Author’s response to reviews

Title: PEP-1-SOD1 fusion proteins block cardiac fibroblast activation and angiotensin II-induced collagen production

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Author’s response to reviews: see over
Dear Dr. Fedak and Fujiu

We thank Drs. Fedak and Fujiu for their careful examination of the manuscript and for their constructive comments. The manuscript has been revised by following the reviewer’s suggestion. Additional experiments were performed and new data were added. We hope that the revised manuscript is acceptable for publishing in BMC Cardiovascular Disorders.

The point-by-point responses to reviewer’s comments are as follows:

**Reviewer #1: Paul Fedak**

**Concern #1:**“The data in Figure 4A shows that the cells express alpha-SMA at baseline. This indicates that the cells are myofibroblasts and not fibroblasts. I would recommend that the cells here referred to as myofibroblasts though out the paper.”

**Response:** We thank the reviewer for the valuable comments. As a matter of fact, a low level of $\alpha$-SMA has been found in fibroblasts although myofibroblasts express it at a much higher level upon the Ang II treatment. Moreover, the untreated cells do not express collagen I, supporting that these cells have not become myofibroblasts yet. Therefore, we hope the reviewer agrees that it is more appropriate to refer the untreated cells as fibroblasts.

**Concern #2:**“How can the authors be certain that cell health or viability is not occurring in after treatment?”

**Response:** To address the reviewer’s concern, we have added new data in Fig 3A, showing that SOD1 and PEP-1-SOD1 does not affect cell viability as measured by CCK-8 assay.

**Concern #3:**“There are spelling and grammar errors though out the text that should be corrected. Examples: • Line 341, change “is” to “are” • Line 369, change “show” to “shows”

**Response:** We thank the reviewer for identifying these errors. We have carefully corrected the spelling or grammar errors in the revised manuscript.

**Concern #4:** “The investigators serum-starved the cells prior to SOD1/PEP-1-SOD1 treatment but replaced the serum prior to Ang-II treatment. The rationale for this is unclear and could be better explained in the manuscript for clarity.”

**Response:** The starving condition was intended to mimic the ischemic damage while the serum-contained medium replacement is to mimic the reperfusion condition. We have added the rationale to the manuscript.

**Reviewer #2: Katsuhito Fujiu**
Concern #1: “Like these reports, authors should evaluate effects of this protein in vivo model like angiotensin II infusion model or other cardiac fibroblast activation model.”

Response: We agree with the reviewer that the in vivo experiment is important. However, due to the time constraint, we may study the in vivo effect in the future.

Concern #2: “In Fig 1A and B, cropped western blotting pictures’ quality is not publication grade, especially PEP-1-SOD1 in 1A and 1B, a-tubulin in 1B.”

Response: We have replaced the Fig 1 with better quality data in the revised manuscript.

Concern #3: “In Fig 1C and 1D, there is no statistical analysis.”

Response: The statistical analysis was added in Fig 1.

Concern #4: “In Fig 2A, control staining or counter staining like nuclei staining or something like that is required, especially in PEP-1-SOD1 groups, to shown the existence of cardiac fibroblast in this slide.”

Response: We appreciate the reviewer’s concern. Because Fig 2A were immunostainings of living cells, we did not stain the nuclei. However, by adjusting the brightness and contrast, we can clearly see that a large number of cells were present in each group (see images below). In order to show high quality and clear images, we have kept the original data for Fig 2A in the revised manuscript.

Concern #5: “In Fig 2B-D, authors used ‘#’ for significant statistical difference compared to angiotensin II administration group. However, there are two angiotensin II administration group in these experiments.”

Response: We have used different symbol for different treatment for indicating the statistical analyses.

Concern #6: “In Fig 5A and C, results of western blotting for collagen type III (5A) is
not correspond to its summarized data (5C).”

Response: The mistake has been corrected.