Peer Review File
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Reviewer A

The paper titled "SLC1A3 knockdown in inhibiting the proliferation, apoptosis resistance, and migration of ovarian cancer cells" is interesting. High expression of SLC1A3 is linked to poor prognosis in ovarian cancer patients. SLC1A3 activity impedes apoptosis while enhancing the proliferation and migration of ovarian cancer cells, suggesting its potential as a therapeutic target for drug development. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There are many detection methods for cell proliferation, apoptosis and migration. Why this study only uses one method? If multiple methods are used, the results may be more reliable. It is suggested to add test results of other methods.

Reply 1: Thank you very much for your professional comments. In this study, we used CCK-8 assay, flow cytometry, and wound healing assay to assess cell proliferation, apoptosis, and migration abilities. These experimental methods are well-established, widely applied, and recognized in related studies, with stable conditions and high reproducibility. The data obtained from these experiments are clear and consistent, allowing us to draw meaningful conclusions based on the current findings. Due to limitations in experimental conditions and resources, we did not conduct additional experiments for further clarification. However, we plan to include more experimental studies in future research.

2) The description of some methods is too simplistic. Suggest providing a detailed description.

Reply 2: Thank you very much for your professional comments. We have revised this section based on your suggestions, and the changes are highlighted in blue.

Changes in the text: After the cells reached full confluence, a 200-μL pipette tip was used to create a linear wound by gently scraping the central part of the cell monolayer. The area was carefully cleared to create a consistent scratch. The cells were then washed with phosphate-buffered saline (PBS) to remove any debris or detached cells. Images of the scratch area were captured using an inverted microscope to document the initial wound width. The cells were subsequently cultured in serum-free medium for 24 hours to allow for migration. After 24 hours of incubation, the width of the wound was measured again using the inverted microscope to assess cell migration and closure. We hope that our revisions effectively address this issue.

3) There are many genes that regulate the ovarian cancer cells. Why did the author choose SLC1A3 for research? Please describe the reason.

Reply 3: Thank you very much for your professional comments. SLC1A3 encodes a glutamate transporter, which may influence the metabolic pathways of cancer cells. Since cancer cells often alter their metabolism to promote proliferation and survival, SLC1A3 may play an important role in the development and progression of cancer through its impact on glutamate metabolism. While existing studies have linked SLC1A3 to various characteristics of other types of tumors, such as

proliferation, migration, and invasion, its role in ovarian cancer has not yet been confirmed. Therefore, we chose this gene as the focus of our study.

4) There are still some weak points in this paper. It is suggested that the author increase the research of signaling pathway. This is more conducive to support the conclusions of this study.

Reply 4: Thank you very much for your professional comments. We recognize that studying signaling pathways is crucial for a comprehensive understanding of the role of SLC1A3 in ovarian cancer and could further support the conclusions of this study. However, due to current limitations in experimental conditions and resources, our focus in this study has been primarily on the direct effects of SLC1A3 on the biological behaviors of ovarian cancer cells. In future research, we plan to further explore key signaling pathways associated with SLC1A3 to more systematically reveal its mechanisms in ovarian cancer and enhance the depth and scientific rigor of the study.

5) It is recommended to increase the study of lncRNA or miRNA regulating SLC1A3, which may make the whole study more complete.

Reply 5: Thank you very much for your professional comments. We understand that investigating the lncRNAs or miRNAs that regulate SLC1A3 could reveal its upstream regulatory mechanisms, thus making the overall study more complete and in-depth. In this study, our primary aim was to explore the direct effects of SLC1A3 on the behaviors of ovarian cancer cells, which is why we did not focus on analyzing its upstream regulatory factors. However, we plan to conduct further research in the future, focusing on key lncRNAs and miRNAs that regulate SLC1A3, to enrich our understanding of its regulatory network in ovarian cancer and enhance the overall depth and clinical significance of the study.

6) Can SLC1A3 be used as a potential biomarker for patient risk stratification and local regional metastasis in ovarian cancer? It is recommended to add relevant content.

Reply 6: Thank you for the reviewer's suggestion. The potential of SLC1A3 as a biomarker for risk stratification and local regional metastasis in ovarian cancer patients is indeed of great significance. While this study primarily focuses on the role of SLC1A3 in ovarian cancer cell behavior, we also recognize that its expression level may be associated with clinical staging, prognosis, and metastasis in ovarian cancer patients.

Based on existing literature, we will expand the discussion section to include an analysis of the potential of SLC1A3 as a biomarker, highlighting its expression differences across various cancer types and its correlation with clinical pathological features. Future studies will further validate the role of SLC1A3 as a biomarker using large patient cohorts, to assess its application potential in patient risk stratification and prediction of local regional metastasis.

Changes in the text: This study reveals the role of SLC1A3 in the biological behavior of ovarian cancer cells, providing preliminary evidence for its potential as a biomarker in ovarian cancer. Previous research has shown that SLC1A3 is associated with invasiveness and metastasis in certain cancers, and its expression level may reflect the degree of cancer progression (22). Therefore, SLC1A3 holds potential as a stratification marker in ovarian cancer patients, helping to distinguish

high-risk from low-risk patients.

Furthermore, the expression of SLC1A3 may be closely related to local regional metastasis. In ovarian cancer, metastatic spread is a major factor contributing to poor prognosis, and whether SLC1A3 can serve as a molecular marker for predicting local regional metastasis still requires further validation. Future studies will require larger clinical cohorts, combined with patient tissue samples, to analyze SLC1A3 expression levels and further explore its correlation with clinical staging, tissue differentiation, metastasis, and prognosis, in order to assess its clinical application value.

In conclusion, these results support that SLC1A3 promotes proliferation and migration, while has an anti-apoptotic effect in ovarian cancer cells. Altogether, SLC1A3 may serve as a potential therapeutic target in ovarian cancer.

Reviewer B

1. **Figure 1**

Please revise “HR” to “HR (95% CI)”.

Response: we have revised it.

2. **Figure 2C**

For cell map, please indicate the staining method in the figure legend.

Response: we have revised it.

3. **Figure 3**

Please explain “***” in figure legend.

Response: we have revised it.

4. **Figure 4**

For cell map, please indicate the magnification (or scale bar) in figure legend.

Response: we have revised it.

5. There are only 21 studies in the reference list, but citation (22) was mentioned. Please check and revise.

Response: we have revised it.