Peer Review File

Responses to Reviewer A

Major issues:

Comment 1: It is not clear how common the Cul7-survivin cascade is turned on in docetaxel-resistant NSCLC. Please consider validating the findings (upregulation of Cullin 7 and survivin) in tumor microarrays or gene expression datasets with treatment-naive and docetaxel-resistant NSCLC tumors (relapsed).

Reply 1: Thank you very much for this important question. We also considered this evidence in our initial experimental design. Please let me explain to you the following reasons for not completing this part of the experiment:

1) According to clinical guidelines, docetaxel is generally used for the second or the third line treatment and needs to be combined with platinum chemotherapy drugs. It is very difficult to confirm the direct relationship between Cul7/Survivin expression and docetaxel resistance.

2) The risk of pathological examination after failure of the second or the third line treatment is high. Furthermore, clinical sampling is also difficult.

Thank you again for your very constructive comment. We hope our explanations satisfy you.
Changes in the text: No.

Comment 2: A different NSCLC cell line, H1299, was used in this experiment Figure 2G and Figures 3-5. Please include H1299 in the experiments described in Figure 1 and Figure 2 (A-F) or H358 in the experiments described in Figures 3-5.

Reply 2: Thank you very much for this important question. We have added the Figure S1, that showed the experimental results about H1299 and H1299DTX cells on pages 26-27 lines 661-676. At the same time, we have also added a description about this result in the first part of Results on pages 9-10 lines 279-284. Please allow us to explain why this part of the data was not included in the results section of the previous manuscript.

1) We conducted three MTS proliferation experiments to detect the sensitivity of H1299DTX cells to docetaxel. The IC_{50} values were 189.051, 59.537, and 64.930 respectively, which are too different, so we did not include these results in the manuscript results.

2) The corresponding IC_{50} values of H1299 were 9.073, 7.854 and 10.673. The drug resistance of H1299DTX cell was increased by 6-fold at least. The construction of drug-resistant cells can be considered successful if the drug resistance factor reaches 5 times or more. Moreover, we found that the mRNA and protein expression levels of Cul7 were significantly increased in H1299DTX cells (S1C, 1D). Survivin was also upregulated at the protein level (S1E).

3) Considering the overall fluency of the manuscript, we described this section of the results as a supplementary FIGURE. Moreover, we will further screen H1299DTX for stable drug resistance by adding drugs, and also consider conducting more in-depth research in three cell lines simultaneously in the following study.

Changes in the text: See pages 9-10, 12, 26-27, lines 279-284, 322-323, 367, 373,661-676.

Comment 3: Figure 4 E-F. Figure legend does not match the data shown in the figure.

Reply 3: We apologize for the error in our manuscript. We have modified the word "Cul7" to "Survivin" in the figure legend of Figure 4 E-F.

Changes in the text: See page 24, line 635.

Minor issues:

Comment 1: Line 51-53. Specify what was detected in A549 and A549DTX cells.

Reply 1: Thank you very much for this important question. We have revised the sentence to "we used flow cytometry to detect the apoptosis rate of A549 and A549DTX cells with the same drug concentration".
Changes in the text: See page 2, line 52.

Comment 2: The findings of the study (lines 102-110) should not be summarized in the introduction. Consider removing it.
Reply 2: Thank you very much for this important comment. We have removed summary about the results and revised the sentence to "We found that Cul7 may play an important role in LUAD docetaxel resistance. Cul7 was coexpressed with Survivin and may promote the occurrence of docetaxel resistance in LUAD by increasing the protein level of Survivin."
Changes in the text: See page 4, line 102-110.

Comment 3: Fig. 3C-D. The difference between Cul7 overexpression and the control vector is subtle. Consider repeating these experiments in the NSCLC cells with Cul7 knockdown.
Reply 3: Thank you very much for this important comment. We have conducted three independent repeated experiments, and the IC_{50} calculation results are shown in the table below.

IC_{50} / Times	A549-pCDNA3	A549-HACul7	H1299-pCDNA3	H1299-HACul7
First	5.226	19.478	17.459	33.307
Second	5.985	21.116	13.445	27.604
Third	7.76	18.326	13.776	28.269

The results of three independent repeated experiments were relatively stable, and the multiple differences of IC_{50} values between the control group and the overexpression group were also relatively constant. Therefore, this part of the experiment was not repeated. We hope to gain your understanding.
Changes in the text: NO.

Comment 4: Figure 4 G-H. Please specify which Cul7 siRNA was used in these experiments.
Reply 4: We ultimately choose siRNA233-Survivin and have provided an explanation "and siRNA233-Survivin small interfering RNA was selected for subsequent experiments." in figure legend of Figure 4.
Changes in the text: See page 24, lines 636-637.

Comment 1: In line 412 and 418, the author described that Cul7 inhibits apoptosis and promotes cell proliferation in a p53-dependent manner (24-26), and Survivin is negatively regulated by wild-type p53 and induces apoptosis in a p53-dependent manner (19,28-30), respectively. Among the LUAD cell lines used in this study, A549 cells harbor wild-type p53; however, H1299 and H358 cells are p53-null cell lines (Oncotarget. 2015 Dec 8; 6(39): 41692–41705.). How to explain the effects of Cul7/Survivin axis modulation in H1299 and H358 cells shown in this study? At least, this issue should be discussed.
Reply 1: Thank you very much for this important comment. In our manuscript, we mentioned "Cul7 inhibits apoptosis and promotes cell proliferation in a p53-dependent manner and Survivin is negatively regulated by wild-type p53 and induces apoptosis in a p53-dependent manner", both Cul7 and Survivin may be correlated with p53. But it was not clear whether Cul7 could lead to the accumulation of Survivin through direct polyubiquitination of p53. For further verification, we selected p53-null H358 and H1299 cells in our study and found that Cul7 could regulate Survivin protein level even through p53 deletion. It will need more experiments to verify the interaction relationship between them. To make the manuscript more complete, we have discussed this issue in the sentence "In addition, we found that the upregulation and

downregulation of Cul7 expression could lead to the corresponding changes of Survivin protein level in wild-type p53 A549 cell or in p53-null H358 and H1299 cells." in lines 434-436.
Changes in the text: See page 14, lines 434-436.

Comment 2: According to Fig. 1F, the increase of Cul7 in H358DTX cells was much less than that in A549DTX cells. However, the increase of IC_{50} value in H358DTX cells was similar to than that in A549DTX cells. How to explain this phenomenon via the hypothesis described in line 364-365 and line 98-99?
Reply 2: Thank you very much for this important comment. Please allow us to explain this result. Firstly, we need to compare the difference of IC_{50} values between docetaxel resistant cells and parental cells. Secondly, the docetaxel IC_{50} value of H358 parental cell was higher than that of A549 parental cell. A549 and H358 cell are two different types of LUAD cells, but docetaxel resistance of them all caused the protein accumulation of Cul7 and Survivin. This phenomenon in line 98-99 can be fully explained by the above results. In order to make the expression of the manuscrip more rigorous, we have revised the sentence to "the results of flow cytometry and Western blotting confirmed our hypothesis that the Cul7/Survivin axis promotes the insensitivity of LUAD cells to docetaxel by inhibiting the activation of the intrinsic apoptotic pathway based on experimental results from A549 and A549DTX." in lines 377-380.
Changes in the text: See page 12, lines 377-380.

Comment 3: According to the data shown in Fig. 5E, the protein level of Bcl-2 was increased and the levels of Bax and cleaved Caspase3 were decreased in A549DTX cells, not in "A549 and A549DTX cells treated with 5 nM docetaxel" as described in line 339-341). Similarly, the description in line 429-431 should be revised to "In our study, BCL-2 was upregulated and the protein levels of p21, Bax, and cleaved Caspase3 were decreased in A549DTX cells, in contrast to A549 cells.". The clause "when cells were stimulated with docetaxel (5 nM)" should be deleted.
Reply 3: Thank you very much for this important comment. We have added the relevant description "Meanwhile, there was no significant upregulation of Bax and Cleaved-Caspase3 protein expression was observed in A549 DTX cells stimulated with docetaxel (5 nM) comparing to A549 cells (Figure 5E)." in line 344-346 and revised the sentence to "the protein expression data showed that the level of the antiapoptotic protein BCL-2 was increased and the levels of the apoptotic proteins Bax and Cleaved-Caspase3 were decreased A549DTX cells comparing to A549 cells (Figure 5E)," in line 349-352 according to your suggestion.
Changes in the text: See page 11, lines 344-346 and 349-352.

Comment 4: For semantic and logical description, it is recommended to revise the "However" in line 362, 380 and 399 to "Moreover", "Accordingly" or another adequate word.
Reply 4: We apologize for the error of logical description in our manuscript. We have revised "However" in line 375, 394 and 413 to "Subsequently", "Meanwhile" and "Moreover".
Changes in the text: See pages 12 and 13, in lines 375, 394 and 413.

Comment 5: The descriptions of IC_{50} values in line 272-275, 291-294 and 303-306 do not match that illustrated in the Figures.
Reply 5: We apologize for the of logical description in our manuscript. We have revised the descriptions of IC_{50} values in line 276-277, 298-300 and 311-313.
Changes in the text: See pages 9 and 10, in lines 276-277, 298-300 and 311-313.

Comment 6: There is a typo "cu7" in line 420.
Reply 6: We apologize for the error in our manuscript. We have revised "cu7" to "Cul7" in line 437.
Changes in the text: See page 14, line 437.

Comment 7: According to the descriptions in line 317-319, "neither knockdown nor overexpression of Cul7 caused a change in the Survivin mRNA expression level" and line 398-

399," Cul7 can inhibit Cul9-mediated ubiquitination and degradation of Survivin", no evidence support the effect of Cul7 on the expression of Survivin protein at either transcription or translation level. In line 320, "the protein expression of Survivin" should be revised to "the protein level of Survivin". Similar revisions should also be done in the "expression" in line 3, 45, 107, 312, 316, 317, 325, 331, 332, 360, 361, 404, 435,443, 609, 611, 612, Highlight box, etc. As such, it is recommended to revise the "expression" in the current title of this paper.

Reply 7: Thank you very much for this important comment. We have revised "the protein expression of Survivin" to "the protein level of Survivin" in line 3, 109, 319, 324, 325, 327, 333, 373, 418, 452, 460, 626, 629 and Highlight box.

Changes in the text: See pages 1, 2, 4, 10-14 and 23, in lines 3, 109, 319, 324, 325, 327, 333, 373, 418, 452, 460, 626, 629 and Highlight box.

Comment 8: Besides G1 arrest, increase of p21, p27 may also lead to G2/M arrest (JS-K induces G2/M phase cell cycle arrest and apoptosis in A549 and H460 cells via the p53/p21WAF1/CIP1 and p27KIP1 pathways, Oncol Rep. 2019 Jun;41(6):3475-3487.). If there was no data supporting the G1 arrest of Cul7 knocked-down A549DTX cells, the "G1 arrest" in line 347 should be revised to "cycle-cycle arrest".

Reply 8: Thank you very much for this important question. There was no confirmed evidence of cell cycle flow cytometry. The expression downregulation of CyclinD1, as a checkpoint protein for G1 phase, may not clearly indicate cell cycle arresting in G1 phase. But to some extent, it could also indicate G1 phase arrest. To be cautious, we have revised "G1 arrest" to "cell-cycle arrest" in line 355 according to your suggestion.

Changes in the text: See page 12, line 358.

Responses to Reviewer C

1. The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

In addition, studies have shown that Cul7 can induce epithelial-mesenchymal transition in human choriocarcinoma cells (17).
Reply: OK, we have revised "studies" to "study" in line 400.

2. The authors mentioned "studies...", while no reference was cited. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

Studies have shown that Cul9 is a downstream molecule in 3-M complex signaling pathways.
Reply: OK, we have cited the reference in sentence "*Studies have shown that Cul9 is a downstream molecule in 3-M complex signaling pathways (15, 23).*" In lines 418-419.
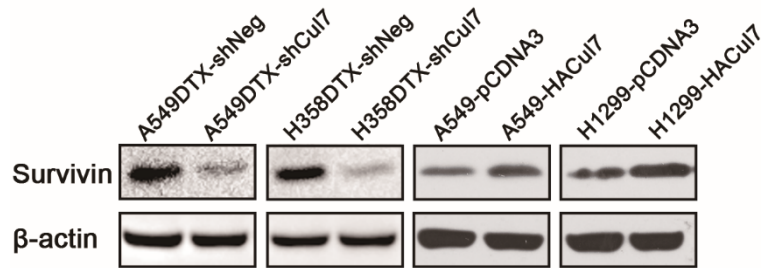
3. Figure 3
No "*" in Figure 3, while it was explained in the legend. Please revise.
Reply: OK, we have deleted the "*" in Figure S1.

4. Figure 4B
Should they be changed to "A549-pCDNA3, A549-HACul7, H1299-pCDNA3, H1299-HACul7". Please check the whole text and figures, and unify accordingly.
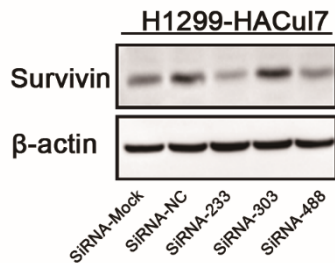
OK, we have revised "A549pCDNA3, A549HACul7, H1299pCDNA3 and H1299HACul7" to "A549-pCDNA3, A549-HACul7, H1299-pCDNA3 and H1299-HACul7" in whole text and all figures.

5. Figure 4F

Should it be changed to "H1299-HACul7". Please check and revise.

Yes, we have revised "H1299HACul7" to "H1299-HACul7" and all figures.

6. IC50 or IC_{50}? Which one is correct? Please check the whole text and all figures, and revise.

OK, we have changed IC50 to IC_{50} in whole text and all figures.

7. Figure S1

No "*" in Figure S1, while it was explained in the legend. Please revise.

OK, we have deleted the "*" in Figure S1.