Author’s response to reviews

Title: Granulocyte colony-stimulating factor attenuates myocardial remodeling and ventricular arrhythmia susceptibility via the JAK2-STAT3 pathway in a rabbit model of coronary microembolization

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Response to Reviewers' comments

Dear Dr. Bazoukis,

We thank you for your careful consideration of our manuscript. We appreciate your response and overall positive feedback and made modifications to improve the manuscript.
We hope that you will find the revised paper suitable for publication, and we look forward to contributing to your journal. Please do not hesitate to contact us with other questions or concerns regarding the manuscript.

Best regards,

Reviewer #1

1. Previous question: There are many errors and suspicions in the description of IHC. The tissue is from the rabbit. Why the sources of primary antibodies were chosen from the rabbit (the same species with the tissue) and the second antibody is goat anti-rabbit? It would result in false positive. The DAB is not conjugated to HRP.

Response: We thank the Reviewer. This is a clerical error that occurred during manuscript preparation and translation. It was corrected.

I am still concerned about the antibodies used in this study. In the revised manuscript (P11), the authors claimed that: "with the goat anti-rabbit anti-p-Cx43 (1:300, SC-101660, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-t-Cx43 (1:200, BA1727, Boster Biological Technology Co., Ltd., California, USA), or anti-G-CSFR (1:200, SC-9173, Santa Cruz Biotechnology, Santa Cruz, CA, USA) primary antibody. The sections were incubated with mouse anti-goat secondary antibody labeled with horseradish peroxidase (HRP)". In fact, the SC-101660 and SC-9713 are all from rabbit (https://www.citeab.com/antibodies/3430728-sc-101660-p-connexin-43-ser-368?des=073947ad8fca2096; https://www.citeab.com/antibodies/794519-sc-9173-g-csf-antibody-h-176; Host: rabbit; Reactivity: Homo sapiens (Human), Mus musculus (House mouse), Rattus norvegicus (Rat).) No information of antibody BA1727 is available in the Internet. Theoretically, this experimental method is not feasible. Therefore, the correct negative control in this experiment should be in the tissue of rabbit which does not contain the detected antigen.

Due to the incorrect information of antibodies and negative controls, it is difficult to determine if the IHC is valid or not.

Response: We thank the Reviewer for the comment. The catalog numbers were indeed incorrected in previous manuscript.

Our research group is doing experiments on rabbits, rats, and mice, so there is a large amount of antibody reserves. We apologize for the mistakes in the previous manuscripts, where we did not check these information clearly. In view of the reviewers' comments, we have re-examined the antibodies in the manuscript and the antibodies used in the experiment, and have made corresponding modifications in the revised paper.
For the anti-p-Cx43 antibody, we consulted a lot of literature and commercial products before starting the experiment. We did not find a suitable antibody for detecting p-Cx43 in rabbits. However, we found that anti-p-Cx43 antibody from Santa cruz's was used in the rabbit’s research by Zhenhao Zhang et al[1]. Therefore, we purchased SC-101660 and SC-25165 for experiments and found that SC-25165 can detect p-Cx43 in our experiment.

2. It is unnecessary to claimed that "Data are shown as the mean± SD of at least 3 independent experiments" if each group has certain number (n = 9 or 10).

Response: We thank the Reviewer for the comment. We have revised it in the paper, “of at least 3 independent experiments” was deleted in the revised paper.

3. P14, Line 30: "the G-CSF blocked the effects of G-CSF"?

Response: We are sorry for this. We meant “that AG490 blocked the effects of G-CSF”.

4. P23, Line 27: Why the authors declared in the "Data Availability" section, " No data were used to support this study".

Response: This is a clerical error. The study data are available from the corresponding author upon reasonable request.

Reviewer #2

Authors responded thoroughly to the comments. They have improved the paper significantly.

Response: We thank the Reviewer for taking the time to review our manuscript and for the constructive comments.

Reviewer #3

Figure 4, the positive staining should be labeled in the picture, otherwise, the readers will be confused which spots are stained positive.

Response: We thank the Reviewer for the comment. We provide the revised Figure 4, in which the arrows point to the enriched area, but please note that since the expression of CX43 is high, we could not indicate all enriched areas or the figure would become a mess of arrows.
For G-CSFR, it is widely expressed in the cell, and it is not possible to mark the specific location.

Reviewer #4

1. Why choose AG490 inhibitor, need to supplement other JAK2-STAT3 signaling pathway inhibitors.

   Response: We thank the Reviewer for the comment. We selected AG490 because it is a JAK2-STAT3 inhibitor that is fairly well described [2] and because this inhibitor has been used in previous studies on Cx43 [3-5], but we agree that there are multiple other JAK inhibitors available. Unfortunately, we presently do not have the resources to perform whole additional sets of experiments in this study. Our future studies will examine multiple inhibitors.

2. Please correct some grammatical errors in this paper.

   Response: The manuscript was proofread.

Reviewer #5

The manuscript is well altered, and I'd recommend to accept.

   Response: We thank the Reviewer for taking the time to review our manuscript and for the constructive comments.

References


