Response to reviewers

Reviewer 03567380**:**

Major Concerns

1) Due to H89 targeting multiple kinases, the authors should use a more specific antagonist (such as KT5720) or genetic approach to ensure the effects of PGE1 are PKA-dependent.

Reply: We agree with reviewer's comments. Although H89 has been the most commonly used PKA inhibitor through competitive inhibition of the adenosine triphosphate site on the PKA catalytic subunit, there are studies showed that H89 has nonspecific effects, includeing actions on other protein kinases and signaling molecules. KT5720 was also found having many nonspecific effects when used at high concentration. Therefore, it is better to use both two inhibitors, we have added the results of the effects of KT5720 on the induction of GRP 78 by PGE1 in figure 5b, KT5720 also inhibited the induction of GRP 78 by PGE1. Therefore, we concluded that the induction of GRP 78 by PGE1 was via PKA pathway.

2) For the apoptosis studies, were the concentration of necrotic cells increased/decreased by any of the treatments? If there was no change, and thus it did not warrant reporting in the manuscript, this technique (line 181) should be removed from the methods.

Reply: After treatment with PGE1, H89 or TG, the concentration of necrotic cells did not significantly increase. So we did not present the results about the necrotic cells and we delete this technique.

please see page 7 line 190~194 in the revised manuscript:

"Flow cytometry (Beckman Coulter Gallios, USA) was performed according to the manufacturer's specifications. The apoptotic index was calculated as the percentage of Annexin V^+ cells divided by the total number of cells in the gated region."

3) Cell viability was only reported on a small subset of experiments performed. The authors should perform MTS assays in all of the groups from experiments 5B, 6D and 6E.

Reply: We have performed MTS assays for 5B, 6D and 6E. Please see figure 5d, figures 6e and 6g.

4) Intracellular calcium should be assessed in both cell lines including all study groups to give a better idea of the specific signaling effects of PGE1 in these in vitro studies.

Reply: Thanks for your valuable comments, TG are known to induce ER stress by blocking ER Ca2+ uptake, which leads to depletion of ER Ca2+ stores. PGE1 can also increase intracellular Ca2+ level by promoting the influx of Ca2+ from the external medium as well as by mobilization of Ca2+ from intracellular stores. A previous study has also shown that cAMP has PKA-independent interaction with Ca2+ stored in lymphocytes. These results indicate that PGE1 and TG might have complex interactions on the intracellular Ca2+ store and need an independent study to clarify their interaction. As the binding of PGE1 with its receptors stimulates the production of the second messenger cyclic 3,5 adenosine monophosphate (cAMP). cAMP may act via distinct intracellular signaling effectors such as PKA and the exchange proteins activated by cAMP. In our study PGE1 significantly induced GRP 78 expression, therefore, we only hoped to clarify whether the induction of GRP 78 and the hepatoprotective effect of by PGE1 dependent on PKA pathways or not. After we used H89 or KT5720 to block PKA pathway, PGE1 lost the induction of GRP 78, demonstrating that the induction of GRP 78 by PGE1 was dependent on PKA pathway. Whether the direct effects of PGE1 and/or it's interaction with TG on intracellular Ca2+ store may also involved in the hepatoprotective effects on ER stress induced apoptosis remain to be clarified in future study.

Minor Concerns

1)Wording and grammar errors exist in the text. An example of this is on line 259 where the text reads, “As showed in Figure 2a…”. This should read “As shown….”. Please carefully proofread entire manuscript and make appropriate corrections.

Reply: Thanks, we have changed it. (please see page 10 line 271 in the revised manuscript). This manuscript has also been edited and proofread by Medjaden Bioscience Limited once again.

“As shown in Figure 2a,”

2) Many of the figures are missing labels or cannot be read. Specific figures that need to be addressed are figure 2C (bars missing/covered), figure 5B (legend only contains 3 of the 5 groups).

Reply: Thanks, we have corrected them, please see figures and figure legends.

3) Line 166, 2820 microM should be converted to 2.82 mM

Reply: Thanks, we have changed it. (please see page 7 line 179 in the revised manuscript)

“PGE1 was dissolved in ethanol at a concentration of 2.82 mM as stock solution.”

4) The fold change values reported for the western blots seem to be scaled incorrectly based on the representative images. For example, in figure 1 GRP78 is essentially absent (with a more intense beta actin band) yet the difference between 0 and 6 hours is only 1.6 fold. sXBP has a much higher fold change though it looks like the change should be equivalent to GRP78. To help explain this better, the authors need to report the fold change +/- standard deviation or standard error of the mean so that variability between the three gels can be determined.

Reply: Thanks, we have corrected them, please see figures.

5) In sections 1 and 2 of the results, the authors report on figures 2a, 2b and 2c (lines 259 through 265) but then come back to them in the next section and report additional data in those figures (lines 268 through 274). These should be combined and not be in different sections (all in section 2 of the results would be best)

Reply: Thanks. Please see the sections of results, we have combined section 1 with section 2 of the results to avoid to describe the same figure in two section. (please see page 10 line 263~264 in the revised manuscript)

1. "TG-induced ER stress and apoptosis in L02 cells and PGE1 protected L02 and HepG2 cells against ER stress-induced apoptosis"

Reviewer 02822428

The authors need to fix the WBs in terms of size and position since some of them are larger than the others. Do the cells change morphologically after TG treatment or they keep the same phenotype when treated with PGE1? Some representative images would help. Moreover, a graphic abstract with a summary of the results would be also valuable. Last, but not least, what happens to JNK activation in presence/absence of PGE1 upon TG-induced ER stress?

Reply: Thanks for your valuable comments. We have adjusted all WBs results to the same size and position, please see figures. We have only observed morphological changes of L02 cells by microscope, after treated with TG, a part of cells presented the morphologically characteristics of apoptosis, unfortunately we did not take the pictures. The other cells did not show significant morphologically changes on cell phenotype when treated with PGE1. For we hope to further explore the mechanisms underlying the hepatoprotective effects of PGE1 on ER stress induced apoptosis, we did not present a graphic abstract to summary our results at this

study. As the reviewer point out whether JNK signal also play some roles warrant further studies. After the mechanisms is further clarified, we will present a graphic abstract to summary our results.