Author Response 1

Reviewer: 1
Comments to the Author
The manuscript was focused on the autophagy inhibition of CD133+ cell promoting cisplatin efficacy in the NSCLC and A549 cells. In my opinion, this story should be improved by addressing the following issues:
1. The CD133+ cells were elevated upon CDDP treatment. From the figure 1a, it seems that both expression level and proportion were increased for the CD133+ cell. Therefore, the authors need to quantify the CD133+ cells by expression intensity and percentage. Also, the increased proportion acquired by Facs and other methods could be a consequence of CDDP sensitive cell death, which needs to be explained.
Response:
Thanks for the reviewer. The expression of CD133 was examined by real-time PCR and Western blot, and the related data was provided in the Figure 1b. Furthermore, total cells and CD133 positive cells were counted according to immunohistochemical staining and the percent positive cells in each high-power field was calculated. Related data was provided in the Figure S1.
The increased proportion of CD133 positive cells after CDDP treatment was attributed to cells death of bulk tumor cells (CD133 negative cells), another possibility is that cisplatin-treatment itself triggers CD133 expression. We discuss the results in the revised manuscript.

2. It would be important to compare CD133 positive and negative cell viability upon the treatment of CDDP and/or autophagy inhibitors.
Response:
Per the reviewer’s suggestion, we examined the cells viability of CD133 positive and negative cells after treated with CDDP and/or autophagy inhibitors. Data was provided in Figure S2 in the Supplemental Information.

3. It would be more confirmative by using other inhibitors or knockdown of key autophagy genes.
Response:
Per the reviewer’s suggestion, we knockdown ATG5, a key autophagy gene, using shATG5, and we find ATG5 downregulation could counteract the increase of CD133 positive cells induced by CDDP. Related data was provided in the Figure S3 in the Supplemental Information.

Reviewer: 2
Title: Autophagy Inhibition of Cancer Stem Cells Promotes the Efficacy of Cisplatin against Non-small Cell Lung Carcinoma
General comment:
This research was conducted to study on the function of autophagy in cisplatin resistant lung cancer stem cells and the effect of autophagy inhibition in reducing the stemness characteristic of NSCLC cancer stem cells and promotes the efficiency of Cisplatin treatment. Overall, all the experimental procedure conducted in this study were able to answer the main objective of the study.
Minor revision:
1. Introduction.
In the introduction section line 56-58, the author write “Elevated autophagy level supplies enough energy for tumor growth and drug resistance”. Please put an explanation on how the autophagy process promote the tumor growth.
Response:
Thanks for the comment. Actually, autophagy supplies metabolic substrates essential for cancer cell survival, which thereby support tumor growth. We have explained in the revised manuscript.

2. Methodology part of the manuscript was written in very simple version and lacking many important point. Please rewrite by adding the details as below:

- The brand and manufacturer for some materials, reagents and kits used in the experiment were not written. For example the cell culture media (Line 45), RNA extraction kit (Line 54), antibodies (Line 52) and so on. Please include the details.

Response:
We have added the brand and manufacturer for all materials, reagents and kits used in the experiment.

Section 2.2: Real time PCR.
- Line 52. “The hippocampal tissues were collected 12 hours after the interventions”. Why the author did used Hippocampal tissue collected for RNA extraction?
- Please include the primer sequence for all genes measured in real time PCR

Response:
We are sorry for the mistake. Actually, the tumor tissues and cell lines were collected for RNA isolation. We have deleted the wrong information.
We have added all the primers sequence for detected genes.

Section 2.3 Western blot
- What is the amount of proteins loaded into each well of the 10% SDS page?
- For all antibodies, please include the brand, catalogue number and the dilution used.

Response:
Total of total of 10 µg protein was loaded into each well of 10% SDS page.
We have added the brand, catalogue number and the dilution for all the antibodies.

Section 2.4 MTT assay.
- What are the different doses of CDDP used?
- When mentioning about “the protocol were performed according to the manufacturer’s protocol”, it’s good for at least briefly explain the steps performed.

Response:
The doses of CDDP are 0, 20, 40, 60, 80, 100 μM, and we have added the information in “MTT assay”
We have added the steps that was performed in MTT assay.

Section 2.5 and 2.6. Immunohistochemistry and Immunofluorescence
- Again, the protocols was not explained in detail. Please rewrite to include all the details of the protocols including the incubation time for each step for example how many minutes incubation in H2O2 and etc. Please also include the catalogue no, brand and antibody dilution for all antibodies used in IHC.

Response:
We have added all the information in “Immunohistochemistry and Immunofluorescence”.

Section 2.7 Colony and sphere forming assay
- Please include the detail compositions of sphere forming medium.
Response:
The medium for colony formation is RPMI 1640 (Gibco) medium supplied with 10% fetal bovine serum (Gibco). We have added all the information in “Colony and sphere forming assay”.

Section 2.8 Flow cytometry
• Please indicate the antibody clone for CD133
Response:
The clone is W6B3C1. We have added it in “Flow cytometry”.

Section 3. In vivo xenograft model
• In the method there is no explanation on how the CDDP/CQ treatment were given to the subcutaneous tumor. Please write in detail the procedure including the dosage and treatment period (incubation time).
Response:
We have added the details of the procedure, includes the dosage and treatment period in “In vivo xenograft model”.

3. Discussion
• Discussion line 5 and 54: Is it correction or correlation?
Response:
Thanks for figuring out the mistakes. We have modified “correction” to “correlation” in the indicated sites.

Suggestion:
The manuscript need to be send for language proof reading.
Response:
Thanks for the suggestion. We have improved language of the manuscript.