Peer Review File

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Reviewer A

In this study, the authors investigated whether YAP overexpression or activation would induce ferroptosis to increase the sensitivity of chemotherapy in retinoblastoma cells. Major and minor concerns are as follows.

Major concerns

1. YAP expression in retinoblastoma. The Introduction is inconsistent in providing the rationale for the study. The authors indicated that YAP was elevated in retinoblastoma with reference 23 in Line 124; on the other hand, they mentioned retinoblastoma is included in YAPoff tumors with low expression of YAP with reference 25 in Line 141.

With these contradictory publications, the expression of YAP should be evaluated in retinoblastoma tissues.

Reply: Thanks for your rigorous and careful review of our submission, we have made correction seriously.

Changes in the text: Please see introduction part from line 115 to line 141, we had a detailed correction for the role of YAP in tumor progression and deleted the inconsistent part and the conflicting references 23 and 25.

2. Ferroptosis. It is unclear whether YAP overexpression induces ferroptosis in retinoblastoma cells from the current manuscript. The Results should provide enough explanations on this aspect regarding Figure 3.

1) What is the meaning of differential levels of glucose, glutamine, and FA with overexpression of YAP? (The difference in glutamine level is not significantly decreased.)

2) Please provide the quantitative data of ROS measurement.

3) What does differential expression of genes related to ferroptosis mean? Do these results imply that YAP overexpression induces ferroptosis in retinoblastoma cells?

Reply: Thanks for your inspection very much. Firstly, we have found our careless result description and immediately take a correction for the mistake of glutamine level no difference. Secondly, it is a good suggestion for providing a quantitative graph of ROS measurement result. However, since the remarkable significance were showed in the flow cytometer graph and the graph layout is limited, we had to provide it into the supplemented material. The last, some elegant publications have confirmed that related genes COX2, ACSL4, PTGS2 and NOX1 were up-regulated, whereas GPX4 and FTH1 were down-regulated when ferroptosis occurrence.

Changes in the text: Please see the context of line 285 to line 289, we had correction for no difference of glutamine level. From line 297to line 303, we added a detailed description for ferroptosis related genes change.

3. PY-60 as a YAP inducer. Please provide the data of YAP induction by PY-60 in retinoblastoma cells.

Reply: PY-60 has been reported as an effective activator in many human cell lines publication on the journal of *Nature chemical biology* (Cite: YAP-dependent proliferation by a small molecule targeting annexin A2). Because of the reply required soon and we do not have enough time for preparing the experiments, here we could not provide the result in RB cells and we will verify the effect of PY-60 in activating YAP on RB cells in the background.

Changes in the text: None

4. Increased sensitivity to cisplatin and etoposide with YAP induction in retinoblastoma cells.

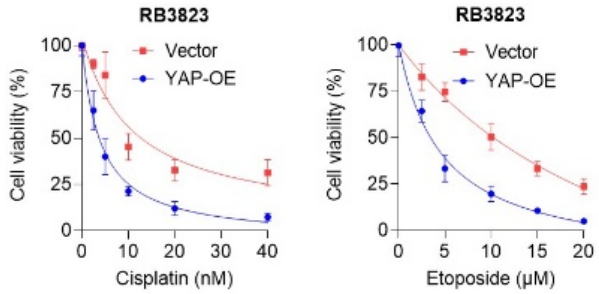
1) In most countries, cisplatin is not currently the primary drug for treating retinoblastoma patients. Please correct the expressions in the Introduction.

2) Etoposide is not a platinum-based drug. The title seems to need a correction.

3) Please show the data on the sensitivity of control and YAP-overexpressing retinoblastoma cells to cisplatin and etoposide.

Reply: Thanks for this precise question for our submission. We have corrected the expression in the introduction and other parts of description, and we apologize for the wrong description of cisplatin and etoposide. Because of the limited times for reply and brief article content requirement, we have provided a result for YAP-overexpression enhanced the sensitivity of cisplatin and etoposide in RB3823 cells. The below MTS assays showed consistent tendency with the result of YAP activator PY-60。

Changes in the text: Please see the title and introduction context of line 93 to line 105, we had correction for expression of wrong descriptions.



4) What is the mechanism of increased sensitivity of retinoblastoma cells to cisplatin and etoposide with YAP induction? Does YAP induction induce the same mechanisms regarding two different drugs, cisplatin and etoposide? Or separate mechanisms? Data on mechanisms linking YAP induction and chemosensitivity might provide essential insights.

Experimental details

1) Please indicate the source of Müller cells.

2) Please indicate what the 'hippocampus experiment' is.

Reply: Thanks for your rigorous and careful review of our submission, we have made correction seriously and apologized for our submission negligence and language mistake.

Changes in the text: Please the methods part context of line 231 to line 236.

Minor concerns

1. Please thoroughly revise the manuscript to correct grammatical and typographical errors. Some paragraphs are difficult to follow.

2. Please indicate what Q2 and Q3 mean in the Y-axes in Figure 2.

3. Please include denotations of statistical analyses in Figure 5.

Reply: Thanks for your elaborative examination and found these grammatical and typographical errors we made. We apologized for the crude writing skill and mistake of submission. We have made a dedicated correction in this time.

Changes in the text: Please see the legend text of figures 1 to 5, we had a correction for the wrong description.

Reviewer B

1) First, the title needs to indicate the experimental research design of this study such as in vitro or in vivo.

   Reply: Thanks for your good suggestion and we have made a more rational title which showed the result qualified in vitro.

   Changes in the text: Please see the title correction.

2) Second, the abstract needs some revisions. The background needs to accurately describe the clinical questions to be addressed by this experimental study and what the potential clinical significance of this study is. The methods need more details of the experiment and what the questions to be answered by these procedures. The results need to report the P values for statistical analysis. The conclusion needs more detailed comments for the clinical implications of the findings.

   Reply: Thanks for your rigorous and careful review of our submission, we have made correction seriously.

   Changes in the text: Please see the abstract from line 31 to line 57.

3) Third, in the introduction of the main text is too long. Please have the review focus on the research focus, review what has been known, analyze the knowledge gaps and limitations of prior studies, and importantly, what the questions to be addressed in this study.

Reply: Thanks for your rigorous review of our submission, we have made correction seriously and apologized for our writing skill.

Changes in the text: Please see the introduction part from line 80 to line 142, we had a simplified description of introduction part.

4) Fourth, the methodology needs to use a flowchart to briefly describe the research procedures. At the beginning of this part, please also have an overview of the research procedures and the questions or hypotheses to be examined. In statistics, there is no need to have the three levels of statistical significance since these are not recommended in modern statistics. Further, please indicate whether $P<0.05$ is two-sided.

Reply: Thanks for your rigorous and careful review of our statistic description and we have correction now.

Changes in the text: Please see the legend text of figures 1 to 5, and statistical methods from lines 247 to 252, where we have provided a simplified description of the selection of statistical methods.

5) Finally, please consider to review and cite several related papers: 1. Wen Y, Zhu M, Zhang X, Xiao H, Wei Y, Zhao P. Integrated analysis of multiple bioinformatics studies to identify microRNA-target gene-transcription factor regulatory networks in retinoblastoma. Transl Cancer Res 2022;11(7):2225-2237. doi: 10.21037/tcr-21-1748. 2. Wang R, Zhu G. A narrative review for the Hippo-YAP pathway in cancer survival and immunity: the Yin-Yang dynamics. Transl Cancer Res 2022;11(1):262-275. doi: 10.21037/tcr-21-1843. 3. Guo N. Identification of ACSL4 as a biomarker and contributor of ferroptosis in clear cell renal cell carcinoma. Transl Cancer Res 2022;11(8):2688-2699. doi: 10.21037/tcr-21-2157.

Reply: Thanks for your good suggestion and we will supplement these excellent publications.

Changes in the text: Please see in the man text on line 85, line 110 and line 131.

Reviewer C

The paper titled “Overexpression of YAP induces ferroptosis/lipid-peroxidation in retinoblastoma to increase the sensitivity of platinum chemotherapy” is interesting. The research results found that the overexpression of YAP induced iron cell apoptosis/lipid peroxidation in retinoblastoma and improved the sensitivity of retinoblastoma to platinum chemotherapy, which provides a new therapeutic target for personalized treatment of retinoblastoma patients. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) In 2022, a study reported a new strategy to eliminate multi-drug resistance retinoblastoma

cells by inducing autophagy-dependent ferroptosis. What are the advantages and uniqueness of this study compared to it? What is the inspiration? Suggest adding relevant content.

Reply: Thanks for this elegant research example. When compare to this recent study, our research had found the intracellular role of YAP in RB cell lines. This finding provided a new sight to verify the lipid peroxidation induced ferroptosis phenomenon, which is important for the chemotherapy effect on RB cell lines.

Changes in the text: Please see the discussion part from line 358 to line 362.

2) Please summarize the main molecular mechanisms associated with platinum chemotherapy in retinoblastoma and the development of new approaches to tackle this clinically relevant problem.

Reply: Thanks for your good suggestion, we have considerate to discuss more detail about the molecular mechanism of platinum chemotherapy in the discussion part.

Changes in the text: Please see the context from line 373 to line 389.

3) What is the clinical value of tumor microenvironment in platinum-resistant retinoblastoma? What roles of YAP in the tumor microenvironment? Suggest adding relevant content.

Reply: Thanks for this suggestion, we will have some detail discussion for the role of YAP in tumor microenvironment, especially in platinum-resistant RB.

Changes in the text: Please see in the man text from line 423 to line 438.

4) What is the role of YAP in maintaining the stemness of retinoblastoma stem-like cells? Suggest adding relevant content.

Reply: Thanks for your kindly suggestion, it need to be researched more on the effect of YAP on RB stem-like cells. And we would like to develop a further project on the role of YAP in RB stem-like cells.

Changes in the text: None.

5) In this paper, it is best to supplement the in vivo research. This is more conducive to support the conclusion of this paper.

Reply: Thanks for this rigorous advice, we plan to perform a further in vivo study to provide a more conductive result to support our conclusion.

Changes in the text: None.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Expression of YAP in endometrial carcinoma tissues and its effect on epithelial to mesenchymal transition, Transl Cancer Res, PMID: 35117328”. It is recommended to quote the article.

Reply: Thanks for this good citation advice, we have cited this excellent publication in our submission.

Changes in the text: Please see in the man text on line 117.

7) What new insights can this study provide for potential chemotherapy pathways that inhibit tumors?

Reply: Our research referred to investigate a intracellular function of YAP in the RB cells, which supported the Hippo pathway signal as a potential target in RB chemotherapy.

Changes in the text: Please see the discussion text from line 438 to line 453.