Peer Review File
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Reviewer A

The article is organized in a traditional format. A thorough investigation into the relationship between microRNA and the effectiveness of PD-1 therapy was conducted using bioinformatics techniques. However, it is crucial to carefully examine the results. Ultimately, it is important to consider the potential implications of these findings for clinical practice.

Reply: Thank you for your positive comments regarding the structure and depth of our investigation into the relationship between microRNA and the efficacy of PD-1 therapy. We appreciate your suggestion to carefully examine the results and consider the potential clinical implications. In the revised manuscript, we have added a more detailed analysis of the findings, emphasizing their relevance to clinical practice and highlighting how these microRNAs may contribute to personalized therapy in gastric cancer.

Changes in the text: We have modified our text as advised (see Page 12-13, line 405-410;418-422).

Reviewer B

Thank you for the opportunity to review your manuscript entitled " The efficacy of plasma exosomal miRNAs as predictive biomarkers for PD-1 blockade plus chemotherapy in gastric cancer" The results of this study are interesting and have the potential to contribute to future gastric cancer treatment. Below are my comments for your consideration:

Major Comments:

1. The authors should provide a clear definition of "advanced gastric cancer" (AGC) as used in this study. Specifying the cancer stage would enhance reader comprehension. For patients with Stage IV disease and distant metastases, details on the metastatic organs and patterns should be included.

Reply 1: Thank you for your valuable feedback. We have clarified the definition of "advanced gastric cancer" (AGC) used in our study and specify the cancer stages for improved clarity. In this study, AGC refers to both locally advanced (stage III) and metastatic (stage IV) cases. Among five patients in the primary cohort, three presented with locally advanced disease (involving abdominal lymph node metastasis and invasion into surrounding tissues), and two had distant metastases (one with thoracic metastasis and one with liver metastasis). In the validation cohort, four patients were locally advanced (with abdominal lymph node metastasis and invasion into surrounding tissues), and two had distant metastases (one with liver metastasis and one with lung metastasis). We hope these clarifications enhance the understanding of our patient selection and the stage definitions applied in our study.

Changes in the text: We have added this information in both Method and Result section of our revised manuscript (see Page 5, line 155-156; Page 8, line 245-249; Page 9, line 301-304).

2. Clarification is needed regarding the timing of the SOX plus camrelizumab regimen in the patients' treatment course. Was this administered as a first-line therapy for treatment-naïve patients, or following other interventions such as surgery, chemotherapy, or radiotherapy? Any concurrent treatments should be explicitly stated.

Reply 2: Thank you for your insightful comment. In our study, all patients received the SOX plus camrelizumab as a first-line therapy. Each patient was comprehensively evaluated following diagnosis and confirmed to have advanced gastric cancer, with no prior interventions administered before this regimen. No concurrent treatments were provided alongside this first-line therapy, ensuring a consistent treatment course for all participants. This approach allowed us to specifically evaluate the efficacy of the SOX plus camrelizumab combination as an initial intervention for treatment-naïve advanced gastric cancer patients.

Changes in the text: We have added this information in the Methods section of our revised manuscript (see Page 5, line 158-162).

3. The authors should delineate the specific indications (e.g., cancer stage, treatment stage, the sequence in the overall treatment strategy) for SOX plus camrelizumab in gastric cancer management within your country's clinical practice.

Reply 3: Thank you for your insightful suggestion. In our country's clinical practice, SOX plus camrelizumab is a standard first-line treatment option for patients with advanced gastric cancer. This regimen is typically selected immediately upon diagnosis of advanced gastric cancer, particularly for patients without other actionable targets, such as HER-2 or high PD-L1 expression. In cases where targetable mutations or biomarkers are absent, PD-1 inhibitors combined with chemotherapy are preferred as the first-line therapeutic approach. We have clarified these specific indications in the revised manuscript to enhance understanding of the treatment strategy within our clinical context.

Changes in the text: We have added this information in the Introduction section of our revised manuscript (see Page 4, line 105-112).

4. The authors state in the Methods section that "Plasma samples were prepared after the patients had undergone treatment to capture the post-treatment changes in exosomal miRNA expression that might be associated with treatment efficacy". Why did you use post-treatment samples to investigate exosomal miRNAs that might predict treatment response? The rationale for using post-treatment plasma samples to investigate exosomal miRNAs as potential predictors of treatment response requires explanation. Consideration should be given to the possibility that post-treatment exosomal miRNAs may have been altered by the therapeutic intervention.

Reply 4: Thank you for your valuable comment. The focus of this study is to identify exosomal miRNAs that can predict treatment response following therapy. The analysis of exosomal miRNA profiles after treatment initiation allows us to capture the dynamic changes induced by the therapeutic intervention. These changes may reflect the

biological processes underlying treatment efficacy and could potentially serve as valuable biomarkers. While it is acknowledged that post-treatment exosomal miRNAs may be influenced by the therapeutic intervention, our objective was to identify miRNAs that reflect the treatment-induced changes, which could potentially serve as predictive markers of treatment efficacy.

Changes in the text: We have modified this information in the Methods section of our revised manuscript (see Page 5, line 162-165).

5. The criteria used to define "responders" and "non-responders" should be supported by relevant references. The timepoint at which this classification was made during the treatment course should be specified. Incorporating established metrics such as Progression-Free Survival (PFS) and Duration of Response (DOR) may enhance the robustness of the response assessment.

Reply 5: Thank you for your valuable suggestion. In this study, patients were classified as "responders" or "non-responders" based on the Response Evaluation Criteria in Solid Tumors (RECIST 1.1) [Eisenhauer et al., New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009]. The classification was performed at the end of the second treatment cycle, which corresponds to a 21-day treatment period per cycle. According to RECIST 1.1, patients were considered "responders" if their treatment resulted in complete remission (CR) or partial remission (PR), and "non-responders" were those with disease progression (PD) or stable disease (SD). While we did not include PFS or DOR as metrics in this study, we believe that the use of RECIST 1.1 provides a reliable basis for assessing treatment response, as it is a widely accepted standard in clinical oncology.

Changes in the text: We have added this information in the Methods section of our revised manuscript (see Page 5, line 165-167).

Minor Comments:

1. Abbreviations used in the tables (e.g., MMR, MSI, MSS) should be provided for clarity.

Reply: Thank you for your suggestion. We have included the full definitions of the abbreviations used in the tables (see Page 17-18, line 535-536, line 538-539).

2. The scale bar for exosome images obtained by Transmission Electron Microscopy (TEM) in Figure 1 should be more clearly delineated.

Reply: Thank you for your helpful suggestion. We have revised Figure 1 to provide a more clearly delineated scale bar, ensuring accurate representation of the exosome size.

Reviewer C

This study is an exploratory investigation into biomarkers for predicting the efficacy of immunochemotherapy in gastric cancer. The authors conducted a comprehensive analysis of exosomal miRNAs in blood samples from five patients with advanced gastric cancer who underwent immunochemotherapy with camrelizumab, S-1, and oxaliplatin. Through differential expression analysis of miRNAs between responders

and non-responders, they identified miR-451a and miR-142-5p as promising predictive biomarkers. Although this study holds potential for advancing clinically useful cancer immunochemotherapy, its reliability is considerably limited because the validation cohort analyzed was as small as six patients. The authors also noted that these two miRNAs are involved in the regulation of proteins related to transcription factors and the cell cycle, based on searches in large databases. However, since many miRNAs are involved in intercellular signaling, these findings alone do not strongly support the potential of miR-451a and miR-142-5p as predictive biomarkers for immunochemotherapy response in gastric cancer. While the reviewer recognizes the exploratory value of this study, it remains incomplete as a scientific report. Resubmission with additional validation data from a larger cohort is recommended.

Reply: Thank you for your detailed and constructive feedback. We appreciate your recognition of the exploratory value of our study and the potential of miR-451a and miR-142-5p as predictive biomarkers in gastric cancer immunochemotherapy. We fully acknowledge the limitations posed by the small sample size in both the primary and validation cohorts, and we agree that a larger and more diverse cohort is needed to confirm the robustness of our findings. Due to current resource limitations, we are unable to supplement the data with a larger validation cohort at this time. However, we are committed to expanding this work as resources permit.

In the Discussion section of revised manuscript, we emphasize the hypothesis-generating nature of this study, clarifying that our findings are preliminary and require validation in future studies. Additionally, we agree with your observation regarding the functional roles of miR-451a and miR-142-5p in intercellular signaling and immune response modulation. While our bioinformatics analysis suggests potential involvement of these miRNAs in transcription and cell cycle regulation, we recognize that further *in vivo* and *in vitro* experiments are essential to comprehensively elucidate their mechanistic roles.

Thank you again for your valuable insights and recommendations, which will help us improve the clarity and focus of our study.

Changes in the text: We have modified our text as advised (See Page 12, line 395-405).

Reviewer D

1. Please indicate the table legends for tables S1-S5.

Reply: Thank you for pointing this out. We have now included detailed legends for Tables S1-S5 in the revised manuscript to improve clarity.

2. As for the special symbols "*, **, ***, ****, ns" in Table S4, please explain their meaning in the legend.

Reply: Thank you for pointing this out. We have added this explanation to the legend of Table S4.

3. As for the special symbols "*, **, ***, ****, ns" in Table S5, please explain their meaning in the legend.

Reply: Thank you for pointing this out. We have added this explanation to the legend

of Table S5.

4. Tables 1 & 2

Please check whether the groups are correct, since " > 5， < 10" is missing.

Reply: Thank you for pointing this out. We have checked and revised.

5. Fig 1C

1) HSP701(main text) or HSP70 (figure)? Which one is correct? Please check and revise.

2) TSG10(main text) or TSG101(figure)? Which one is correct? Please check and revise.

Reply: Thank you for pointing this out. We have checked and revised.