**Peer Review File**

Article Information: https://dx.doi.org/10.21037/tcr-24-292

**Reviewer A**

The paper titled "Upregulation of miRNA-450b-5p targets ACTB to affect drug resistance and prognosis of ovarian cancer via the PI3K-Akt signaling pathway" is interesting. High expression of miRNA-450b-5p might affect drug resistance and prognosis in OC by targeting thirteen co-expressed genes of ACTB directly through the PI3K-Akt signaling pathway. Thus, miR-450b-5p might provide a new therapeutic target for drug resistance in OC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the correlations between OC drug resistance-related genes and tumor immune infiltration, tumor mutation burden, and microsatellite instability? How to evaluate? It is recommended to add relevant content.

Reply: We greatly appreciate the reviewer's professional and positive comments, rigorous reading, insightful and constructive suggestions. We are committed to addressing each of your comments diligently. In summary, the reviewer's professional opinion is a very innovative idea for us, and we will use it to guide further research. However, our current study has not yet addressed the direction of tumor immunity, so we have added some relevant content in the discussion section.

(a) Tumor immune infiltration: Based on the reviewer's suggestion, we have added relevant content to the Introduction section. (see Page 3, lines 48-49)

**Assessment methods:**

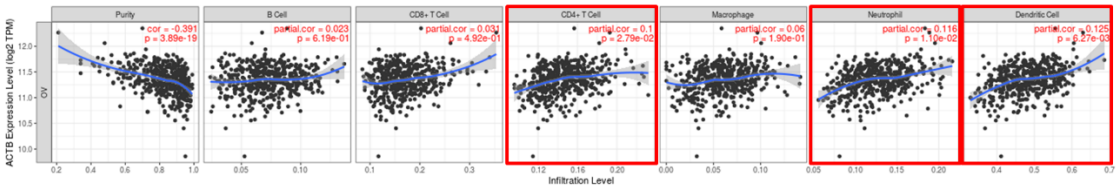
**Bioinformatics analysis:** Utilize large-scale transcriptomic data from public databases (e.g., Tumor IMmune Estimation Resource, The Cancer Genome Atlas) to analyze the expression levels of resistance-related genes (identified through literature) across different patient cohorts and immune cell subtypes.

**Immunohistochemistry:** The level and distribution of infiltration of T cells, macrophages, suppressor immune cells, etc. in tumor tissues were assessed using immunohistochemical methods.

**Flow cytometry:** Phenotyping of immune cells in individual tumor samples to understand the proportion of different types and subpopulations of immune cells in the tumor.

**Inspiration based on reviewers' suggestions:**

The constructive comments from reviewers are valuable for our research. Therefore, we attempted to analyze the relationship between ACTB (Beta-Actin) and immune infiltration using the TIMER (Tumor IMmune Estimation Resource) database. As shown in the figure as below, we observed a significant correlation between ACTB expression levels and the infiltration levels of $CD4_+$ T cell, Neutrophils, and Dendritic cells, which was a surprising discovery. The reviewers' suggestions are valuable guidance for our further research in the future.




(b) Tumor mutation burden: We have added relevant content to the Introduction section. (see Page 3, lines 49-51)

**Assessment methods:**

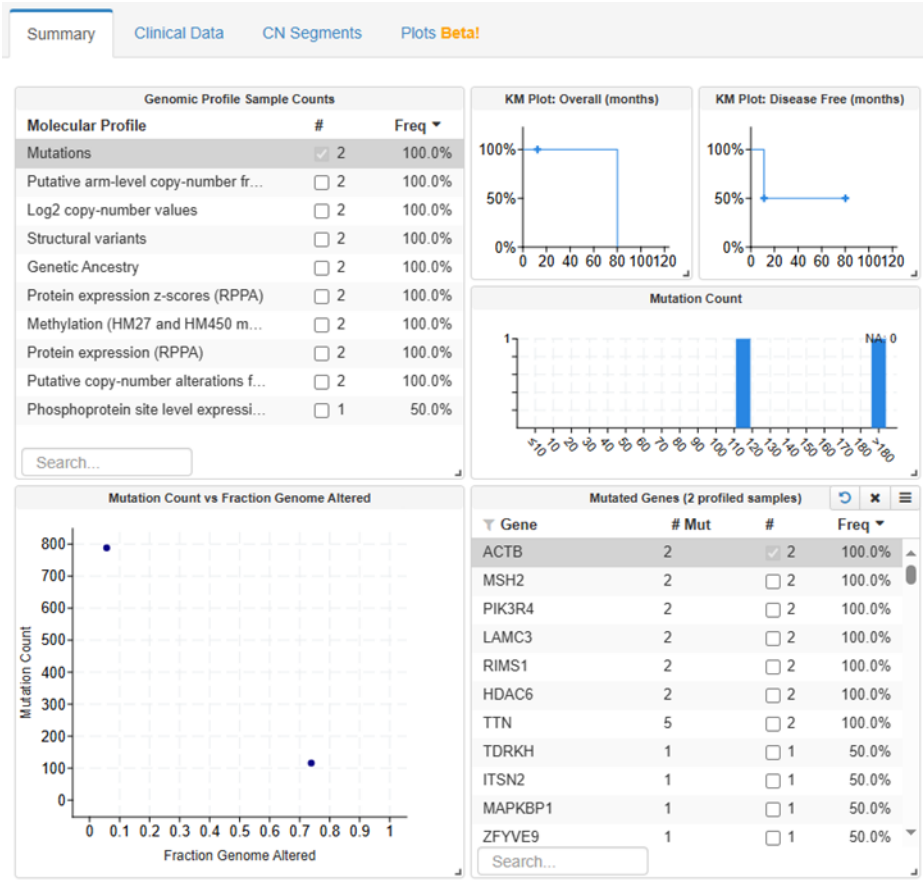
**Bioinformatics analysis**: Large-scale sequencing data from public databases, such as cBioPortal (cBioPortal for Cancer Genomics) and TCGA (The Cancer Genome Atlas Project) data, were used to analyze the relationship between TMB **(**tumor mutation burden**)** and drug resistance-related genes in ovarian cancer samples.

**Genome sequencing and analysis**: **Whole genome or exome sequencing of the patient's tumor samples was performed to assess TMB levels (i.e., number of mutations).**

**Clinical Data Correlation Analysis:** Analyze the clinical data, especially the relationship between treatment response and patient prognosis and TMB levels.

**Inspiration based on reviewers' suggestions:**

**The relationship between ACTB and tumor mutation burden was analyzed through the cBioPortal for Cancer Genomics database. As illustrated in the figure below, a smaller proportion of OC samples exhibited a correlation between ACTB and tumor mutation burden. This suggests that further investigation is required to elucidate the correlation between ACTB and tumor mutation burden.**




(c) Microsatellite instability (MSI): We have added relevant content to the Introduction section. (see Pages 3-4, lines 51-53)

**Assessment methods:**

**Bioinformatics analysis**: The cBioPortal for Cancer Genomics database allows for the analysis of mutation frequency, co-occurrence of related genes, and gene expression.

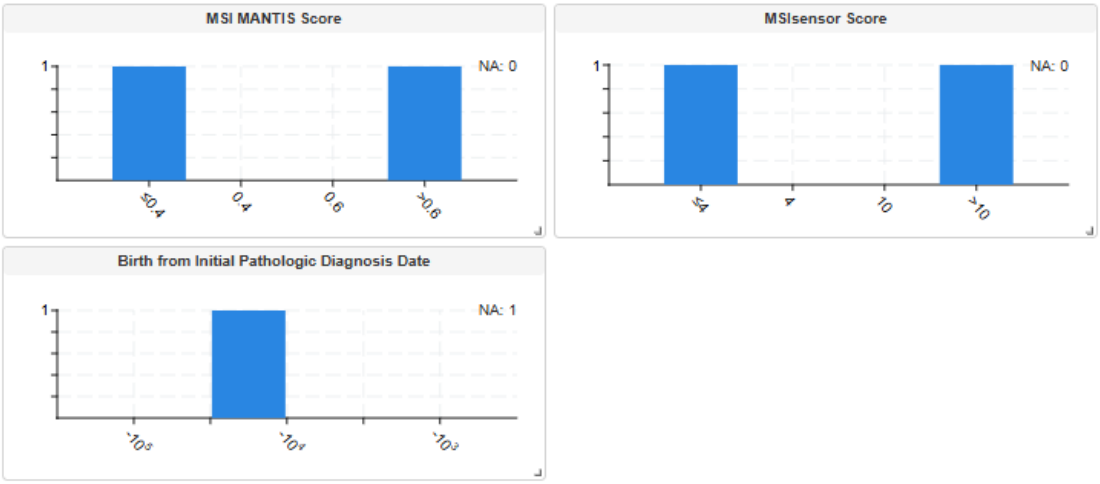
**MSI Detection:** Detection of MSI in tumor samples by PCR (Polymerase Chain Reaction) or other molecular methods.

**Genome sequencing analysis:** Perform whole genome or exome sequencing was used to evaluate the relationship between MSI and OC resistance-related genes (e.g., genes associated with DNA repair mechanisms, immune escape).

**Inspiration based on reviewers' suggestions:**

We analyzed the correlation between ACTB and MSI through the cBioPortal for Cancer Genomics database, and we observed a result similar to the one described above, i.e., only a

very small number of ovarian cancer samples suggested a correlation between the ACTB and MSI.




**2)** There have been many studies on OC. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

**Reply 2:** Thank you for your professional comments. Based on the reviewer's suggestions, we have incorporated relevant content into the Introduction section. (see Pages 4-5, lines 62-75).

**3)** It is recommended to add in vivo experiments to verify the results of this study, which may be beneficial to better application in clinical research.

**Reply 3:** We appreciate your suggestion regarding conducting in vivo experiments. While we acknowledge the importance of in vivo experiments for application in clinical research, conducting such experiments may require several months or more to conclude. Due to my impending graduation and the academic review by the university (which requires acceptance notification of manuscripts by July 8 for the issuance of the master's degree), completing animal experiments may be challenging based on experimental and time constraints. We hope to report these findings in a future report. Therefore, we seek for the reviewer's understanding, and we hope the revised manuscript could be acceptable for you. Many thanks for your kind suggestions!

**4)** What is the important clinical value of miR-450b-5p-medicated PI3K-Akt signaling pathway in OC? What is its potential as a therapeutic target for OC? It is recommended to add relevant content to the discussion.

**Reply 4:** We sincerely appreciate your valuable suggestion. Accordingly, the content you suggested has been added to the Discussion section. (see Pages 11-12, lines 221-229, 247-252).

**5)** There are many miRNA that regulate the drug resistance and prognosis of OC. Why did the author choose miRNA-450b-5p for research? Please describe the reason.

**Reply 5:** Thank you for your question. In a previous program, our laboratory conducted miRNA tissue microarray detection on drug-resistant and sensitive tissues of OC. It was found that miR-450b-5p was upregulated approximately 1.8-fold in drug-resistant OC tissues. Based on the results, the miR-450b-5p was selected for further study.

**6)** What are the relevant characteristics of the tumor microenvironment of OC? What is the correlation between miRNA-450b-5p and the tumor microenvironment? What are the possible goals of future drug development? It is recommended to add relevant content to the discussion.

**Reply 6:** Thank you very much for your constructive suggestions. As suggested by the reviewer, we have included additional discussions in the Discussion section. (see Pages 8-9, lines 194-195, 200-203, 249-252)

Currently, there is limited research on miRNA-450b-5p, especially in cancer. Thus, we have not found literature linking miRNA-450b-5p to the tumor microenvironment. The reviewer's professional suggestion has provided us with new insights. Although our current study does not focus on immunology, we intend to explore this direction in the future, which aligns more closely with the original intent of our research — to translate the results of basic research into clinical applications.

**Reviewer B**

1. ***Ref 12*** and ***Ref 42*** seem duplicates. Please check and revise.

Reply 6: Thank you very much for your careful correction. We sincerely apologize for our careless mistakes. We have updated reference 12 and carefully reviewed the entire manuscript. (see Page 6, lines 99-100)

2. **All abbreviations** in figures/tables and legends should be explained. Please check all your figures and tables.

Reply 8: Thank you for the reminder. We have reviewed all figures/tables and legends in the manuscript and made corresponding additions where necessary. (see Caption for Figures 1-4, Figures 6-7 and Table 1)

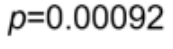
3. In the manuscript (including figures and tables), the description of the P value should be in the uppercase format, i.e., "P". Please check through and revise.

Reply 10: Thank you for your thorough review. We have checked the entire manuscript and all figures, making necessary revisions accordingly. (see legends for Figures 2-3 and Figure 5)

**4. Figures and Tables**

(1) If P value <0.001, report "P<0.001" to avoid reporting unnecessarily excessive precision (except hypothesis tests that include correlations or studies with exponentially small P values, such as genetic association studies, which can be reported exponentially, e.g., P=1×10-5).

In Figure 2A, please consider presenting the P value as P<0.001.



Reply (1): Thank you for your professional suggestion. We have changed “P=0.00092” to “P<0.001” in the legend of Figure 2A. (see Figure 2A)

(2) Do not round P values, do not report "not significant" simply because the data is greater than an arbitrary value, and do not report only vague bounds such as P<0.05, as described above, but report the exact P value. For example, report P=0.007 rather than P<0.01 for Figure 2B in your text.

Reply (2): We sincerely appreciate your guidance. We apologize for our oversight. Following the editor's suggestion, we have made the necessary modifications in the text. (see Page 10, line 195)

(3) There seems no mention of Figure 2C in your text.

Reply (3): Thank you for your comments. In the initial version of the manuscript we submitted, Figure 2B included information on both OS (overall survival) and PFS (progression-free survival). In this revised version, we have separated them into distinct labels, added Figure 2C, and referenced Figure 2C in the text accordingly. (see Figure 2; Page 10, line 195)

(4) It is suggested to supplement a proper space to “PValue” in Figure 5.

Reply (4): We sincerely appreciate your professional suggestion. We have modified the legend of Figure 5 as requested. (see Figure 5)