

Dear editors and reviewers:

Thank you very much for considering our revised manuscript entitled “**Lnc524369 promotes Hepatocellular carcinoma progression and predicts poor survival by activating YWHAZ-RAF1 signalling**” (Manuscript NO: 69092, Basic Study) .

We appreciate the careful review and constructive comments provided by the reviewers of our manuscript. We have studied the comments carefully and provided answers in a point-by-point manner to each of the reviewers’ questions. We had made corrections which we hope are suitable for publication in *World Journal of Gastrointestinal Oncology*. Below are our answers for questions raised by the reviewers.

Reviewer #1:

Specific Comments to Authors:

the queries of the reviewer (reviewer ID: 05038589) is very important: a. Results figures are cited in the introduction HCC tissue sampling process should be clearly mentioned in the methodology before Cell lines section. b. In the methodology section, it is written that "According to the sequence of Lnc524369 gene (provided by the customer)," What customer? I think you are the customer that should provide the company and your readers by the sequence. It is copied and paste from the pamphlet of the company and this is unacceptable. c. Sentences should not start with numbers in figures, they should be written in letters. d. It makes more sentences to start your results section with the screening of the HCC tissues "YWHAZ mRNA and protein was tested in our included ten HCC samples by real time-PCR and western blot. e. " what is the exact number of samples used in study 41 or 10 or 5 as shown in your figure!!!! Correlation between Lnc524369 level expression and pathological grade should be presented as a correlation analysis linear figure not a table with P-value. f. Figures are captioned screening in-vitro and in vivo?? What in-vivo?? you did not use any in-vivo models in your study I think you should revise your paper well before submitting it to avoid this type of confusion.

a. Results figures are cited in the introduction HCC tissue sampling process should be clearly mentioned in the methodology before Cell lines section.

Response a: Thank you for your advice. Results figures have been clearly cited in the methodology before cells line section.

b. In the methodology section, it is written that "According to the sequence of Lnc524369 gene (provided by the customer)," What customer? I think you are the customer that should provide the company and your readers by the sequence. It is copied and paste from the pamphlet of the company and this is unacceptable.

Response b: We apologize for the mistakes in the manuscript, the wrong description of (provided by the customer) has been deleted, and its sequence was attached in table 1.

c. Sentences should not start with numbers in figures, they should be written in letters.

Response c: Thank you for your advice. It has been revised---41 change into "Forty-one" .

d. It makes more sentences to start your results section with the screening of the HCC tissues "YWHAZ mRNA and protein was tested in our included ten HCC samples by real time-PCR and western blot.

Response d: Thank you for your advice. We have given more detailed description of our results section with the screening of the HCC tissues.

e. " what is the exact number of samples used in study 41 or 10 or 5 as shown in your figure!!!! Correlation between lnc524369 level expression and pathological grade should be presented as a correlation analysis linear figure not a table with P-value.

Response e: Thank you for your advice. We apologize for the mistakes problems in the original manuscript. Forty-one included human HCC sample from Zhejiang Provincial People's Hospital were used to test Lnc524369 level. Due to limited human

HCC sample tissue, five of forty-one HCC sample was used to YWHAZ and RAF1 protein or mRNA level. We confused the number of HCC sample (n=5) and para-HCC sample (n=5) into ten by mistake. Correlation between lnc524369 level expression and pathological grade has been presented as a correlation analysis linear figure in Figure 4d. thank you very much for your comments and suggestions.

f. Figures are captioned screening in-vitro and in vivo?? What in-vivo?? you did not use any in-vivo models in your study I think you should revise your paper well before submitting it to avoid this type of confusion.

Response f. Thank you for your advice. We apologize for the mistakes in the manuscript, we already have revised the wrong expression of “in vivo” .

Editorial comments:

a. In the materials and methods section the authors have described the transwell migration assay twice. Is there a specific reason for this? Because I have read them, and they both described almost the same methodology. b. It is not clear from methodology how the authors detected the cytoplasmic and nuclear expression of lnc524369 in HCC cell lines. In the results section, the authors have stated that they have chosen Huh-7 for transfection experiments. HepG2 cell line has the lowest expression of lnc524369 and Huh-7 has the highest expression levels. Wouldn't it be more important for your hypothesis to work on the low expression cell line and determine the changes in cell biology? c. Figures are not cited orderly throughout the results section? d. Why did the authors analyze the expression of 14-3-3 protein zeta/delta (YWHAZ) and RAF1 in the specimens of only 10 patients rather than all 41 patients? These points are valid and should be addressed by the Authors.

a: In the materials and methods section the authors have described the transwell migration assay twice. Is there a specific reason for this? Because I have read them, and they both described almost the same methodology.

Response a : Thank you for your advice. In the materials and methods section, we describe the two different transwell assays (one for migration without Matrigel, the other one for invasion with Matrigel).

b. It is not clear from methodology how the authors detected the cytoplasmic and nuclear expression of lnc524369 in HCC cell lines. In the results section, the authors have stated that they have chosen Huh-7 for transfection experiments. HepG2 cell line has the lowest expression of lnc524369 and Huh-7 has the highest expression levels. Wouldn't it be more important for your hypothesis to work on the low expression cell line and determine the changes in cell biology?

Response b : Thank you for your advice. Methodology of the cytoplasmic and nuclear expression of lnc524369 in HCC cell lines has been added into the manuscript. Due to HepG2 cell line with the lower expression of lnc524369, which is more difficult to difference the level of lnc524369 in the cytoplasm and nucleus by qt-PCR. That is why we choose Huh-7 cells.

c. Figures are not cited orderly throughout the results section?

Response c: Thank you for your advice. Figures have been cited orderly throughout the results section.

d. Why did the authors analyze the expression of 14-3-3 protein zeta/delta (YWHAZ) and RAF1 in the specimens of only 10 patients rather than all 41 patients.

Response d: Thank you for your advice. We are so sorry for it, due to limited research funding, we just test the expression of 14-3-3 protein zeta/delta (YWHAZ) and RAF1 of ten patients.

The authors have provided non-native speaker English editing certificates. However, there is still a need for significant language polishing.

Response: Thank you for your advice. The entire manuscript has been thoroughly revised.

The “Article Highlights” section is provided but it should be a concise review of the cardinal findings of the study; this seems inappropriate. Please revise the “Article Highlights” section;

Response: Thank you for your advice. The “Article Highlights” section has been thoroughly written again. The more detail information have been added into our manuscript, thank you very much for your comments and suggestions.