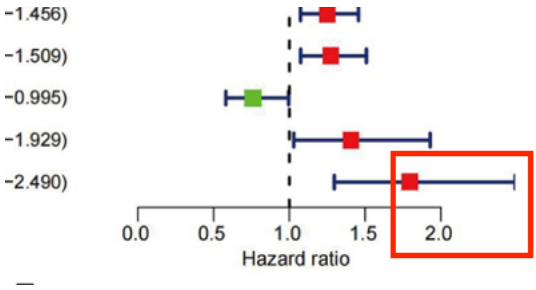
1. Figures

- Figure 1B: Please revise Hazard ratio to **Hazard ratio (95%CI)**.

Author response: Revision completed.



- Figure 1B: Please **extend the axis**.

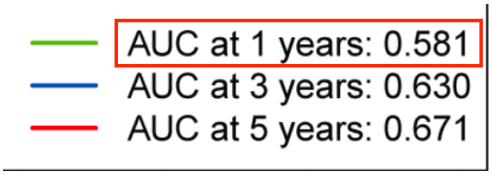


Author response: Revision completed.

- Figure 3A-F: there is a typo.

Author response: Revision completed.

- Figure 3J-3L: Please revise 1 years to **1 year**.



Author response: Revision completed.

- All abbreviations in figures and the legends should be explained. HR, LASSO in Figure 1 for example. Please check all abbreviations and provide the full names in the corresponding legends.

Author response: Revision completed.

- Figure 4C, Figure 8: Please indicate the meaning of *, ** and *** in the legends.

Author response: Revision completed.

- Figure 4D, 8G, 9A, 9B: Please indicate the staining methods and the magnification.

Author response: Revision completed.

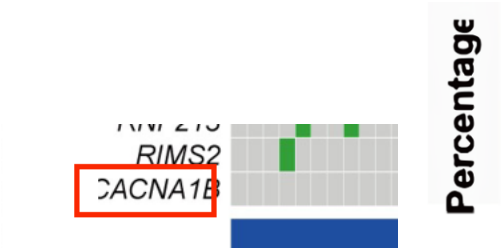
- Figure 4E: Please indicate the meaning of ** and *** in the legends.

Author response: Revision completed.

- Figure 4F: Please indicate the meaning of *** in the legend.

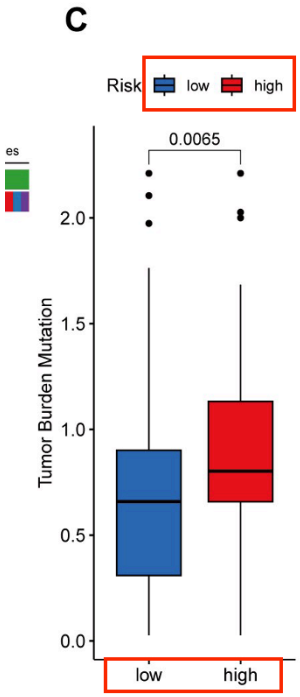
Author response: Revision completed.

- Figure 5A, 8B: The following word can't be seen fully. Please check through and revise.



Author response: Revision completed.

- Figure 5C: The following symbols are repeated. Please check and revise.



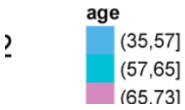
Author response: Revision completed.

- Figure 6C: Please revise all p-value to **P value**.

	p-value
95774	0.027

Author response: Revision completed.

- Figure 6A: Please add a unit to the age.



Author response: Revision completed.

- Figure 6B: Please indicate the meaning of *** in the legends.

Author response: Revision completed.

- Figure 7B, 8G, 9A-B: Please indicate the **staining/observation method and magnification** in the legends.

Author response: Revision completed.

- Figure 7B-C legends don't match with your Figure 7B-C.

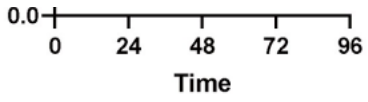
Author response: Revision completed.

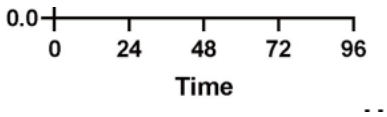
Figure 7: RAB27B Knockdown or Overexpression Regulates Exosome Secretion. (A) The expression of Alix, CD9, TSG101 and Calnexin in cell and exosome explored by western blotting. (B) Nanoparticle tracking analysis result of our exosome sample. (C) Image of transmission electron microscopy of our exosome sample. (D) Protein level of RAB27B in 6 pancreatic cancer cell lines. (E) Efficiency of

- Figure 7G: Please indicate the meaning of * and ** in the legends.

Author response: Revision completed.

- Figure 8E-8F: Please add a unit to the Time.





Author response: Revision completed.

- Please remove the legends from the separate supplementary figures and list them after the reference list in the manuscript.

Author response: Revision completed.

- Figure S3: Please indicate the meaning of the red arrows and the **, ***.

Author response: Revision completed.