Peer Review File

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Reviewer A

The paper titled "Identification of the key miRNA-mRNA regulatory network in lung adenocarcinoma" is interesting. This study used transcriptome sequencing and a bioinformatics analysis to identify key genes and construct the miRNA-mRNA regulatory network in LUAD. The functional analysis showed that immune response, cell tumorigenesis, and tumor cell proliferation play central roles in the overall regulatory network. In addition, we speculated that miR-5698, miR224-5p, and miR4709-3p may be important biomarkers for the occurrence and development of LUAD and have great potential in the prognosis of LUAD patients and the development of new therapeutic targets. However, there are several minor issues that if addressed would significantly improve the manuscript.
Response: Thank you for your positive and constructive comments about our work. We have made corresponding revision.

1) How to analyze the immune infiltration pattern of LUAD based on the results of this study? What other bioinformatics analysis methods can be used? It is recommended to include relevant descriptions in the discussion.
Response: Thank you for your constructive suggestion about our work. To analyze the differences in the proportion of immune cells between samples, immune cell infiltration analysis of the datasets could be performed by the Timer and Cibersort methods respectively in future's work. We have added corresponding information in the discussion (see Page 12, line 382).

2)"Additionally, 129 differently expressed genes were found to be involved in immediate response, of which 107 were downregulated, such as Wnt3a, and 22 were regulated, such as Wuc5b. The analysis also identified the following 10 key regulatory motors: IL1 β, toll like receptor 4 (TLR4), PECAM1, CCL5, CXCL12, SELE, C-X-C motif chemokine receptor 2 (CXCR2), EGFR, CXCR1, and selectin P ligand (SELPLG) (Figure 8)". Should the result be Figure 9? The author is requested to carefully review the results and make corrections.
Response: Sorry for the mistake. We have carefully checked and revised (see Page 10, line 314).

3) Figure 8 is not clear enough. It is recommended to provide clearer figure again.
Response: Thank you for your reminding. We have provided new figure.

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

Response: Thank you for your constructive suggestion about our work. Most of previous studies focused on protein-coding genes or single signaling pathway in LUAD. In this study, we extended understandings of the molecular mechanism of LUAD by constructing a miRNA-mRNA regulatory network to analyze the key genes involved in the development of LUAD. We have made corresponding revision in the instruction (see Page 4, line 116).

5) Is there any research on lncRNA? How to construct a ceRNA network of LUAD related genes? What is the author's next plan?
Response: Thank you for your constructive suggestion about our work. Recently, some research focused on the lncRNA–miRNA–mRNA ceRNA network in LUAD. Jiang et al. characterized relevant functional roles of the lncRNA GSEC/miR-101-3p axis in the setting of LUAD. Bi et al. found GMDS-AS1 and LINC01128 function by targeting miR-6077 as competing endogenous RNAs regulating CDKN1A and KEAP1 expression, thereby stimulating cell-cycle arrest in G2/M phase or ferroptosis when the LUAD cells were treated with CDDP/PEM and facilitating chemoresistance. To further explore the upstream regulating mechanisms of miRNAs and construct a ceRNA network, we could screen candidate lncRNAs by integrating the results from miRNA pull-down and the online predictive tool LncBase. We will perform immune infiltration analysis and screen candidate lncRNA to construct a ceRNA network in future's work, biofunctional verification of the sequencing results both in vivo and in vitro to verify the findings are also warranted (see Page 12, line 382).

6) It is recommended to increase the weighted gene co-expression network analysis to determine the key modules related to LUAD.
Response: Thank you for your constructive suggestion about our work. We will perform the analysis in future's work.

7) This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.
Response: Thank you for your constructive suggestion about our work. We will perform biofunctional verification of the sequencing results both in vivo and in vitro to make our conclusion solid (see Page 12, line 384).

Reviewer B

The authors performed mRNA and miRNA sequencing and bioinformatics analysis to find key regulatory network in lung adenocarcinoma (LUAD). There some concerns regarding this manuscript:
1. The authors only collected 3 data samples. You need to increase the data samples to give meaningful results

Response: Thank you for your kind suggestion about our work. We agree with reviewer that more samples could make results much more confident. We will collect more samples in our future's work.

2. This research involved patients. I believe you should obtain the ethical approval from the ethics committee(s) or institutional review board(s). Then you need to state the approvals (name of committee, approval ID) in your paper
Response: Thank you for your kind reminding. The study was approved by the Institutional Ethics Committee of Binhai County People's Hospital of Yancheng (see Page 5, line 136).

3. "The patients' clinical information, such as age, gender, tumor node metastasis (TNM) classification, and stage, was basically consistent". What do the authors mean consistent? It is better to give a tablne that shows the mean of age, number of female/males, number of T1, T2, T3, T4, N0, N1, N2, N3, M0, and M1, and other summary descriptions of the patients.
Response: Thank you for your kind reminding. We have added the above information in new table 1 (see page 21, line 597). Cancer tissues and their adjacent tissues of these patients were collected for sequencing analysis.

4. Before DEG analysis, did the authors perform normalization to adjust for GC-content effect, distributional difference between lanes or other additional factors that interfere with intra-sample comparisons? No information about the normalization procedure in the methods action.
Response: There was no interference from other factors in the data analysis, and the sample clustering (as shown in PCA and Heatmap) met expectations without additional processing of the data.

5. There is also no information about filtering procedures to remove mRNAs and miRNAs with low signals across samples. For points no 3 and 4, the authors can check the references: "TCGA Workflow: Analyze cancer genomics and epigenomics data using Bioconductor packages" or "A survey of best practices for RNA-seq data analysis".
Response: Before conducting differential gene expression analysis, we removed mRNA/miRNA with counts mean values less than 2 in both the experimental and control groups, reducing the impact of detecting false positives.

6. Why use maximal clique centrality (MCC) algorithm to determine hub/top genes? Is there any reference that showed MCC can identify hub genes? Please include the reference and add it to the discussion.
Response: Chia-Hao Chin et al. implement the network scoring methods, MCC, MNC and DMNC, and eight other popular methods into a Cytoscape plugin, cytoHubba. Among the 11 methods, the newly proposed method MCC performs better than the others (BMC Syst Biol,2014). It is widely used to identified hub genes (Front Genet.

2022,13: 950136; Int J Med Sci. 2020; 17(14): 2063–2076). We have added corresponding information in the methods and discussion (see Page 7, line 231 and Page 11, line 344).

7. What methods did the authors use to perform gene functional annotation? Please state it in the methods section

Response: Ingenuity Pathway Analysis (IPA, QIAGEN Redwood City, USA) software was used to analyze the molecular function of the DEGs, the methods of Z-score calculation can be found in causal analysis approaches in IPA (Bioinformatics, 2014). We have added corresponding information in the methods (see Page 7, line 220).

8. The authors used raw p value to determine significant mRNA and miRNA. The authors should use adjusted P value using Bonferroni or FDR correction as significance threshold.

Response: Thank you for your kind suggestion about our work. we are sorry for the mistake. The significance threshold of miRNAs is q value<0.05 (see Page 8, line 238).

Reviewer C

The authors investigated the key genes and construct the miRNA-mRNA regulatory network in LUAD. Herein, the authors provided evidence that miR-5698, miR224-5p, and miR4709-3p may be important biomarkers of LUAD. However, the underlying mechanism of miRNAs for the occurrence and development of LUAD are not clear which need further investigation. Besides, I have some suggestions, which required further attention before the article should be considered suitable for publication.

1. The conclusion of abstract should be further summarized.

Response: Thank you for your kind suggestion about our work. We have added corresponding information in the abstract (see Page 3, line 57).

2. Line 238, page 7, 'the default filter criteria were a P value <0.05. Please check the filter criteria of miRNAs.

Response: Sorry for the mistake. The significance threshold of miRNAs is q value<0.05. We have added corresponding information in the methods (see Page 8, line 238).

3. The figure 9-11 are missing in the results of the manuscript. Please check it carefully.

Response: Sorry for the mistake. We have added corresponding information in the manuscript (see Page 10, line 314).

4. All the relevant information must be presented in the legend of figures, for example the meaning of different colors.

Response: Thank you for your kind suggestion about our work. We have added corresponding information in the figure legends (see Page 16-20, line 522-588).