Author’s response to reviews

Title: S1PR2 antagonist ameliorate high glucose-induced fission and dysfunction of mitochondria in HRGECs via regulating ROCK1

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Author’s response to reviews:

Thank you again for your comments concerning our manuscript entitled“S1PR2 antagonist ameliorate high glucose-induced fission and dysfunction of mitochondria in HRGECs via regulating ROCK1” (ID: BNEP-D-18-00806).

Those comments are all valuable and very helpful. It is very important to improve our paper, and optimize this research. We have studied comments carefully and made correction which we hope meet with approval. Revised portion are marked in red in the new manuscript. The main corrections in the paper and the responds to the reviewer’s comments are as following:
Responds to the reviewer’s comments:

1. Response to comment (Reviewer 1): (Authors block S1PR2 and demonstrate benefits, particularly in mitochondrial function, in HG treated endothelial cells. However, they need to demonstrate that S1P is indeed secreted by the cells under high glucose conditions and acting in paracrine manner. A time course would possibly justify the duration of HG exposure (72hr, which seems very long considering intervention was only for 30 minutes if I understand correctly)

Response:

(1) We have modified certain descriptions in the introduction that addressed out focus on the role of S1PR2 in the endothelial dysfunction without the effect of S1P.

(2) The HRGECs were pretreated with JTE-013 and Y27632 30min, indicted that JTE-013 and Y27632 intervention were 30min longer than HG intervention. For the time point selected, we observed a long intervention time point based on previous report (see reference [26]).

2. Response to comment (Reviewer 1): (The discussion is very superficial and basically reiterates the results without discussing them in the context of diabetes and its complications. Authors also need to discuss the potential mechanism driving S1P secretion and the possible implications for blocking it.)

Response: To support our findings in the context of clinical importance, we have added sentences in the discussion section to address the reason and importance of our findings in diabetic nephropathy in page 14, paragraph 3.

3. Response to comment (Reviewer 1): (Is the cell death mechanism apoptosis? Ann/PI does not discriminate other forms of cell death. Caspase 3 should be measured.)

Response: In this article, we focus on the fission and dysfunction of mitochondria in HRGECs. We explore the role of S1PR2 in HRGECs dysfunctions which conclude the permeability, apoptosis, and migrations. Apoptosis is one of the cell dysfunctions we researched. And we picked Annexin-V/PI as the reagent for detecting apoptosis to reflect the cell dysfunction.
4. Response to comment (Reviewer 2) :(In figure 1A and 3B, although the overall mitochondrial shape seems to be restored by the JTE-013 or Y27632 treatments, the low magnification pictures provided by the Authors make it difficult to observe the mitochondria cristae morphology. Larger magnification pictures would be helpful to elucidate whether the mitochondria are undergoing loss of cristae or are not. Better quality images should be provided for the figures 1D and 3D as well. The pictures provided do not match the graphs.)

Response: Thanks for your good suggestions. We have modified as you advised. Please see the following figures 1,3.

5. Response to comment (Reviewer 2) :(In abstract: I suggest to the Authors to change "diabetic kidney disease" by "high glucose milieu" in Conclusion. The data provided do not support this conclusion.)

Response: We modified as you advised.

6. Response to comment (Reviewer 2) :( Authors should provide data regarding the mitochondrial outer membrane potential in the different groups (e.g. using tetramethylrhodamine ethyl ester dye). It would be helpful to understand the real role of the S1PR2/ROCK1 in harming the mitochondria integrity.)

Response: To detect variation of the mitochondrial outer membrane potential we used the lipophilic cation 5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolcarbocyanine iodide (JC-1). JC-1 is capable of entering selectively into mitochondria, since it changes reversibly its color from green to red as membrane potentials increase. This property is due to the reversible formation of JC-1 aggregates upon membrane polarization that causes shifts in emitted light from 530 nm (i.e., emission of JC-1 monomer) to 590 nm (i.e., emission of J-aggregate) when excited at 490 nm; the color of the dye changes reversibly from green to red as the mitochondrial membrane becomes more polarized. But from figure 5, the JC-1 monomer showed the color couple green with red. We cannot figure out the variation of the mitochondrial outer membrane potential from the color change. So we measured the mitochondrial function by levels of ATP, ROS, and Ca2+.

7. Response to comment (Reviewer 2) : (Page 1, line 3 - lysophospholipid not "lysosphospholipid").

Response: We modified as you advised.
8. Response to comment (Reviewer 2) :(What was the software used to perform the statistical analysis?)

Response: We add the statistical analysis software (Prism (version 5, GraphPad)) in page 11.

9. Response to comment (Reviewer 2) :(Figure 2D is not convincing. The RhoA WB provided does not match the graph.)

Response: We re-measured the results of WB, and showed the changed graph in figure2D.

10. Response to comment (Reviewer 2) :( In page 12 "Moreover, the increased levels of cell permeability and apoptosis were markedly reduced and the level of migration was significantly elevated in the HG+JTE-013 group compared to the HG-treated group..", please review this sentence.)

Response: We rewrite it as “HG increased cell permeability, induced cell apoptosis, and inhibited cell migration. However, the pretreatment of cells with JTE-013 significantly alleviated these HG-mediated effects”.

11. Response to comment (Reviewer 2) :(The English quality sometimes undermines the understanding of the text.)

Response: Have edited.

We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.