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

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Reviewer: Nissar Darmani

Reviewer's report:

Manuscript: Inhibition of PI3/Akt molecular pathway….
Authors: Shi et al.
MS#: PHAT-D-19-00260

Shi and co-workers have studied the anti-proliferative effects of cryptotanshinone (CPT), an active compound of traditional Chinese medicine Danshen) and its molecular mechanisms on nuclear estrogen receptor (nER) but GPER positive breast cancer SKBR-3 cells. Their finding indicate that: i) proliferation of SKBR-3 cells was blocked by CPT in a dose- and time-dependent manner, and ii) CPT arrested cell cycle progression in G1 phase as it down-regulated the expression of Cyclin A, B and D as well as the cyclin-dependent kinase (CDK2). Their pharmacological studies demonstrate that the anti-proliferative of CPT was potentiated by the GPER agonist G1, and the GPER antagonist, G15 blocked the effect. Moreover, PI3K and pAkt expression was down-regulated by CPT via the GPER which was further confirmed by the GPER gene silence test. The authors have concluded CPT exerts its anti-proliferative effect on SKBR-3 cancer cells by inhibiting the GPER-mediated PI3K/Akt signaling pathway.

Overall, the experiments are well designed and appropriate techniques have been applied. My major and minor concerns are written below:

1. The first major problem with the manuscript is the use of English language in the manuscript. Although the authors' English is far superior than this referee's Chinese, it is of critical importance that the authors ask an English speaking colleague of theirs to review the manuscript and make appropriate changes, especially in the introduction and discussion sections.

2. The second major issue appears to be a lack of a concise hypothesis driven proposal regarding the effects of CPT on PI3K/Akt signal transduction system. It should be clearly stated in the manuscript whether CPT may act as an agonist or antagonist. So far published literature have demonstrated that activation of GPER by either its selective synthetic agonist G1, or endogenous E1 (estrone) or E2 (estradiol-17b) agonists of GPER lead to increased pAkt and pPI3K expression (Kamanga-Sollo et al., 2017; Wang et al., 2019; Oliveira et al., 2018). Since combined exposure to CPT+G1 in the current study decreases, while a combination of CPT+G15 increases the expression levels of pI3KpAkt; how do the authors reconcile their finding with those of published literature. In addition, the direct effects of G1 alone and the antagonist G15 by itself, on PI3K and p-AKT have not been investigated in the current which would be very helpful to demonstrate the direct regulation of GPER on PI3K/AKT pathway.
3. In the third paragraph of their discussion, the authors have stated that CPT is capable of activating GPER....What is the possible mechanisms by which CPT activates GPER? Authors should give it a discussion.
1. Authors have not mention which phosphorylation site of AKT was tested in their work.
2. Beta-actin blots shown in Fig. 4 and 5 appear to be the same. Were all proteins running on the same gel?
3. Page 6, line 41, please change Chinese characters to English words.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

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?
If not, please explain in your comments to the authors.

Unable to assess

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I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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