

Answering Reviewers

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Title: Erlotinib combination with a mitochondria-targeted ubiquinone effectively suppresses pancreatic cancer cell survival

Reviewer #1:

Thank you for inviting me to evaluate the Basic Study titled “Erlotinib combination with a mitochondria-targeted ubiquinone effectively suppresses pancreatic cancer cell survival”. Increased activity of the EGFR is often observed in pancreatic cancer, however, the antitumor effect of the small molecule EGFR inhibitor erlotinib alone is limited. In this report, they demonstrated that erlotinib can elevate the mitochondrial membrane potential ($\Delta\psi_m$) in pancreatic adenocarcinoma (PDAC) cells, facilitating tumor cell uptake of the mitochondria-targeted ubiquinone (MitoQ) to the level to break the mitochondrial homeostasis and induce lethal responses, and MitoQ combination with erlotinib can synergistically increase lethal effects in PDAC cells in culture. The paper is well arranged and the logic is clear, and the cited literature is comprehensive and modern. The provided figure and tables are well composed and understandable. The quality of language of the manuscript is quite acceptable for me. So, I recommend to you that this manuscript may be accepted.

Response: Thank you very much for accepting our report and rating our study highly with such enthusiasm.

Reviewer #2:

1. The full name of the 462 and 670 cell lines can be written on the labels without any abbreviation.

Response: Thank you for pointing that out. We have put the full names of 462 and 670 cell lines in figures.

2.

2-1. Only the lower-right picture in picture 1A depicts the groups; if the groups are identical, they should all be designated similarly.

Response: In Figure 1A, the groups are identical for all four figure panels. Therefore, we labeled only the last panel to make the figure concise. The text and figure legends are straightforward, and we believe the readers would be able to figure out this easily.

2-2. This conclusion is insufficient: "the functional moiety of MitoQ CoQ10 (ubiquinone) did not suppress cell viability, while the vehicle moiety TPP mildly decreased cell viability only at higher doses."

Response: These two chemicals are the controls for MitoQ. We believe this description sufficiently indicates that MitoQ is superior to these two controls in suppressing tumor cell viability.

2-3. Figure 1C's IC₅₀ value is excessively high for a three-dimensional culture; an explanation of why this is the case is required.

Response: It is generally known that cells in organoid cultures are more drug-resistant than cells in 2D cultures because cells in organoid cultures are packed in 3D, which decreases drug accessibility. Besides, organoid cultures contain extra protein supplements, which drugs will adsorb. Therefore, the relatively high IC₅₀ values are not unexpected. There is no concern about the data, given that they rationalize the preclinical study.

3. Each group should be shown in a different color in Figures 2A and 3B.

Response: In response to this suggestion, we have adjusted the colors in Fig 2A and 3B.

4. Figures 4A and D have been redone and clearly noted.

Response: We have adjusted the colors and patterns in Fig 4A and D.

5. HIF1 α is typically detectable in hypoxic conditions for 15 minutes; please add to the short time data shown in Fig. 4C.

Response: The purpose of testing HIF1 α is not to show how fast the drugs induced HIF1 α but to confirm that cells were under hypoxic conditions during the drug treatment. Cells were treated sequentially with erlotinib and MitoQ over 72 hours, and the HIF1 α Western blot data confirmed hypoxia during this time frame.