

Reviewer #1: we revised with interest a manuscript by dr Zhou Y et al , entitled (Sirolimus increasing the anti-cancer effect of Huai Er by regulating the HIF-1 α mediated glycolysis in HCC), we have some comments:

- Language needs corrections

Reply: Thank you for the kindly suggestion. We have revised and edited the language of the article for readers to read and understand by a medical English specialist. The revised contents marked with red. If the editors consider that this manuscript needs to be modified by the language company, we will accept this suggestion.

- Page 6: images were analyzed by Image J” needs reference

Reply: Thanks for your good suggestion, we have added the reference in the manuscript.

- The relation between HIF-1 α , mTOR, autophagy needs to be addressed

Reply: Thanks very much for taking your time to review this manuscript. I really appreciate your comments and suggestions! This study preliminarily investigated the molecular mechanism of the sirolimus application with Huai Er in inhibiting proliferation of HepG2 cell lines by hypoxic dependence. Reviewing experts put forward on the research of autophagy may help to further clarify the molecular mechanisms of cancer growth, we will intervene in vitro and carry out mice orthotopic liver cancer model in the future to investigate the correlation between autophagy and hypoxic, and explore whether it plays a role through the mTOR pathway.

- How can you explain the suppressed HIF-1 α and mTOR in S50, H8 and S50+H8

Reply: Thank you for the valuable suggestion. We have discussed the experimental results under normoxic and hypoxic conditions which are marked as red in the manuscript, and concluded the possible signaling pathways of monotherapy and combination therapy.

- Page 21: “ Most anticancer drugs decrease the downstream activation effect by down-regulating or reducing the activity of PI3K-Akt-mTOR pathway[28,29], which can up-regulate the increase of PTEN,”..its supposed that PTEN affects Akt-mTOR pathway as a negative regulator not the reverse ? please clarify

Reply: Thank you for the review of our manuscript. I really appreciate your comments and suggestions. We carefully checked the content of the article. Due to our negligence in writing, the regulatory role of PTEN was not clearly explained. We consulted relevant literature and made modifications which marked red in the manuscript.

- I suggest that the author can represent the different signaling pathways involved in their research in a diagrammatic way (graphical presentation), will simplify the data acquisition for the readers.

Reply: Thanks very much for the kindly suggestion. We have presented the different signaling pathways involved in our research in a diagrammatic way with Fig S-4.

Reviewer #2: I would like to thank the authors for their work to verify the role of Sirolimus and Huai Er in anti-cancer effect through regulating the HIF-1 α mediated glycolysis in HCC. I would suggest that the bar graphs should be tabulated with numbers and p values in the tables for easier reading and comprehension of significance and difference between each variables. This would save the highly crowded bars. The statistical analysis could not be evaluated due to the absence of tables with actual numbers.

Reply: Thanks very much for taking your time to review our manuscript. I really appreciate your well suggestions! We have revised the bar graphs with numbers and p values in a separate table at the supplementary materials named table S2.