

Dear Editor:

Thank you very much for your comments for our manuscript entitled “Propofol induces ferroptosis and inhibits malignant phenotypes of gastric cancer cells by regulating miR-125b-5p/STAT3 axis”. The reviewer’s and editors’ comments are very helpful for revising and improving our paper. We carefully revised our manuscript according to the editors’ comments point by point. Please find attached reply to comments.

We state that the material is original research, has not been previously published and has not been submitted for publication elsewhere while under consideration. No conflict of interest exists in the submission of this manuscript, and manuscript is approved by all authors for publication.

Thanks for your kind consideration.

Sincerely,

En-You Li

Point by point response to referee comments

Reviewer #1 (Comments to the Author (Required)):

1. Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing) Conclusion: Major revision

Specific Comments to Authors: The study by Liu and colleagues investigates the inhibitory effect of propofol on the progression of gastric cancer which is associated with the regulation of ferroptosis. They presented that propofol decreased the growth and promoted apoptosis of gastric cancer cell lines. Propofol also impaired invasion and migration of gastric cancer cells, which were closely related with the induction of ferroptosis. Furthermore, propofol suppressed STAT3 expression which was mediated by miR-125b-5p. These findings could provide novel insights for gastric cancer

prevention and treatment. The study is well conducted and methods used are appropriate. These findings will be of interest to researchers involved in the treatment of gastric cancer. However, I regret to inform you that your manuscript could not be considered for publication in its present form. My comments are as follows. Major comment;

1. There have been a variety of papers describing the understanding mechanism for the effect of propofol on the proliferation of gastric cancer. Certainly, the data of ferroptosis is novel, but it is doubtful whether ferroptosis is a more important factor for the effect of propofol on malignant phenotypes of gastric cancer compared with apoptosis. How the authors translate this question? Targeting ferroptosis is promising strategy for gastric cancer therapy?

Response: Thank you for your professional and constructive comments. You raised a crucial issue. Ferroptosis is one of the critical malignant phenotypes mediated by propofol in gastric cancer progression. The importance between ferroptosis and apoptosis should be compared by more complicated investigations. Meanwhile, it has been reported that the regulation of ferroptosis may benefit the inhibition of gastric cancer development and targeting ferroptosis may be a promising strategy for gastric cancer therapy. We added the related discussion in the revised manuscript.

2. Overexpression of STAT3 reversed expression of ferroptosis associated proteins, it would be good to see they were also regulated by miR-125b-5p.

Response: Thank you for your professional and constructive comments. We added the related analysis according to the comment in the revised manuscript (Fig. S2).

3. The results of proliferation, colony formation and invasion assays of two different gastric cancer cell lines are so similar. The author should explain why the differences between two cell lines are so similar in most experiments.

Response: Thank you for your professional and constructive comments. Sincerely sorry about the mistakes and we re-performed the analysis in the revised manuscript (Fig. 1 and 2).

4. Figure 1 A & B; In order to assess the growth effects, the proliferation assay should be done in various dose of propofol. The effects were obtained in a dose dependent manner?

Response: Thank you for your professional and constructive comments. We added the related analysis and description according to the comment in the revised manuscript (Fig. S1).

5. The reason determining in vitro concentration of should be described.

Response: Thank you for your professional and constructive comments. We added the related analysis and description according to the comment in the revised manuscript (Fig. S1).

6. Inhibitory effect of propofol on invasion (about 50% at 20nM) looked higher compared to growth inhibition. But it is likely that inhibitory invasion ability by propofol only results in impaired growth of cell lines. How the author explains this question? The possibility cannot be denied that this effect is induced by the inhibition of cell growth. To address this, authors should attempt to isolate factors involving in cancer invasion accelerated by propofol, by doing additional experiments, such as measurement of proteolytic activity.

Response: Thank you for your professional and constructive comments. We added the related analysis according to the comment in the revised manuscript (Fig. 2E).

7. Figure 3&4; The quality of bands seems to be low, so this is hard to tell without densitometry and quantitation.

Response: Thank you for your professional and constructive comments. We added the related analysis according to the comment in the revised manuscript.

8. The evidence showing that transfection of pmirGLO- STAT3 plasmid effectively induced STAT3 expression is required.

Response: Thank you for your professional and constructive comments. You raised a crucial issue. The pmirGLO- STAT3 contained the STAT3 3'UTR, but not CDS region.

9. The wound healing assays, shown in Figure 2 C & D, need to be compared when the control wound is completely closed. The photographs do not show the clear significance by propofol treatments.

Response: Thank you for your professional and constructive comments. We reformed the analysis according to the comment in the revised manuscript (Fig. 2C and D).

10. In vivo experiments need to be repeated at least twice and with at least 10 randomized mice for each experimental group. Did the authors report any toxic effect, as body weight loss, during the experiments?

Response: Thank you for your professional and constructive comments. You raised a crucial issue and provided a kind advice. We added the related analysis according to the comment in the revised manuscript (Fig. 7 and S3). The mice numbers were similar to many previous reports and the body weight had no significant difference in the model (data not shown).

Minor comments:

1. Please provide more detail information of experimental procedure of invasion and migration assay. Matrigel was coated in the bottom of chamber?

Response: Thank you for your professional and constructive comments. We added the related information according to the comment in the revised manuscript.

2. Page 8 line 3-4; Please change "5mmol/L erastin or ferrostatin (1mmol/L)" with "5mmol/L erastin or 1mmol/L ferrostatin" or "erastin (5mmol/L) or ferrostatin (1mmol/L)"

Response: Thank you for your professional and constructive comments. Sincerely sorry about the mistake and we corrected it in the revised manuscript.

3. Page 12 line 12; Please change “by inhibiting miR-125b-5p in ...” with “by up-regulating miR-125b-5p in...” I think it would be serious negligence.

Response: Thank you for your professional and constructive comments. Sincerely sorry about the mistake and we corrected it in the revised manuscript.

Reviewer #2:

Scientific Quality: Grade B (Very good) Language Quality: Grade A (Priority publishing) Conclusion: Accept (General priority)

Specific Comments to Authors: very much interesting and novel research idea. Congratulations.

Response: Thank you for your professional and constructive comments.