

Peer Review File

Article information: <https://dx.doi.org/10.21037/jgo-23-462>

Reviewer A

The authors present complete evidence about how CD44⁺/CD24⁻ CSC resist hypoxia-induced apoptosis, implying the CHOP-mediated ERS apoptosis pathway and activation of the mitochondrial apoptosis pathway.

The results are precise; however, the cytometry data looks very small, and the fluorescence images could be more straightforward. It is suggested to work on their sharpness and contrast.

Reply 1: the sharpness and contrast of the image has been adjusted.。

Reviewer B

The paper titled “Esophageal cancer stem cells reduce hypoxia-induced apoptosis by inhibiting the GRP78-perk-eIF2 α -ATF4-CHOP pathway” is interesting. CD44⁺CD24⁻ tumor stem cells in EC cell line EC9709 can resist hypoxia-induced apoptosis. The molecular mechanism of this resistance lies in the inhibition of CHOP-mediated ERS apoptosis pathway by CD44⁺CD24⁻ tumor stem cells in a hypoxia environment and further inhibit the activation of the mitochondrial apoptosis pathway. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) The description of some methods in this study is too simplistic, please describe in detail.

Reply: I have checked the method section and it is unclear which method descriptions are relatively simple. Can the reviewer point out and I will make corresponding changes.

2) In the results of this study, it was mentioned that "Further detection of stem cell markers SOX2 and OCT4 in cells shown that the expressions of SOX2 and OCT4 in CD44⁺CD24⁻ cells were significantly higher than those in non-sorted cells and non CD44⁺CD24⁻ cells (P<0.05, Figure 1D, 1F)", but 1F did not appear in the figure. Please carefully review and make corrections.

Reply: I mistakenly wrote 1E as 1F, which has been corrected in the results section of the text.

3) Some of the figures in this study have scale bars added, while others have not. Please unify the standards and make corrections.

Reply: I have uniformly added a scale bar to all images in the text.

4) Please discuss the analysis in depth rather than just listing the data.

Reply: The data has been analyzed and explained in the results section of the article.

5) In this paper, it is best to supplement the in vivo research. This is more conducive to support the conclusion of this paper.

Reply: Due to time and funding limits, in vivo experiments will be further conducted in the future.

6) It is recommended to add the molecular mechanism of esophageal cancer and analysis of a wide range of pathological factors.

Reply: the molecular mechanism of esophageal cancer and analysis of a wide range of pathological factors have been added in the introduction section.

7) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as “Long non-coding RNA HAGLROS regulates the proliferation, migration, and apoptosis of esophageal cancer cells via the HAGLROS-miR-206-NOTCH3 axis, J Gastrointest Oncol, PMID: 34790377”. It is recommended to quote the article.

Reply: This reference has been cited.

8) Many of the figures in this study are not clear enough, please adjust and upload again.

Reply: The clarity of the image has been adjusted and reuploaded.

Reviewer C

1) First, the title needs to indicate the experimental research design of this study, i.e., in vitro or in vivo.

Reply: In vitro experiments have been indicated in the title.

2) Second, the abstract needs some revisions. The background did not indicate the knowledge gap on this research focus and the methods did not describe the research questions to be answered by these experimental procedures. The results did not quantify the findings by reporting statistics such as the expression levels and accurate P values. The conclusion should not repeat the main findings and please provide comments for their clinical implications.

Reply: The abstract section of the article has been modified.

3) Third, in the introduction of the main text, the authors need to briefly review what has been known on the molecular mechanisms in EC, have comments on the limitations of prior studies, and explain why the CSCs pathways deserved to be studied. A more detailed comment on the potential clinical significance of this research focus is needed.

Reply: It has been described in the introduction.

4) Fourth, in the methodology of the main text, please describe the research design and have an overview of the experimental procedures, as well as the research questions to be answered by these procedures. In statistics, please indicate the P value for statistical significance.

Reply: the research design, the overview of the experimental procedures and P value have been added in the main text.

Reviewer D

1. Figure 4

Please explain FADD in the legend.

Reply: Have been revised

2. Figure 5

Please explain JNK in the legend.

Reply: Have been revised

3. Figures

Please check the word "Normoxia" in all figures, they should be the same in the figure. Please check figure 7-10, 13-15.

Reply: Have been revised.

