
Peer Review File

Article information: <https://dx.doi.org/10.21037/jtd-23-187>

Review Comments-Reviewer A

The paper titled “YTHDC2-mediated m6A mRNA modification of Id3 suppresses cisplatin resistance in non-small cell lung cancer” is interesting. To regulate the activity of Id3, YTHDC2 requires modifications to m6A, which ultimately inhibit cisplatin resistance in NSCLC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the roles and prognostic significance of m6A-associated regulatory genes in NSCLC? It is suggested to add relevant contents.

Response: Thanks for your valuable guidance. We tried to improve this point as suggested and have added the roles and prognostic significance of m6A-associated regulatory genes in NSCLC to improve our introduction section. ([introduction/paragraph 2, p2-3/line93-103](#))

2) The identifications in the figure are inconsistent with those in the figure legends, for example, a and b are used in Figures, but A and B are used in the figure legends. Uniform identification is recommended.

Response: We have standardised the identifications in the text and figure legends to lower case letters. Thank you for reading the manuscript so carefully.

3) Please supplement the statistical results of cell apoptosis detected by flow cytometry.

Response: We supplemented the Figure 3b and Figure 3c with the new bar graph containing the statistical results. We appreciate the reviewer’s rigor concerning this point.

4) What is the relationship between the low expression of YTHDC2 and poor differentiation, lymph node metastasis, tumor size and stage of NSCLC? It is suggested to add relevant contents.

Response: Thank you. We have added the additional information regarding the relationship between the low expression of YTHDC2 and poor differentiation, lymph node metastasis, tumor size and stage of NSCLC in the revised manuscript. ([discussion/ paragraph 2, p12/line401-404](#))

5) Figures 3A and 3B are not clear enough. It is recommended to provide clearer figures again.

Response: We thank the reviewer for this important comment and agree that the original version of the manuscript did not provide clear figures 3a and 3b. The figure 3 in text has been replaced.

6) There are many detection methods for proliferation, apoptosis, invasion, and migration. If multiple methods are used, the results may be more reliable. It is suggested to add test results of other methods.

Response: Thank you very much for pointing out this important issue. We agree with your opinion. Unfortunately, due to the limited time and funding, we are unable to argue the conclusion from multiple perspectives at this time. In the later stage, if conditions permit, we are willing to conduct a more comprehensive experimental demonstration on this issue.

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Increased m6A modification of lncRNA DBH-AS1 suppresses pancreatic cancer growth and gemcitabine resistance via the miR-3163/USP44 axis, PMID: 35433957”, “The m6A methyltransferase METTL3 regulates autophagy and sensitivity to cisplatin by targeting ATG5 in seminoma, PMID: 33968659”. It is recommended to quote the articles.

Response: We now include the papers suggested by the reviewers and refined out presentation of the introduction. ([introduction/paragraph 2, p2/line85-93](#))

8) How does YTHDC2 affect the progression, chemoresistance and eventual recurrence of NSCLC? It is suggested to add relevant contents.

Response: Thank you. This part had been supplemented and marked in red in the text. ([discussion/ paragraph 3, p12/line395-399, line411-412](#))

Review Comments-Reviewer B

After reading the article, I have the following suggestions:

1. YTHDC2 has been studied in lung cancer, and this article lacks innovation.

Response: We understand your concern regarding the novelty of our study. However, we would like to clarify that our study focuses on the specific role of YTHDC2 in cisplatin resistance in lung cancer, which has not been extensively investigated in previous studies. While previous research has explored the involvement of YTHDC2 in lung cancer, our study sheds light on a specific mechanism of drug resistance in this context. We have revised our manuscript to better emphasize the unique contribution of our study. We hope that these revisions address your concerns.

2. Figures 1C and 2B have been published in other articles.

Response: The two aforementioned figures were created by us using data from publicly available databases Gepia2 and Kaplan-Meier plotter, and were not copied from elsewhere. It was indeed our oversight that we did not realize that similar figures had already been published in other literature. Currently, regarding Figure 1c, we have re-analyzed the data using R language and 1410 clinical samples from TCGA & GTEx, obtaining consistent results with larger intergroup differences. As for Figure 2b, unfortunately, we could not reproduce the same conclusion after changing the data source, thus we have decided to remove the image and its corresponding results.

3. Add the following research: Id3 expression in drug resistant patients and drug resistant cells.

Response: Thank you for your comments on our manuscript. We appreciate your suggestion to improve our experiment. We plan to collect more cases and then conduct additional experiments to further investigate this issue.

4. Add the following research: the effect of Id3 on the phenotype of drug resistant cells.

Response: We appreciate your suggestion to include additional experiments in our study. However, due to the significant time and resources required to complete these experiments, we plan to conduct them in the future. We believe that these experiments will provide valuable insights and we will work to ensure they are included in our future studies.

5. Add the following research: YTHDC2 expression in drug resistant patients.

Response: We appreciate your suggestion to include additional experiments in our study. However, at present, we do not have access to suitable clinical specimens for conducting the proposed experiments. We plan to collect more cases in the near future and incorporate these experiments in our study.

6. Add the following research: YTHDC2 regulates the phenotypic effect of ID3 on drug-resistant lung cancer.

Response: We appreciate your suggestion to include additional experiments in our study. However, we would like to inform you that the experiment you suggested requires a comprehensive and sophisticated design, and thus, we will need more time to complete it. We plan to conduct this experiment in the future and ensure that it is designed with the necessary rigor to provide robust results.

7. Add the following research: the correlation between the expression of Id3 and YTHDC2 in patients with drug resistant lung cancer.

Response: Thank you for your thoughtful comments on our manuscript. We acknowledge that our experiment has limitations and we plan to address these issues in future studies. We will

collect more cases and conduct more experiments to validate our findings and provide a more comprehensive analysis.

8. Add the following studies: overexpression of YTHDC2, and detection of Id3 expression changes by PCR and WB.

Response: Thank you for your helpful comments and suggestions. We appreciate your feedback and have carefully considered your recommendation to improve our experiments. As you have pointed out, the expression of Id3 is indeed reflected in Figure 1d and 1e, which were obtained through qPCR and WB analysis after overexpression of YTHDC2 in A549/DDP cells.

9. The English spelling is poor. English spelling needs improving and polishing.

Response: Thank you for your comments regarding the English spelling in our manuscript. We appreciate your feedback and would like to explain that we have carefully checked our manuscript and made the necessary corrections to improve the language quality. We understand the importance of clear and concise communication in scientific writing and have taken great care to ensure that our manuscript is as clear and accurate as possible. We apologize for any inconvenience caused by the initial errors and hope that the revisions we have made have addressed your concerns.

10. The quality of the picture is poor, and needs to be improved.

Response: Thank you for your feedback regarding the image quality in our manuscript. We have addressed your concerns by replacing some of the unclear images with high-resolution versions. We apologize for any confusion caused by the initial images and believe that the new images provide a more accurate representation of our findings.

Review Comments-Reviewer C

1. This is a single cell line study on functional one, and clinical data of their own is only 9 cases though database seems to support some of the authors' claim.

Is it possible to test using another cell line system to corroborate the findings?

Response: We appreciate your suggestion to include another cell line to better support our conclusions. However, due to time constraints and the need to complete the paper for graduation, we are unable to carry out additional experiments at this point. We acknowledge that our study can benefit from further investigations with a larger sample size and different cell lines. We plan to pursue this direction in future research and will take your feedback into consideration.

2. Several important reference like ALKHB and lung cancer is missing (PMID: 35318440,

PMID: 34408926)

Response: We have carefully considered your recommendation and would like to inform you that we have already included similar references in our manuscript. However, we are open to considering any additional references that you may suggest, provided they are relevant to the topic and contribute to the overall quality of the paper.

3. The limitation of this study should be addressed.

Response: We appreciate your valuable feedback on our manuscript. We recognize that our study has certain limitations, including the small sample size and the lack of long-term follow-up data. To address these limitations, we plan to collaborate with other research teams to collect more data and conduct a meta-analysis in the future.

Review Comments-Reviewer D

1. Please check all abbreviations in the main text, such as “NSCLC” below. All abbreviated terms should be full when they first appear.

93 target to overcome seminoma cisplatin resistance. The mechanism of METTL3-
94 mediated autophagy in reversing gefitinib resistance of NSCLC cells by β -elemene was
95 revealed by Liu et al.(8). In contrast, erasers in m6A-modified mRNA counteract the

Response: Thank you for the request to review all abbreviations in the main text. I fully understand the importance of maintaining consistency and clarity in written communication, particularly in scientific writing. I have reviewed the text and made corrections to ensure that the writing adheres to the best practices of academic writing.

2. Please check if any more references need to be added in the below sentence since you mentioned “Studies”, but only one reference was cited. If not, “studies” should be changed to “a study/a previous study”.

544 adjacent normal tissues. However, some studies have shown that YTHDC2 promotes
545 colon cancer metastasis via enhancing HIF-1 α mRNA translation (25). The results from

Response: I would like to express my gratitude for the reminder provided. At present, there is no necessity to incorporate additional references so I have rectified my statement.

3. Figure 1:

- 1) Please indicate the meaning of * in the legend.
- 2) Please indicate the full name of “NC” in the legend.

Response: We have now provided the necessary indications for the meanings of the symbol and abbreviation, and we would like to express our regret for any prior oversight in this matter.

4. Figure 2:

1) Please check Figure 2C legend. There is no “NotI” in your Figure 2C, but you indicated it in the legend.

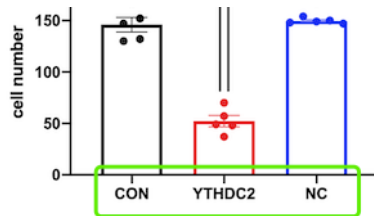
709 (KM) plots. (c) Structure of YTHDC2 overexpression vector pCDH-CMV-MCS-
710 EF1A-GFP-T2A-Purois 8196bp in length and contains EcoRI and NotI cleavage sites.

2) Please indicate the full name of “NSCLC”, “NC” in the legend.

Response: In the course of constructing the target plasmid, the 3Flag tag protein was added and subsequently followed by insertion of the target gene sequence utilizing the NotI restriction enzyme site. It is noteworthy that the resulting sequencing data analysis file indicates the presence of the NotI digest site downstream of the Flag tag protein and immediately following the stop codon, which is uploaded in the supplemental file. In addition, we have indicated the full names of "NSCLC" and "NC" in the legend.

5. Figure 3:

1) Please check whether the 3 groups names are correct.



2) There is no *** in your Figure 3, but you indicated it in the legend. Please check.

2) Please indicate the full name of “NSCLC”, “OD”, “NC” in the legend.

Response: We appreciate you reminding us and asking this question. We would like to inform you that we have made the necessary corrections to the group name of the blank group, changing it from "CON" to "Blank," which is a more precise and accurate representation. Additionally, we have eliminated any extraneous symbols and have included the complete names of "NSCLC," "OD," and "NC" in the legend for clarity and precision.

6. Figure 4:

1) Please revise all “m⁶A” to “m6A”.

2) Please revise all “input%” to “input, %”.

3) It looks like your main text below don’t match with your Figure 4C and D. Please check.

Response: We would like to express our appreciation for your guidance and would like to inform you that the “m⁶A” and “input%” in the figures has been revised. Additionally, we have taken steps to address and correct any errors in the expression within the result section. Thank you for bringing these matters to our attention.

7. Reference 23 and 27 are the same. Please delete one of them and update the citation in both the main text and reference list.

Response: We would like to express our gratitude for your guidance in reviewing our work. As per your feedback, we have rectified the errors in the citation of references. Thank you for bringing this to our attention.