Peer Review File

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Comment 1) In the introduction and discussion the authors describe DNA damage, often elevated in response to oxidative stress, as an important inducer of cell senescence. Hence, can the authors investigate if DNA damage was also present in their experimental conditions (H2O2 and HGP). Either a comet assay or a western blot or immunofluorescence for gH2AX and P-p53 can be performed to address this.

Reply 1) Thank you for your considerate comments. We agree with the reviewer's points. We know that LDH release assay can investigate only cellular cytotoxicity, not DNA damage. It would be our mistake in experimental design to omit the detection of damaged DNA. If we add this information to our manuscript, it will contribute to the improved completeness of experimental logic. We will add this experiment in our future researches. Again we would like to express deep thanks for your wise and invaluable advice.

Comment 2) Can the authors provide more details on how palmitate was prepared please? Was this through a conjugation reaction with BSA or using EtOH to get palmitate into solution?

Reply 2) Thank you for your good comments. The conjugation reaction was carried out using methanol to get palmitate into solution. As the reviewer recommended, we have modified our text.

Changes in the text 2): We have modified our text as advised (see Page 3)

Comment 3) Can the authors mention where anagliptin was purchased from? And was it dissolved in water, DMSO or ethanol?

Reply 3) Thank you for your good comments. Anagliptin was contributed from SANWA KAGAKU KENKYUSHO CO., LTD. (Nagoya, Japan). The conjugation reaction was carried out using DMSO to get anagliptin into solution. As the reviewer recommended, we have modified our text.

Changes in the text 3): We have modified our text as advised (see Page 6)

Comment 4) Fig. 2E: figure legend describes a WB for p16; however this is not

shown!?

Reply 4) Sorry, We made an editing mistake. We provided Fig 2E at the bottom of Fig 2.

Changes in the text 4): We added Fig 2E in Fig 2.

Comment 5) Legend of Figure 4 mentions "Western blotting assay of NOX4 in HUVECs. The bar graphs show the relative protein expression of NOX4 from HUVECs after H2O2 and HGP treatment" Firstly, the legend should mention C), secondly, no bar graphs are shown in figure 4, and thirdly, it looks like the 4-fold increase in NOX4 expression as described in the text seems true for H2O2 treatment, but not for HGP but quantification of the WBs should bring more clarification.

Reply 5) We apologize again, it also was an editing mistake. We mentioned (C) at the Fig 4 legend and provided quantification bar graphs in Fig 4.

Changes in the text 5): We added (C) at the Fig 4 legend and we have modified figure 4.

Comment 6) The WB for ER-stress markers (Fig. 3) are not very convincing. Can the authors provide new, more convincing WB? And also include quantifications of the WB? Hence, another method to assess ER-stress (e.g. detection of mRNA of spliced XBP1) is vital to examine the importance of the ER-stress pathway in these conditions.

Reply 6) Thank you for your considerate comments. As the reviewer recommended, we provided quantification analysis of the Wenstern blotting of the ER stress markers.

Changes in the text 6) We have modified figure 3.

Comment 7) Can the authors include quantification of the SABG stainings please?

Reply 7) Thank you for your wonderful comments. As the reviewer's recommendation, we include quantification of the percentage of SA-β-Gal (+) cells.

Changes in the text 7): We added and modified figure 2C and 2D.

Comment 8) Can the authors describe the FISH (fluorescent in situ hybridization)

experiment in more detail? Based on the information provided, it seems that the authors only performed a immunofluorescent staining and not FISH! If using FISH, then which co-localisation was assessed and which hybridization probes were used?

Reply 8) Thank you for your considerate comments. We agree with reviewer's points, and we have modified our text as the recommendation.

Changes in the text 8): We have modified our text as advised (see Page 6)

Comment 9) Figure 2: immunofluorescence for p16. Given that p16 is a nuclear protein, can the authors explain why the immunofluorescence for p16 gives a cytoplasmic staining?

Reply 9) Thank you for your wonderful comments. However, previous studies have shown that not only nuclear p16 but also cytoplasmic p16 protein expression increase with ageing process. Also, there was a study about the cytoplasmic-nuclear trafficking of G1/S cell cycle molecules and adult human β-cell replication. Here we prepared some references about the cytoplasmic p16 protein expression. (1-3)

1. Idda ML, McClusky WG, Lodde V, Munk R, Abdelmohsen K, Rossi M, et al. Survey of senescent cell markers with age in human tissues. Aging (Albany NY). 2020;12(5):4052-66.
2. Melk A, Schmidt BM, Takeuchi O, Sawitzki B, Rayner DC, Halloran PF. Expression of p16INK4a and other cell cycle regulator and senescence associated genes in aging human kidney. Kidney Int. 2004;65(2):510-20.
3. Fiaschi-Taesch NM, Kleinberger JW, Salim FG, Troxell R, Wills R, Tanwir M, et al. Cytoplasmic-nuclear trafficking of G1/S cell cycle molecules and adult human beta-cell replication: a revised model of human beta-cell G1/S control. Diabetes. 2013;62(7):2460-70.

Comment 10) It wasn't clear to me from the M&M and figure legend which marker was used to detect mitochondrial ROS in cells?

Reply 10) Thank you for your considerate comments. We used Mito SOX (Invitrogen, OR, USA) for measuring ROS generation, but there weren't any mitochondrial markers and we couldn't perform DAPI staining due to the HUVECs are living cells. We change that mitochondrial ROS to just ROS as the reviewer's comment.

Changes in the text 10): we have modified our text as advised (see Page 6)

Comment 11) The authors describe that H2O2-treated or HGP-treated HUVECs undergo cell death and show mitochondrial ROS. Hence, can the authors investigate if the cells died via the mitochondrial-dependent pathway?

Reply 11) As we replied at comment 10, we tried to measure mitochondrial ROS generation with mitoSOX, but we don't have more evidence. Mitochondria or mitochondrial pathway is another specific hot research field. As the reviewer's comment, we agree to need additional study focusing to mitochondria in the future.

Comment 12) Can the authors please specify in text and figure 5, which NLRP was examined? NLRP1 or NLRP3?

Reply 12) It was NLRP3. We already described it in method and results section. We modified NLRP to NLRP3 in fig 5.

Changes in the text 12): We have modified fig 5.

Comment 13)
What do the authors mean by "Differences between groups were compared by one-way analysis of variance, followed by Student's t-test' (page 6). A one-way ANOVA is used to test if the means of 3 groups or more are equal or not. How can this be followed by a Student- t-test whereas a t-test is used to test the difference in mean between 2 groups? Please clarify.

Reply 13) Thank for your good comments and we agree with the reviewer's point. As the reviewer recommended, we corrected the manuscript.

Changes in the text 13): We have modified our text as advised (see Page 6)

Comment) Page 7: the authors wrote: "To confirm the protective effect of anagliptin against cell death induced by H2O2 or glucolipotoxicity" ; this should be "to TEST the effect of anagliptin against cell death induced by H2O2 or glucolipotoxicity" as now you are already assuming that there will a protective effect, while you still need to investigate that!

Reply) Thank you for your comment. We changed that ward to "test" according to your comment.

Changes in the text): We have modified our text as advised (see Page 7)

Comment) Page 7: “Identical patterns of changes were also seen under conditions of HGP stress (Figure 2B)”. The staining patterns are not identical but similar. Please change accordingly.

Reply) Thank you for your comment. We changed that identical” to “similar” according to your comment.

Changes in the text): We have modified our text as advised (see Page 8)

Comment) Page 9 discussion: the authors wrote “These findings indicate that the protective effects of anagliptin against ER stress partly contribute to its anti-senescence effects.” What evidence do the authors provide to make this statement? Yes, anagliptin protects against ER stress, and yes, anagliptin has anti-senescence effects, but you haven’t investigated whether these effects are linked. Hence, I would recommend to rewrite this sentence.

Reply) Thank you for your wonderful comment, and we rewrite the text according to your recommendation.

Changes in the text): We have modified our text as advised (see Page 10)