Peer Review File
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Reviewer A

The paper titled “Contribution of sphingomyelin phosphodiesterase acid-like 3B to the proliferation, migration, and invasion of ovarian cancer cells” is interesting. The study propose SMPDL3B as a prognostic marker for ovarian cancer, with implications for potential therapeutic intervention targeting the ACER2-SMPDL3B axis. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The abstract is not sufficient and needs further modification. The research background did not indicate the clinical needs of the research focus.

Reply: Thank you for the review comments. The background and clinical significance of the study have been added to the abstract background.

Changes in the text: line28-32:Background: Cancer has the highest mortality rate among gynecological cancers and poses a serious threat to women's lives. However, the treatment options for Ovarian cancer are still limited, and exploring effective targeted biomarkers is particularly important for predicting and treating Ovarian cancer. Therefore, it is necessary to explore the molecular mechanisms of the occurrence and development of ovarian cancer.

2) There are many detection methods for cell proliferation, migration, and invasion. If multiple methods are used, the results may be more reliable. It is suggested to add test results of other methods.

Reply: Thank you for the review comments. There are two reasons for not adding experiments in this study: 1. The experiments on cell proliferation, migration, and invasion in this study have already proven the conclusions, so there is no need to use other validation methods. However, our laboratory will use multiple research methods for multi-dimensional argumentation in subsequent research. 2. Due to the tight schedule, it cannot be added in a timely manner.

Changes in the text: None.

3) What is the correlation between the expression of SMPDL3B and the prognosis of ovarian cancer patients? How to gain in-depth understanding through bioinformatics? It is recommended to add relevant content.

Reply: Thank you for your suggestion. The first and second parts of the results show the relationship between the expression of SMPDL3B and the prognosis of ovarian cancer patients through the public database TCGA. Firstly, we obtained four candidate genes by differential analysis, and then we identified SMPDL3B as a candidate gene for this study by comparing the

degree of prognostic imaging of these genes in patients (forest plot). Meanwhile, we also presented the prognosis of SMPDL3B high and low expression groups by KM survival curve, and found that the high expression group had poor prognosis ($p<0.05$).

Changes in the text:None.

4) There are many genes that regulate the ovarian cancer. Why did the author choose SMPDL3B for research? Please describe the reason.

Reply: Thank you for your suggestion. The first part of the article describes why we chose SMPDL3B for our study. First we screened secreted proteins that may affect ovarian cancer patients by differential analysis. Among them, there were four candidate genes worthy of our attention. Then we compared the relationship between the expression of the four genes and the prognosis of patients through the public database TCGA and drew a forest plot, and found that the effect of SMPDL3B was more significant, so this study focused on SMPDL3B for further analysis and research.

Changes in the text:None.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Inactivation of Wnt-LRP5 signaling suppresses the proliferation and migration of ovarian cancer cells, PMID: 35116545”. It is recommended to quote the articles.

Reply: Thank you for the review comments. This study only explores the regulatory mechanism of SMPDL3B on Ovarian cancer. Therefore, the introduction mainly focuses on SMPDL3B for citation. The teacher pointed out that the inactivation of Wnt LRP5 signal inhibits the proliferation and migration of ovarian cancer cells. Although PMID: 35116545 is a preface related to ovarian cancer, it is not related to the target gene of this study, and therefore it is not necessary to cite it. Please understand, reviewer.

Changes in the text:None.

6) What are the relevant characteristics of the tumor microenvironment of ovarian cancer? What is the correlation between SMPDL3B and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply: Thank you for the review comments. The aim of this study is to explore the mechanism of ACER2-SMPDL3B axis on the proliferation, migration, and invasion of ovarian cancer cells. The relevant characteristics of the tumor microenvironment in ovarian cancer, the correlation between SMPDL3B and the tumor microenvironment, and the potential goals of future drug development are not closely related to the content of this study. However, this part of the content has already been involved in later research in our laboratory, including the regulatory mechanism of SMPDL3B on the immune microenvironment in ovarian cancer. Thank you to the reviewer for their feedback.

Changes in the text: None.

Re-review comments

What is the correlation between the expression of SMPDL3B and the prognosis of ovarian cancer patients? It is recommended to add relevant content. There are many genes that regulate the ovarian cancer. Why did the author choose SMPDL3B for research?

Reply: Thank you for your suggestion. The aim of this study is to reveal that the expression of SMPDL3B can serve as a predictive indicator for ovarian cancer. The result of the first part of the article is a bioinformatics analysis of the relationship and survival analysis between SMPDL3B and ovarian cancer. To determine the functional significance of SMPDL3B in OV, GO annotation and KEGG pathway enrichment analysis were performed on the co expressed genes of SMPDL3B, as shown in Supplementary Figure 1.
Changes in the text: Line 261-263

What is the correlation between SMPDL3B and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

Reply: Thank you for your kindly suggestion. SMPDL3B indirectly alters the homeostasis of the tumor microenvironment by influencing the biological behavior of tumor cells. The relevance of SMPDL3B to the tumor microenvironment and future drug development have been supplemented in the discussion.
Changes in the text: Line 416-443

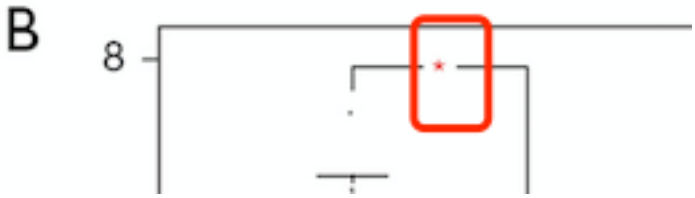
Reviewer B

1. Figure 1

a. Please revise to “Hazard ratio (95% CI)”.

Reply: Thanks, we have modified.

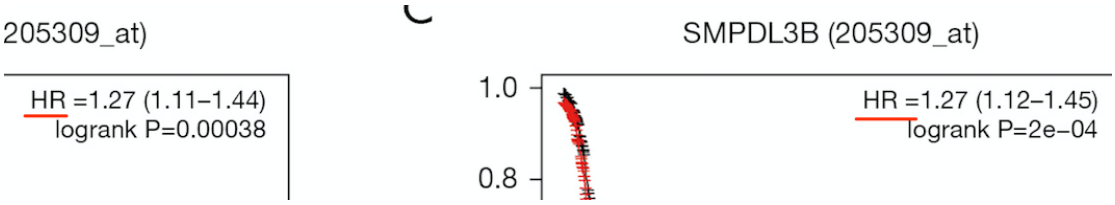
b. Please explain * in figure legend.



Reply: Thanks, we have modified. Changes in the text: Line 521

2. Figure 2

Please revise 'HR'to 'HR (95% CI)'.

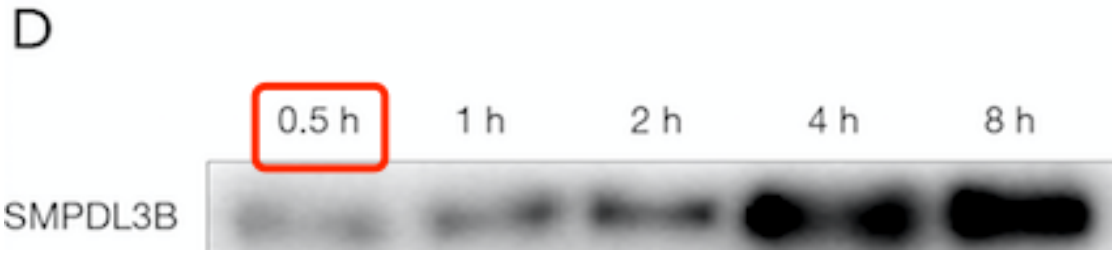


Reply: Thanks, we confirmed.

3. Figure 3

a. It seems that '0, 5' should be '0.5' in line 279. Please revise.

279 for 0, 5, 1, 2, 4, and 8 hours, after which the supernatant was collected to extract exosomes for the
280 assessment of *SMPDL3B* protein levels via Western blotting. Additionally, as the culture time of the
281 primary cells obtained from ovarian cancer tissues elapsed, the *SMPDL3B* protein levels increased
282 (**Figure 3D,E**; P<0.05). Taken together, these results suggest that the transcription and translation

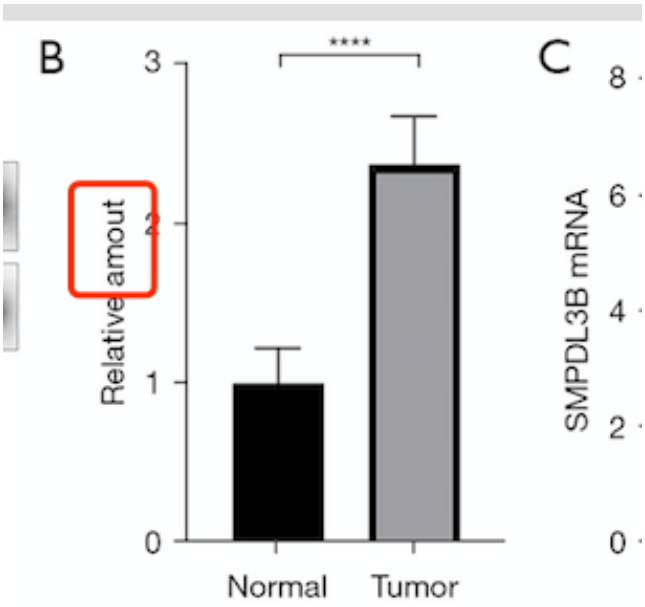


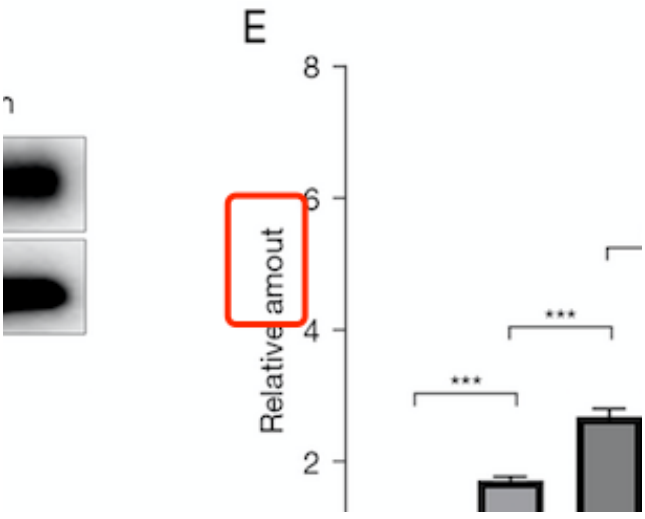
Reply: Modifications have been made. Changes in the text: Line 284

b. Please also explain **** in figure legend (C).

Reply: Modifications have been made. Changes in the text: Line 536

c. Please correct the typo to “amount”.



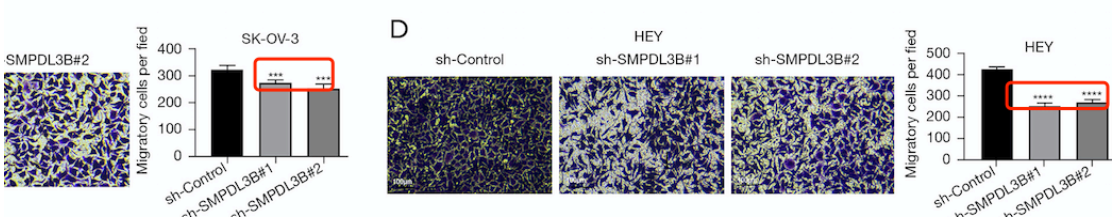


Reply: Thanks, we have modified.

4. Figure 5

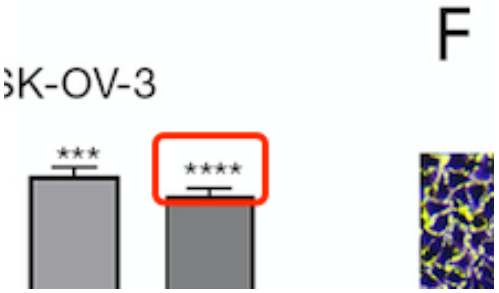
a. No ** in figure 5(C, D), but it was explained in figure legend. Please explain ***, **** in figure legend.

537 **Figure 5 SMPDL3B promoted ovarian cancer cell migration and invasion.** (A,B) Cell scratch assay
538 assessing the migration of SK-OV-3 and HEY cells following SMPDL3B knockdown; **, P<0.01.
539 (C,D) Migration assay further confirming the reduced migration of SK-OV-3 and HEY cells after
540 *SMPDL3B* knockdown; **, P < 0.01. (E,F) Invasion assay evaluating the decreased invasion of SK-



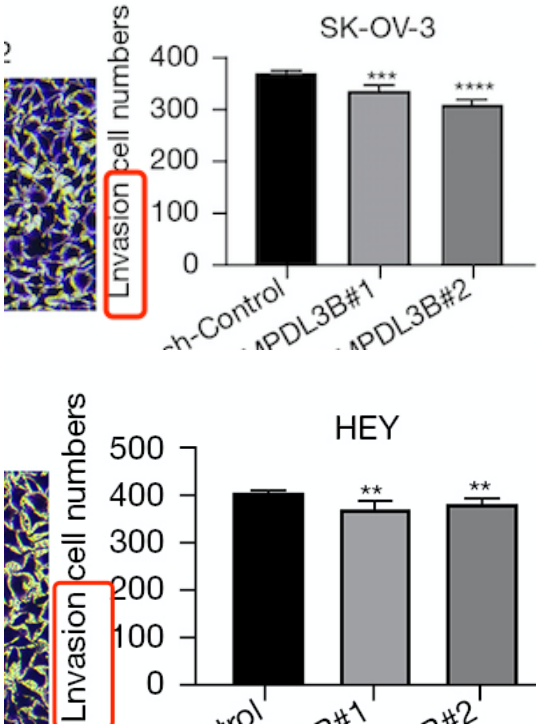
Reply: Modifications have been made. Changes in the text: Line 550

b. Please also explain **** in figure legend (E).



Reply: Modifications have been made. Changes in the text: Line 552

c. Please correct this typo to “Invasion”.



Reply: Thanks, we have modified.

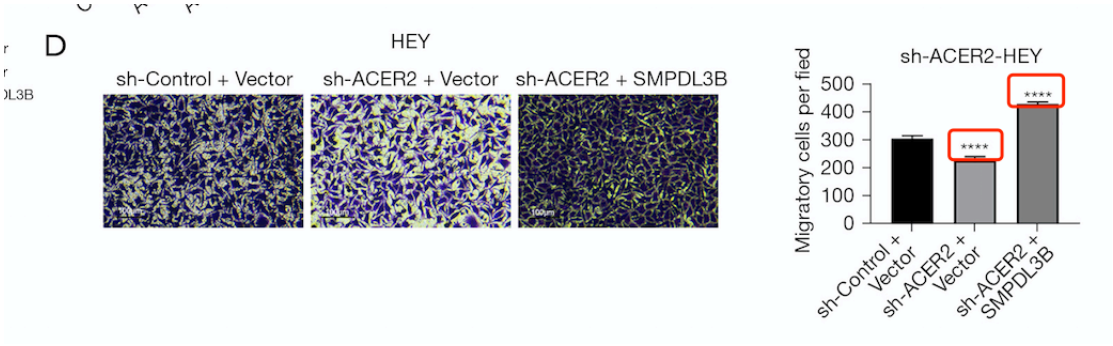
d. For cell map, please indicate the staining method in the figure legend. Please check figure 5C-F.

Reply: Modifications have been made. Changes in the text: Line 553

5. Figure 6

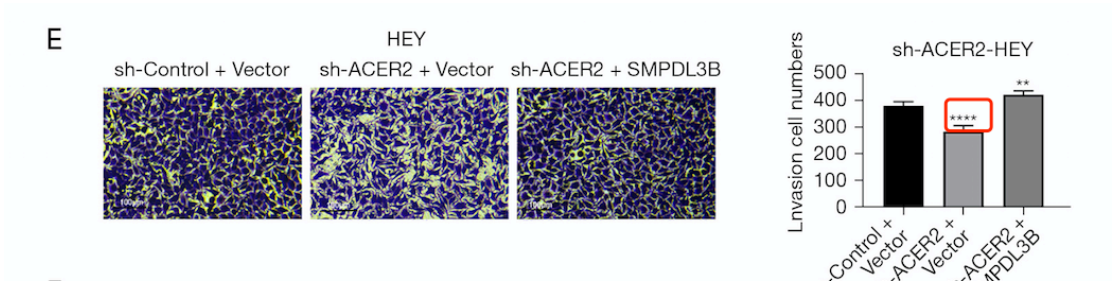
a. Please explain **** in figure legend 6D, and there are no ** and *** in figure 6D. Please revise.

547 assessing the effect of *ACER2* knockdown on the proliferation of HEY cells; *, P<0.5; n=3. (D)
548 Migration assay assessing the migration of HEY ovarian cancer cells after *ACER2* knockdown; P<0.01
549 (**) and P<0.001 (***) n=3. (E) Invasion experiment evaluating the invasion of HEY ovarian cancer



Reply: Modifications have been made. Changes in the text: Line 560

b. Please also explain **** in figure legend 6E.

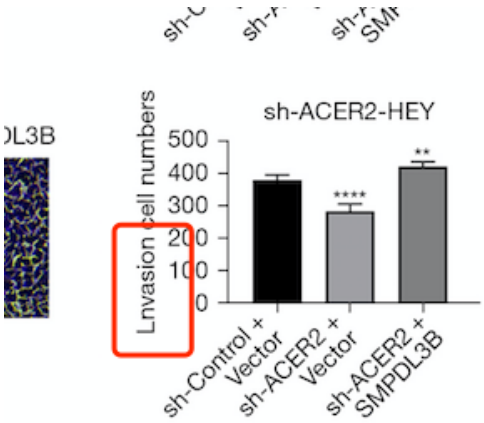


Reply: Modifications have been made. Changes in the text: Line 562

c. For cell map, please indicate the staining method in the figure legend. Please check figure 6D-E.

Reply: Modifications have been made. Changes in the text: Line 565.

d. Please correct this typo to “Invasion”.



Reply: Modifications have been made.

6. Reference

a. References (20,21) are duplicated. Please revise.

20. Gorelik A, Heinz LX, Illes K, et al. Crystal Structure of the Acid Sphingomyelinase-like Phosphodiesterase SMPDL3B Provides Insights into Determinants of Substrate Specificity. J Biol Chem 2016;291:24054-64.

21. Gorelik A, Heinz LX, Illes K, et al. Crystal Structure of the Acid Sphingomyelinase-like Phosphodiesterase SMPDL3B Provides Insights into Determinants of Substrate Specificity. J Biol Chem 2016;291:24054-64.

Reply: 21 references have been replaced. Changes in the text: line469.

b. The authors mentioned “studies...”, while only one reference was cited. Please revise.

Recent studies have clarified the role of ACER2 in the regulation of cellular ceramide hydrolysis. It has been observed that ACER2 positively regulates the expression of SMPDL3B, a known carcinogenic gene, at the mRNA level, and that SMPDL3B acts downstream of ACER2 (11).

Reply: Modifications have been made. Changes in the text: line 320.