Peer Review File

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Reviewer A

The paper titled “Establishment of the LCMT1-AS2/RPS6KA5 regulatory axis by constructing carcinoembryonic antigen-associated competitive endogenous RNA networks and exploring its clinical significance in colon adenocarcinoma” is interesting. The LCMT1-AS2/RPS6KA5 axis may play an important role in tumor progression and is an important prognostic factor for predicting the clinical outcome of COAD. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) Please clarify the significance of preoperative serum carcinoembryonic antigen on DFS in colon adenocarcinoma and attempt to propose a new prognostic grouping system.

Reply: Sincerely thanks for your constructive suggestions on improving our manuscript. Significant revisions have been made to both the introduction and discussion sections of the manuscript. The initial descriptions of CEA protein and CEACAM5 gene were not sufficiently clear, potentially leading to some degree of misinterpretation. Although CEA is also known as CD66e or CEACAM5, in our manuscript, CEACAM5 refers to a gene. CEACAM5 is a Protein Coding gene. It encodes a cell surface glycoprotein, which represents the founding member of CEA protein family. CEA is synthesized in the cytoplasm and then secreted through the cell membrane into the extracellular space, entering the surrounding body fluids. Our research was focused on constructing a CEACAM5-related ceRNA network, not a CEA-related ceRNA network. Serum CEA is not the subject of our study.

Changes in the text: To differentiate between the CEA protein and the CEA gene, we have revised the manuscript. In this context, "CEA" refers to serum CEA and cell-bound CEA, whereas "CEACAM5" denotes the CEA gene.

2) The figures in this study were not presented, please supplement. Figures S1 and S5 are not clear enough. It is recommended to provide clearer figures again.

Reply: Thank you for your correction and suggestions. Figures S1 and S5 are the original images downloaded directly after TCGA data analysis via cBioPortal, which cannot be adjusted as needed.

Changes in the text: Figures S1 and S5 were not adjusted.

3) There is an issue with the section on "Establishment of CEA associated ceRNA networks" in the manuscript. Please make careful corrections.

Reply: We have edited the text to correct this error. The changed part is shown in red-colored font in the revised version.

Changes in the text: We generated a simpler URL that redirects to the correct site (see Page 4, line 127).

4) This study is based on bioinformatics analysis. It is recommended to increase in vivo and in vitro experimental studies, which may be more meaningful.
Reply: We agree with your comments that preliminary experiments are also necessary. Your suggestion provides a direction for our next research. Unfortunately, due to the limited time and funding, we did not supplement experimental validation. In this study, we aimed to explore the biological behaviors and clinical significance of CEACAM5 by bioinformatics methods.
Changes in the text: Unfortunately, we did not add experiments to validate our results, and then we revised the discussion and refined the limitations.

5) What is the correlation of serum carcinoembryonic antigen level and tumor histopathology of colon adenocarcinoma? What role does the regulatory axis of this study play in this process? It is recommended to add relevant content.
Reply: Thank you for your correction and suggestions. Serum CEA is encoded by CEACAM5 gene. CEACAM5 encodes CEA, a clinical biomarker for CRC, and contributes to tumorigenesis as a cell adhesion molecule. Considering the critical role of CEACAM5, we endeavor to construct a CEACAM5-associated ceRNA network utilizing bioinformatics techniques, with the objective of identifying potential promising biomarkers or therapeutic targets. Consequently, serum CEA is not a primary focus of our study. Nonetheless, we highly value and appreciate your suggestion.
Changes in the text: To differentiate between the CEA protein and the CEA gene, we have revised the manuscript. In this context, "CEA" refers to serum CEA and cell-bound CEA, whereas "CEACAM5" denotes the CEA gene.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Construction of a co-expression network and prediction of metastasis markers in colorectal cancer patients with liver metastasis, J Gastrointest Oncol, PMID: 36388701”. It is recommended to quote the articles.
Reply: The reference has already been cited in the introduction section (Citation: 14).
Changes in the text: See Page 3, line 87.

7) How to judge the prognostic characteristics of colon adenocarcinoma based on the results of this study? How to provide candidate targets for the treatment of colon adenocarcinoma? It is recommended to include relevant descriptions in the discussion.
Reply: We constructed a CEACAM5-related ceRNA network, thereby establishing a regulatory axis of LCMT1-AS2/RPS6KA5. Next, the study used bioinformatics methods to explore the function of this regulatory axis from a multi-omics perspective. In the discussion section, we

summarized the relationship between RPS6KA5 and the prognosis of colon adenocarcinoma (see Page 12, line 400-402): RPS6KA5 is downregulated in COAD tissues, and low expression indicates a poorer OS.

In addition, we also found that: 1. The abnormal expression of RPS6KA5 may play a role in colon cancer through the PI3K-Akt signaling pathway. 2. The down-regulation of RPS6KA5 affects iron drooping, inhibiting the progression of colon cancer. 3. A significant decrease in RPS6KA5 expression may lead to a decrease in the m6A methylation modification and demodification/modification levels. 4. The RPS6KA5low25% group responded more poorly to 5-FU therapy possibly.5. RPS6KA5 may affect the immune infiltration of COAD. 6. RPS6KA5 may affect the response of colon cancer to ICI treatment. In summary, the LCMT1-AS2/RPS6KA5 regulatory axis plays an important role in colon adenocarcinoma, and RPS6KA5 may be a potential therapeutic target. These are all detailed in the discussion section.

Changes in the text: We have made significant revisions to the discussion section, while also retaining the detailed elaboration in this part.

Reviewer B

The topic and subject is nice and interesting.

1、The title is too long; it should also be corrected to avoid misleading. Please make it shorter and add a professional word related to what you have done here for this non-experimental manuscript.

I would add at least bioinformatics in the title…..

Like: Bioinformatics-based establishment of the…..

Reply: Thank you for your review and valuable suggestions. We have streamlined the title of the paper as per your request (see Page 1, line 2-3).

Changes in the text: The title of the paper has been changed to: Establishing a Carcinoembryonic Antigen-Associated Competitive Endogenous RNA Network and Forecasting the Principal Regulatory Axes in Colon Adenocarcinoma Patients.

2、About the key findings: please define COAD and other terms?

Reply: We have made the corrections as requested.

Changes in the text: We have made substantial revisions on pages 2 and 3.

3、Abstract, please straightforwardly highlight the main conclusion of the finding.

Reply: We have made the corrections as requested (see Page 2, line 46-48).

Changes in the text: Additional content has been added and some modifications have been made: Subsequent investigations have indicated that this regulatory axis

could potentially participate in the progression of COAD and exert influence on the therapeutic outcomes of chemotherapy and immunotherapy. It may be involved in the PI3K-Akt signaling pathway and may modify the tumor immune microenvironment and influence the course of COAD. Additionally,, it may be related to ferroptosis, N6-methyladenosine (m6A) methylation, and tumor stemness and interfere with the sensitivity of tumor cells to 5-fluorouracil (5-FU) and immunotherapy.

4、Lines 75-77, unclear. Please divide the sentence in two separate clear ones.

Reply: We have made the corrections as requested (see Page 3, line 79-82).

Changes in the text: Certain lncRNAs can function as ceRNAs, harboring the same miRNA response elements (MREs) as mRNAs. Consequently, they establish a competitive interaction for the same type of miRNAs. In this manner, lncRNAs indirectly modulate the expression levels of mRNAs, thereby orchestrating cellular functions (12).

5、Discussion part is poorly written. Please do not sue introductory sentence here. Revise this part very harshly.

Lines 367-372, remove from here and use for your introduction. The same for lines 377-380.

Reply: We have made the corrections as requested.

Changes in the text: see Page 11, line 368-378.

6、Figures are inconsistent with the related captions. Some figures are poor presented and unreadable. In the captions of the figure please always mention numeration and the repeatability of this non-experimental work…. Please harshly revise these points

Reply: We have made the corrections as requested.

Changes in the text: see Page 17-18, line 590-643.