Peer review file

Article information: http://dx.doi.org/10.21037/jtd-20-966

Reviewer A

Comment 1: The detail of EBSS or 3-MA treatment (such as concentration) is missing.

Reply 1: We thank the Reviewer for providing this helpful suggestion. We had unfortunately ignored this important step when writing our manuscript. We have added the details of EBSS and 3-MA to the results and the figure legend sections.

Changes in the text: Please see the revised manuscript; the revisions are marked in red.

Comment 2: Statistical analysis: How many times did the authors repeat the independent experiment? The authors have to add the information about sample size of each data.

Reply 2: We thank the reviewer for reading our manuscript and providing helpful comments. We have added the number of samples and the number of times the experiments were repeated.

Changes in the text: Please see the revised manuscript, Figure legends and Material and Methods, marked in red.

Comment 3: Fig. 3: The authors have to examine whether ectopic overexpression of WWC3 enhances the EBSS-induced apoptosis by Annexin V/PI staining.

Reply 3: Thank you for your relevant and insightful suggestion. There is no doubt that adding Annexin V / PI staining assay to detect apoptosis is more intuitive for

observing the effect of WWC3 on EBSS-induced apoptosis. In response to your suggestion, we adopted the Annexin-V APC/PI staining method, because WWC3 plasmid contains GFP tag, which has a similar absorption wavelength to FITC. The results showed that WWC3 could promote EBSS-induced apoptosis of lung cancer cells. We made the appropriate changes in the Results and Figure legend sections.

Changes in the text: Please Supplementary Figure S1, revised-manuscript, Results (Lines 260-264), and Supplementary Figure legend, marked in red.

Comment 4: Figure 4F and G. The representative cytograms should be included.

Reply 4: Thank you for reading our manuscript carefully. Initially, we did not add an apoptotic cell chart for aesthetic reasons, and only the statistical chart was included. The representative cytograms are now included in Supplementary Figure S2.

Changes in the text: Please see the Supplementary Figure S2, Supplementary Figure legend marked in red.

Comment 5: Although EBSS dramatically increased the cleaved caspase-3/7 expression in DMSO-treated WWC3 knockdown cells (Fig. 4B), no clear increase in apoptosis was observed among them (Fig. 4F). I think that these results imply no involvement of caspase-3/7 in EBSS-induced apoptosis. The authors have to confirm whether caspases inhibitor suppress the EBSS-induced apoptosis.

Reply 5: Thank you so much for providing constructive and helpful comments. We used siRNA to knock down WWC3 expression to observe the effect of decreased expression of WWC3 on the EBSS-induced apoptosis rate and the apoptosis-related protein expression in the A549 cell line. The results showed that silencing WWC3 could attenuate EBSS-induced apoptosis. Besides, we also observed that EBSS could

increase the cleaved caspase-3/7 expression in DMSO-treated WWC3 knockdown cells. We believe that this may be related to the action time of EBSS. The best time for detecting protein changes in signal pathways in Western blotting is different from that in an apoptosis assay. In general, the change in protein levels will precede the appearance of cell phenotype. Therefore, we believe that the apoptosis-related protein caspase-3/7 can be dramatically upregulated in lung cancer cells after 24 hours of EBSS treatment, while the degree of apoptosis may not change significantly. We also calculated the statistical differences in DMSO-treated WWC3 knockdown cells after EBSS treatment (Figure 4F), and the results showed statistical significance. When these results are combined with those shown in Figure 3E , F, it is suggested that caspase 3/7 may be closely related to EBSS-induced apoptosis. As the reviewer suggested, adding caspase 3/7 inhibitor is the most direct way of proving this theory. Due to time limitations, we did not have enough time to perform this experiment. However, we will explain and verify it in our further research. We have added this to the discussion section. Once again, we sincerely thank the reviewers for raising this key issue.

Changes in the text: Please see Lines 354-372 in the Discussion section.

Comment 6: There are some careless mistakes such as “wwc3” in Line 44 and “..” in Line 155. The authors should check the manuscript carefully.

Reply 6: We feel so sorry for our carelessness, we have checked the manuscript thoroughly and have corrected our mistakes in the revised manuscript.

Changes in the text: The corrections are marked in red in the revised manuscript.

Comment 7: I recommend that a native speaker of English reviews the manuscript to

improve word choice, sentence structure, and grammar.

Reply 7: The revised manuscript has been edited by a native English editor.

Reviewer 2

Comment 1: L 155: Double dots.

Reply 1: Thank you for reading our manuscript carefully and we apologize for our carelessness, and we have corrected our mistakes in the revised manuscript.

Changes in the text: The corrections in the revised manuscript are marked in red.

Comment 2: Statistical analysis: Please note what kind of statistical software was used.

Reply 2: Thank you. We take used SPSS22.0 software to perform the corresponding statistical analysis, and we have added the detailed information to the materials and methods marked in red.

Changes in the text: Please see Lines 197-198 in Materials and Methods.

Comment 3: Figures: Figure 4 was included “n.s.”. However, other figures were omitted “n.s.”.

Reply 3: We apologize for our carelessness. As suggested by the reviewer, each figure has been modified accordingly.

Changes in the text: Please see Figure 3C and 3D, Figure legend, Line 532.