Peer Review File

Article Information: https://dx.doi.org/10.21037/cdt-21-732

(1) Most importantly, tissue levels of ROS need to be shown, as Txnip is primarily a pro-oxidant molecule. It needs to be determined whether all effects that Txnip exerts are ROS-dependent or -independent. Specifically, the regulation of angiogenesis could be just a secondary effect to changes in thioredoxin activities.

Reply: Thank you very much for your advice. TXNIP is a pro-oxidant molecule and it plays multiple important roles in ROS regulation. It is very necessary to detect ROS in myocardial tissue of mice in each group. On this basis, we can further explore whether the effect of TXNIP on angiogenesis is ROS-dependent or independent. However, since the experiment requires fresh heart tissue, this critical experiment was not designed at the beginning of this experiment. So, it is difficult for us to provide this experimental result at now. We measured the SOD activity and MDA level of each group of mice 4 days after MI. The details are shown in Figure 1. And the reviewer provided us with a very useful proposal, we will further verify it in subsequent experiments.

Changes in the text: "Methods", line 230-247; "Results", line272-281; "Discussion", line 379-391. Figure 1.

(2) Introduction, Page 3 Line 82: Increased expression of HIF-1α during MI can induce the expression of vascular endothelial growth factor (VEGF) and increase

angiogenesis (11-13). Hoever, References 11-13 do not describe the relationship between myocardial infarction and HIF-1, and mainly focus on the link between cancer and HIF-1.

Reply: Thanks for your suggestion. I have revised references 11-13.

Changes in the text: “References”, line 538-548

(3) The reviewer could not find the detail of of Txnip KO and KI mouse models at the GemPharmatech website. Please provide a simply summary of which exons were deleted in KO and how KI was made. Specifically, hich promoter regulates Txnip expression in KI? This is important, as upregulation of Txnip in KI seems only apparent in MI hearts.

Reply: Thanks for your comments. The details of the TNNIP KO and KI mouse models I have put in the Supplementary Appendix and briefly explained in the Methods section. Also, the expression of TXNIP in the KI-Sham group was significantly higher than that in the WT-Sham group ($p<0.05$, n=5). These data were meant to be statistically significant, but I previously did not label it due to limitations of my own understanding.

Changes in the text: “Supplementary Appendix”, Figure S2; “Methods”, line 106-112; “Results”, line 263-264; Figure 1.

(4) It is known that Txnip is a glucose regulator and systemic Txnip KO mice have lower blood glucose levels (Circ Res. 2007 Dec 7;101(12):1328-38.). How were

blood glucose levels in these KO and KI mice? Those systemic phenotypes may affect cardiac function under MI. This is because the heart switches its substrate preference from fatty acids toward glucose during ischemia.

Reply: Thanks for your comments. The blood glucose levels in KO and KI mice I have added in the Supplementary Appendix. Referring to the echocardiography results of each group in the Sham group, we found that although the blood glucose of each group of mice was different, it has not yet caused cardiac left ventricular dysfunction. Therefore, the differences in left ventricular function between the groups of mice after MI have nothing to do with the systemic phenotype of blood glucose.

Changes in the text: "Supplementary Appendix", Figure S3; 393-403.

(5) All data should be analyzed by ANOVA among MI groups (WT, KO, and KI), rather than t-tests (WT vs KO, and WT vs KI).

Reply: Thanks for your suggestion. I have changed the statistical method to ANOVA.

Changes in the text: "Methods", line 251.

(6) Please provide the other echocardiographic values such as LVIDd, anterior and posterior left ventricular wall thickness. Also, without HR, LV functional parameters mean nothing.

Reply: Thanks for your suggestion. I have provided the other echocardiographic

values and HR in Figure 2.

Changes in the text: Figure 2

(7) Figure 4: How did the authors identify the "cardiomyocyte" apoptosis? Unless triple staining was performed with DAPI and a cardiomyocyte marker, it is not appropriate to express the index as "Cardiomyocyte nuclei".

Reply: Thank you very much for your advice. I have provided the immunohistochemical staining of α-actin to confirm that TUNEL staining was from cardiomyocytes.

Changes in the text: "Supplementary Appendix", Figure S4.

(8) In general, HIF1 is translocated from the cytoplasm to the nucleus under hypoxia, where it acts as a transcription factor to regulate expression of many genes including VEGF. Measuring expression level of cytoplasmic HIF1 is not suitable to support the hypothesis. The authors should show the expression/activity of "nuclei"-located HIF1.

Reply: Thank you very much for your advice. I have done this experiment. I measured the expression of HIF-1α in nuclear proteins by WB. The specific content has been presented in the text.

Changes in the text: "Methods", line 158-160; "Results", line 340-344; "Discussion", line 448-451; Figure 6.

(9) The recent study (J Mol Cell Cardiol. 2021 Jun;155: 36-49) suggests that the

UCHL1-HIF1 axis is involved in the prognosis of MI in addition to decreasing oxidative stress in Txnip C247S mutation model. How about the expression of UCHL1 in your mouse models?

Reply: Thanks for your suggestion. We are really interested in the suggestion proposed by the reviewer and we will study it in a different subject. Thank you very much!

(10) Discussion Page 12 Line 401-403 and page 13 Line 431-431: The expressions of HIF-1α in the KI-MI and KI-Sham groups were significantly reduced, suggesting that TXNIP's regulation of HIF-1α is non-oxygen-dependent. The reviewer does not find any data supporting this statement including Figure 6A.

Reply: Thanks again for your suggestion. I have a misunderstanding of how non-oxygen dependent is regulated. I have removed that part of the description.

Changes in the text: Discussion

(11) Method: Please add the depth of anesthesia during the echocardiography.

Reply: Your advice was greatly appreciated. I have added the depth of anesthesia during the echocardiography.

Changes in the text: "Methods", line 204-212.

(12) Please define the symbols (*, #, +, ^, etc) in all figure legends.

Reply: Your advice was greatly appreciated. I have added it in figure legends.

Changes in the text: Figure legends.

(13) In most figures, the unit of y-axis is unclear.

Reply: Your advice was greatly appreciated. I have modified it.

Changes in the text: Figure.

(14) Please add the explanation in Figure 2a. Indicate Left ventricular anterior wall, left ventricular posterior wall, LVIDs, or LVIDd, etc.

Reply: Your advice was greatly appreciated. I have added the explanation in Figure 2 legends.

Changes in the text: Figure 2 legends; Figure 2.

(15) Figure 3C: Was there any difference in BW between the WT, KO, and KI groups? Since they are systemic KO and KI. The baseline BW may be different.

Reply: Your advice was greatly appreciated. I have added a statistical graph of body weight in Figure 3.

Changes in the text: Figure 3.

(16) Figure 3C: Please check the values on the y-axis. What does HW/BW 0.5% mean?

Reply: Thanks for your kind reminding. I have read the relevant literature and found that the unit of HW/BW most people use was mg/g, that is, all values

should multiplied by 1000. I have changed it.

Changes in the text: Figure 3.

(17) Figure 4A and 5A: The microscope images are unclear. Please add higher magnification images.

Reply: Thanks for your kind reminding. I have changed it.

Changes in the text: Figure 4A and 5A.