Peer Review File

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Reviewer A

More validation is needed before the paper can be accepted. There are some suggestions:
1) There are more public databases available. Why GEO, ArrayExpress, SRA, and other sources were not considered?
Reply 1: Thank you so much for your comment. Because the TCGA database has more advantages compared to other databases: ① The database has a large sample size, with over 100 samples for each type of cancer; ② Abundant omics, transcriptome (RNA seq, gene chip, small RNA seq), genome (miRNA expression, mRNA expression, Copy Number, etc.) and epigenetic analysis were conducted for each sample; ③ High data quality; ④ Complete clinical information; ⑤ Free access. Therefore, we ultimately chose to conduct further data analysis based on the TCGA database.
Change in the text: None.

2) In-house validation is necessary. Please validate the current findings based on the clinical samples from the authors' institute.
Reply 2: Thank you so much for your suggestion. This study is mainly based on bioinformatics analysis methods, and explores the correlation between CDCA4 and LIHC from multiple databases such as TCGA, as well as the potential role and related mechanisms of this molecule in LIHC. This study did not involve clinical data related to our hospital. In future research, we will design relevant retrospective or prospective clinical studies to further clarify the clinical research value of CDCA4.
Change in the text: None.

3) "CDCA4 is a potential biomarker for the diagnosis of LIHC". The data were based on tissue samples. How much is it possible that we need to detect the CDCA4 levels to diagnose LIHC in the clinic?
Reply 3: Thank you so much for your comment. Currently, we mainly use bioinformatics analysis methods to determine the value of CDCA4 as a biomarker for LIHC. However, before applying it to clinical applications, further histological and cytological validation is required; Furthermore, further retrospective and prospective clinical trials are needed to determine the scientific and clinical value of this molecule. In future clinical applications, we can further predict the prognosis of patients by detecting the expression level of this molecule in tumor tissue, thereby better guiding the implementation of clinical interventions.
Change in the text: None.

4) The English writing needs to be seriously improved.
Reply 4: Thank you so much for your comment. Relevant language has been polished to make it easier to read and more professional.
Change in the text: full text.

Reviewer B

Primary liver cancer has become the second most lethal cancer in the world, and it has the second highest fatality rate among digestive system malignancies in China. In the manuscript "The LncRNA-LINC00638/has-miR-29b-3p axis-mediated high expression of CDCA4 is correlated with tumor immune infiltration and hepatocellular carcinoma progression", authors explored the role of cell division cycle-associated protein 4 (CDCA4) in hepatocellular carcinoma patients.
Couple questions are required to be answered before it will be accepted.
(1) In the whole text, it was my suggestion to change "liver hepatocellular carcinoma (LIHC)" into "hepatocellular carcinoma (HCC)", and "has-miR-29b-3p" into "hsa-miR-29b-3p".
Reply 1: Thank you so much for your suggestion. Although HCC is a common expression mode, this study is mainly based on the analysis of the TCGA database. Firstly, its expression in hepatocellular carcinoma is LIHC, and secondly, the results obtained from database analysis are all LIHC. Therefore, the abbreviation of LIHC was used throughout our research process. And we also changed "HCC" to "LIHC" in the introduction to maintain consistency.
About the second issue, we apologize for our clerical error. We have changed all "has miR-29b-3p" in the article to "hsa miR-29b-3p".

(2) It was advised to add related reference (DOI: 10.21037/jgo-21-110) about the cell division cycle-associated protein family.
Reply 2**:** Thank you so much for your suggestion. We have added relevant reference (DOI: 10.21037/jgo-21-110) to the article.
Change in the text: reference 9.

(3) It was showed that CDCA4 RNA expression was elevated in the LIHC tumor tissues and linked adverse clinical characteristics. How to obtain the conclusion that low expression of CDCA4 significantly improves the prognosis of HCC patients?
Reply 3**:** Thank you so much for your comment. Because in the TCGA database, we found upregulation of CDCA4 expression in non paired and paired LIHC tumor tissues. Moreover, LIHC patients with high expression of CDCA4 have poorer AFP, pathological grading, N staging, pathological staging, T staging, vascular infiltration, and poorer nutritional status. Based on these results, we can conclude that CDCA4 plays the role of a "oncogenic gene" in the biological events of LIHC. Therefore, based on data analysis in the database, we can conclude that reducing the expression of CDCA4 can improve the prognosis of patients. Of course, in future research, we will further add clinical research data and analyze the correlation between CDCA4 expression and the OS, DSS, and PFI of LIHC, thereby making the research conclusions more deterministic and credible.
Change in the text: None.

(4) It was a bioinformatics analysis in the study. And the LncRNA-LINC00638/has-miR-29b-3p/CDCA4 was the crucial topic. It was more convincing to validate the correlations between LncRNA-LINC00638/has-miR-29b-3p and CDCA4 by experiments. At least to test their expressions in HCC.
Reply 4**:** Thank you so much for your comment. The main purpose of this study is to quickly obtain a prognostic biomarker through bioinformatics analysis, proposing a potential target for the development of LIHC anti-cancer strategies. Secondly, we have derived the possible mechanisms of action, and these results provide good reference value for our future cytological research. However, due to the lack of experimental funding and conditions, we will improve these shortcomings in our future research.
Change in the text: None.

(5) The figure 9 was not clearly enough. And in the figure 10 legend, what was the meaning of “hsa-miR-29b-correlation between 3p expression and potential lncRNAs in LIHC”? please state clearly.

Reply 5**:** Thank you so much for your comment. We have replaced Figure 9 with a clearer image. We have changed the legend in Figure 10 to 'The correlation between hsa-miR-29b-3p expression and potential LncRNAs in LIHC'. .

Change in the text: Figure 9 and the legend of the Figure 10.

(6) How to identify the LncRNA-LINC00638? Please provide related data.

Reply 6**:** Thank you so much for your comment. This is our clerical error, and LINC00638 is the LncRNA we obtained. We have changed “LncRNA-LINC00638” to “LINC00638”.

Change in the text: the whole text.

(7) The CDCA4 is one of cell division cycle-associated protein. Why to think about the correlations between CDCA4 and immune infiltration? Please state in the discussion.

Reply 7**:** Thank you so much for your comment. The standard treatment methods for LIHC include surgical resection, chemotherapy, radiation therapy, radiofrequency ablation, vascular embolization, or liver transplantation. However, with the progress of medical research, immunotherapy for LIHC is currently in full swing. The tumor immune microenvironment, that is, a large number of immune cells are often gathered inside and around the tumor. These immune cells have complex interactions and regulation with tumor cells. Therefore, when we are looking for biomarkers available for tumors, we hope that this molecule can not only predict the prognosis of patients, but also be related to the tumor's immune microenvironment, so as to provide a reference therapeutic target for future tumor immunotherapy. We have added statement in the discussion.

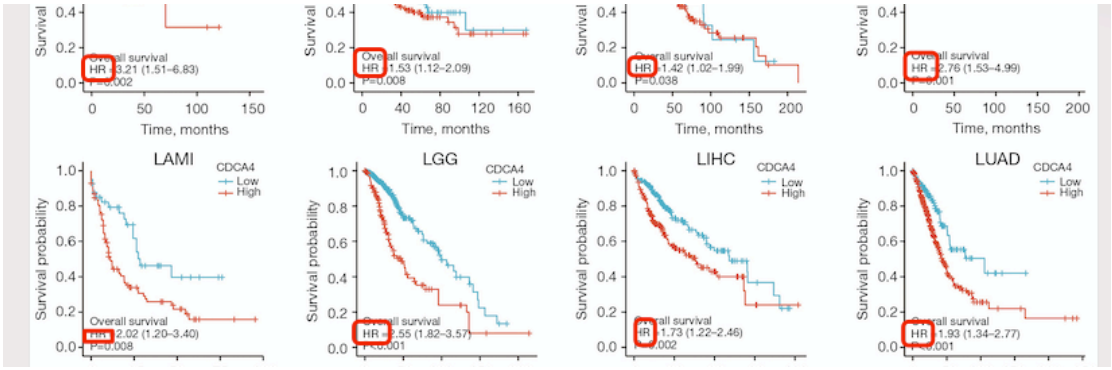
Change in the text: Page 11/Line 357-365.

(8) In the discussion, please supplement the roles of LncRNA-LINC00638/has-miR-29b-3p in HCC.

Reply 8**:** Thank you so much for your suggestion. This study mainly adopts the method of bioinformatics analysis, and the obtained research results are also based on big data analysis. Our research goal is not only to obtain a biomarker that can be used to predict prognosis, but also to predict and analyze the possible mechanism pathways that this molecule plays a role in tumor biological events. In future research, we will conduct relevant basic cytological experiments to analyze the correlation between LINC00638 and has miR-29b-3p, and conduct knockdown or overexpression of one of the two to further observe the expression of the other and its impact on LIHC biological events, in order to better increase the credibility of the article.

Reviewer C

1. Figure 1: Please revise “HR” to “HR (95% CI)”.



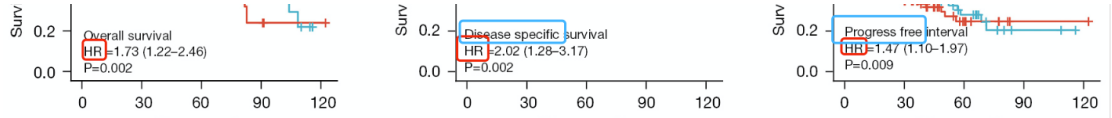
Reply: We have revised “HR” to “HR (95% CI)” and resend the update figure to you. Thank you.

2. Figure 4:

a. Please revise “HR” to “HR (95% CI)”.

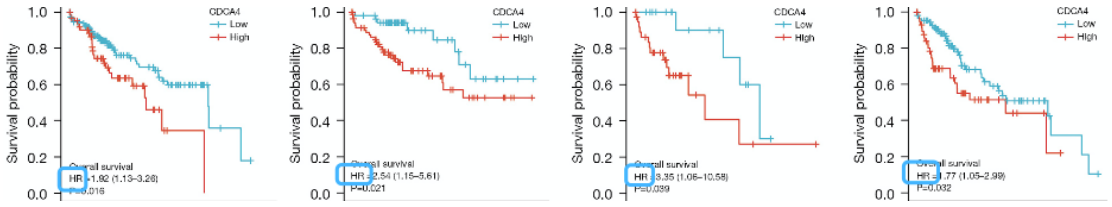
b. Please revise “Disease specific” to “Disease-specific”.

c. Please revise “Progress free” to “Progression-free”.



Reply: We have revised figure 4 as suggested and resend the update figure to you. Thank you.

3. Figure 5: Please revise “HR” to “HR (95% CI)”.

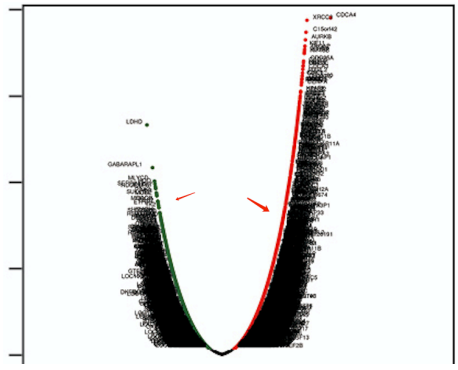


Reply: We have revised “HR” to “HR (95% CI)” and resend the update figure to you. Thank you.

4. Figure 6:

a. Please define those green and red dots either inside the figure or in figure legends.

b. And words in figure 6A are not clear enough to be identified, please resend us higher resolution version as separate file.



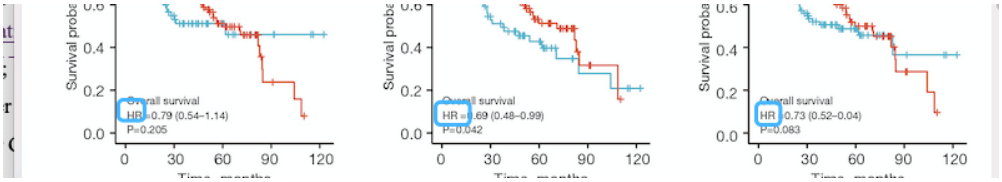
Reply: We have defined green and red dots in figure legend and resend the higher resolution version figure to you. Thank you.

5. Figure 9: Please revise “HR” to “HR (95% CI)”.

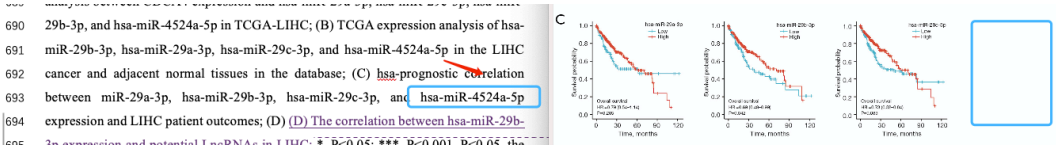
Reply: We have revised “HR” to “HR (95% CI)” and resend the update figure to you. Thank you.

6. Figure 10:

a. Please revise “HR” to “HR (95% CI)”.

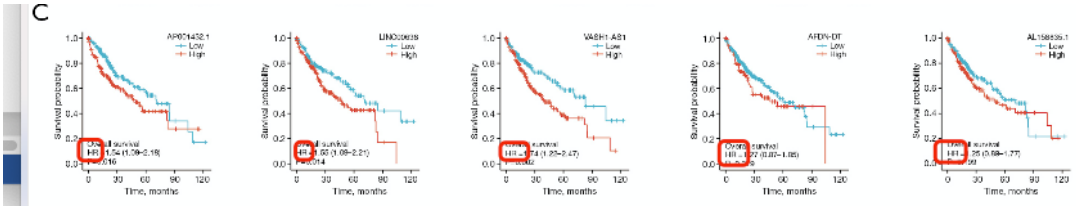


b. Please check the legends of Figure 10C, “hsa-miR-4524a-5p” was not shown in the figure.


690 29b-3p, and hsa-miR-4524a-5p in TCGA-LIHC; (B) TCGA expression analysis of hsa-
691 miR-29b-3p, hsa-miR-29a-3p, hsa-miR-29c-3p, and hsa-miR-4524a-5p in the LIHC
692 cancer and adjacent normal tissues in the database; (C) hsa-prognostic correlation
693 between miR-29a-3p, hsa-miR-29b-3p, hsa-miR-29c-3p, and hsa-miR-4524a-5p
694 expression and LIHC patient outcomes; (D) (D) The correlation between hsa-miR-29b-

Reply: a. We have revised “HR” to “HR (95% CI)” and resend the update figure to you.
b. We have deleted “hsa-miR-4524a-5p” in the legends of Figure 10C.
Thank you.

7. Figure 11: Please revise “HR” to “HR (95% CI)”.



Reply: We have revised “HR” to “HR (95% CI)” and resend the update figure to you. Thank you.