Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-23-192

Reviewer A

The paper titled "Identification and validation of the microRNAs and hub genes for pancreatic ductal adenocarcinoma by an integrated bioinformatic analysis" is interesting. The identified DEMs (has-miR-21-5hashsa-miR-135hasp, hsa-miR-222- 563p) and potential target genes (MYC, PTEN, PARP1, VHL, and FOXP3) may serve as promising prognostic markers and therapeutic targets for PDAC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There have been many studies on pancreatic ductal adenocarcinoma. What is the difference between this study and previous studies? What is the innovation? These need to be described in the introduction.

Reply 1: Our study is mainly to identify the key miRNAs and predict their possible target genes, and then verify the key miRNAs through experiments. There are several novel ideas in the paper:

a. Different and more data sets and overlapping candidate miRNAs in our study: the mentioned article (DOI: 10.1042/BSR20190625) includes four data sets, GSE41368, GSE43795, GSE55643, and GSE41369. However, only GSE41369 was used to screen differentially-expressed miRNAs, the other 3 datasets were used for screening differentially expressed genes, so the differentially-expressed miRNAs have not been cross-verified by other datasets. Our study included 2 miRNA microarray data sets GSE41372 and GSE32688; The overlapped miRNAs in both the data sets were identified as candidate differentially-expressed miRNAs.

b. More samples in our study: in the mentioned article (DOI: 10.1042/BSR20190625), a total of 9 PDAC samples and 9 normal samples were employed for miRNA-seq analysis; in our study, a total of 34 PDAC samples and16 normal samples were employed for miRNA-seq analysis.

c. Experimental verification in our study: We carried out experiments to preliminarily verify the function of the selected differentially-expressed miRNA in PDAC. The mentioned article (DOI: 10.1042/BSR20190625) have not verified the screened miRNAs.

Changes in the text: We have modified our text in the introduction as advised (see Page 4, line 95-107).

2) Figure 3 is not clear enough. It is recommended to provide clearer figure again.

Reply 2: A clearer Figure has been provided both in the manuscript and in the revised file of Figure 3.

Changes in the text: see Page 18, line 493.

3) The description of some methods in this study is too simplistic, please describe in detail.

Reply 3: We have modified the Methods section and provided more detailed methods.

Changes in the text: see Page 5, line 147-149, 150-152; Page 6, line 158-160, 179-181.

4) There are many detection methods for cell proliferation, migration and apoptosis. 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 4: Thank you for your advice. In the current study, we mainly aimed to identify the key

miRNAs and potential target genes involved in PDAC using bioinformatic analysis and verify the key miRNAs by simple experiments. Works of more experimental methods and regulation mechanisms will be carried out in our next work plan.

5) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Screening of potential pain genes in pancreatic ductal adenocarcinoma (PDAC) based on bioinformatics methods, J Gastrointest Oncol, PMID: 36915423". It is recommended to quote the article.
Reply 5: We have added related documents about diagnostic and/or therapeutic biomarker in the text and reference list. We carefully studied the document (Screening of potential pain genes in pancreatic ductal adenocarcinoma (PDAC) based on bioinformatics methods, J Gastrointest Oncol, PMID: 36915423) and found that that research mainly aimed to identify cancer pain genes in PDAC. Our research topic is mainly related to miRNA, and does not involve tumor pain. Therefore, we did not cite this document.
Changes in the text: We have supplemented some progress of PDAC diagnostic biomarkers in the Introduction section of the manuscript (see Page 4, line 95-108).

6) The bioinformatics analysis in this study is too simple. It is recommended to conduct WGCNA analysis on the data to determine the key modules, which may be more meaningful.
Reply 6: Thank you for the comment, we agree that WGCNA analysis is a powerful approach. In our current analysis, we mainly focused on tumor-specific miRNAs on PDAC progression, the miRNA microarray human data sets were acquired from the GEO database, while the potential target genes of the selected DEMs were predicted using miRNet. In this case, it is not suitable to conduct WCGNA analysis to determine the key modules. Undeniably, it may also be limited by our knowledge. Inspired by the suggestion, we want to try WCGNA analysis in our next work.

Reviewer B

First, the abstract needs some revisions. The background did not indicate the significance of this research focus, as well as its knowledge gaps in the literature. The methods need to describe the prognosis outcomes in the databases and how the prognostic roles of the identified biomarkers were analyzed. The results need to describe the findings on the prognostic roles of the identified biomarkers. In the conclusion, the authors need to have more detailed comments for the clinical implications of the findings, not to repeat the significance of this study again.
Reply 1: Thank you for your advice. We have modified the abstract according to the suggestions. In more detail, the sentence "In the progression of pancreatic ductal adenocarcinoma (PDAC), aberrant micro RNAs (miRNAs) expression plays a crucial role" was added to the Background of the Abstract section; the sentence "The prognostic value of the DEMs was accessed using the online server Kaplan-Meier plotter" was added to the Methods section; the sentence "High expression levels of hsa-miR-21-5p, hsa-miR-135b-5p, or hsa-miR-222-3p predicted poor overall survival in PDAC patients" was added to the Results section; the Conclusions section has been revised to "This study constructed the miRNA-hub gene network, which provides novel insights into the PDAC progression. Although further research is required, our results offer clues for new potential prognostic markers and therapeutic targets of PDAC."
Changes in the text: see Page 1, line 31-32; Page 2, line 38-39, line 48-49, line 58-60.

Second, in the introduction of the main text, it is wrong to describe "their prognosis remains very poor due to the lack of effective biomarkers for early diagnosis, recurrence, and prognosis", which should be the lack of effective treatments. The authors need to further explain on this. The authors need to explain why they focused on tumor-specific miRNAs and what the knowledge gaps and strengths of the research focus on tumor-specific miRNAs in comparison

to other known biomarkers. This is also important for this part.

Reply 2: Sorry for the confusion. We originally wanted to express that the lack of effective biomarkers leads to untimely treatment, which leads to a poor prognosis. To avoid confusion, the sentence has been revised to "due to the lack of effective biomarkers for early diagnosis, recurrence, and prognosis, sometimes timely medical treatment was not available, which leads to a poor prognosis". In addition, we have modified the introduction section according to the suggestions.

Changes in the text: see Page 4, line 88-89, line 94-108.

Third, in the methodology of the main text, the authors need to indicate the research design, details of the databases used, the clinical and prognosis outcome variables in the databases, how the prognostic roles of the miRNAs were statistically analyzed, and how to ascertain the independent prognostic roles of miRNAs. The statistical analysis should describe all the statistical methods used in this study and ensure P<0.05 is two-sided.

Reply 3: Thank you for your advice. We have added the detailed methods and statistical analysis in the methodology of the main text

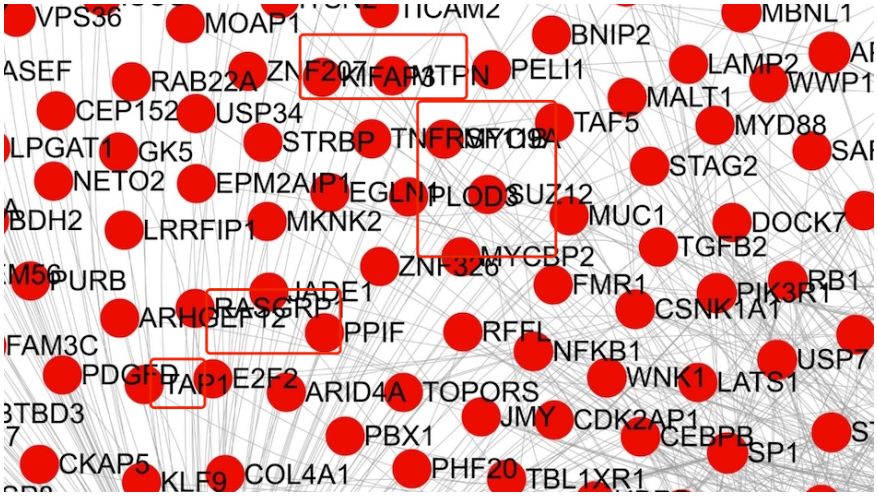
Changes in the text: see Page 5, line 147-149, 152-153; Page 6, line 158-160, 165, 179-181; Page 8, line 221-226.

Reviewer C

1. Please define ALL abbreviations shown in Tables in table footnote.

Reply: ALL abbreviations in Tables have been defined in table footnote.

2. Figure 3: Some words got covered, please revise and resend us updated figure.



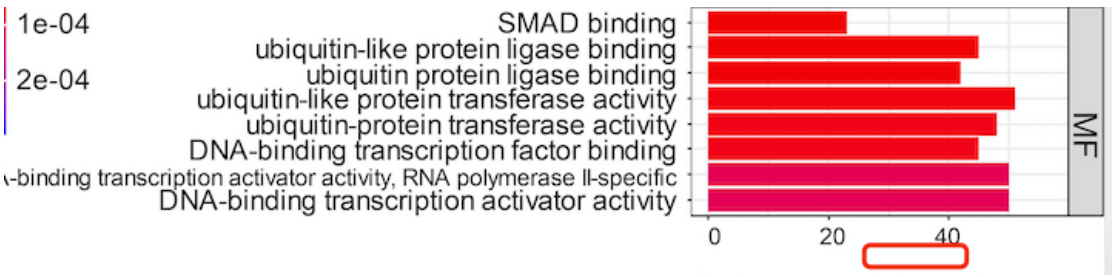
Response: Words of overlaps are modified by both automatic and manual methods.

3. Figure 4

a. Please define all these abbreviations in figure legends.

Figure 4 GO and KEGG analysis of the potential target genes for the DEMs. (A) Enriched BPs, CCs, and MFs of the potential target genes. (B) KEGG pathway enrichment analysis

b. Please supplement description for X-axis in Figure 4A.



Reply:

a. All the abbreviations in figure legends have been defined.

b. The description for X-axis in Figure 4A has been added.

4. Figure 5: Please define PPI in figure legends.

Reply: The abbreviation PPI in Figure 5 has been defined in figure legends.

5. Figure 7: Please define "****" in figure legends.

Reply: Figure 7 has been revised.