Reviewer’s report

Title: Inhibition of PI3K/AKT molecular pathway mediated by membrane estrogen receptor GPER accounts for cryptotanshinone induced antiproliferative effect on breast cancer SKBR-3 cells

Version: 0 Date: 06 Oct 2019

Reviewer: Konstantinos DiMas

Reviewer's report:

In the manuscript entitled "Inhibition of PI3K/AKT molecular pathway mediated by membrane estrogen receptor GPER accounts for cryptotanshinone induced antiproliferative effect on breast cancer SKBR-3 cells" authors report some data on the effect of CPT against SKBR3 breast cancer cells. However the manuscript suffers from severe flaws and I would consider the whole study as actually a preliminary study.

My main concerns are discussed below

A major concern is that the impact of GPER in the mechanism of action (MoA) of CPT is very little as the compound keeps being quite active even after the silencing of the receptor (fig2B). This shows that GPER is only partially involved in the MoA and its role seems to be not that significant. This is further supported by the results presented in fig 2C where all the changes in viability after co-treatments are absolutely marginal. What is the effect of the compounds G1 and G15 alone? This is not shown at all.

Second authors don't show any experiments with the silenced, for GPER, SKBR3 cells in cell cycle nor in PI3K studies which actually is the most specific manner to study the impact of the receptor in CPT MoA. They also don't show the cell cycle phase changes after co-treatment with the agonist or the antagonist they use. They furthermore don't report the exact conditions of the co-treatments but only the concentrations. Again what is the effect of the two compounds alone? What are the effects of co-treatment with 10uM of CPT?

In fig 3 and since the viability is reduced, according the MTT results, dead cells should be present but they are not. So seems that there is a conflict between Fig 2 and Fig 3.

Authors also don't show the total AKT levels to understand if the change in the p-AKT (what is the phosphorylation the analyze?) is the effect of de-activation or change in the protein levels. They further don't mention how many independent experiments have been performed (they only mention replicates).

What is the post hoc test for statistical analysis? is not reported. The software used for cell cycle analysis is not reported as well.

The manuscript needs also to be seriously revised for linguistic errors.
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

No

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No

Are the conclusions drawn adequately supported by the data shown?
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No

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