Peer Review File

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Reviewer A

In their article "The m6A demethylase FTO protects against pyroptosis and inflammation in doxorubicin-induced heart failure through the TLR4/NF-κB pathway" Weiling Tu et al. presented their novel results regarding the molecular aspects of doxorubicin-induced cardiotoxicity. In the comprehensive and time-consuming experiments the provided the excited results of the role of FTO-TLR4/NF-κB pathway.

The Results are clear, interesting and deserve a publication. The whole article is written in satisfying style and English language. The results have been properly discussed.

Finally, I would congratulate this results and recommend the acceptance of this article in the current form.

Reply 1: Thank you very much for your recognition of our team's research results.

Reviewer B

The authors could show in their analysis that patients with Doxorubicin encounter cardiotoxic effects of the therapy. The main mediator in this context is the protein Fat mass and obesity-associated protein (FTO) being the first discovered RNA m6A demethylase. The authors could show in their study including 20 patients and 20 controls that FTO alleviated Doxorubicin-induced HF by blocking the TLR4/NF-κB pathway.

The main issue the authors should add, as most of the readers might not be familiar with the document is the definition of heart failure in the document: “Guidelines for Diagnosis and Treatment of Heart Failure in China 76 2014”. This is of importance to better elucidate the heart failure patients included in the study.

Reply 1: Thanks for your kindly reminder. We have added the relevant content in the serum sample collection part, as following: ***“The guidelines define HF as a complex group of clinical syndroes in which ventricular filling or ejection ability is impaired due to any structural or functional abnormality of the heart. The main clinical manifestations are dyspnea and fatigue (limited activity tolerance), and fluid retention (pulmonary congestion and peripheral edema). HF is a serious and terminal stage of various heart diseases, with a high incidence and is one of the most important cardiovascular diseases today.”***.

Changes in the text: Page 4, line 84 ~ 89.

Reviewer C

I read with great interest and enthusiasm the basic research study of our Chinese colleagues Tu et al on The m6A demethylase FTO protects against pyroptosis and inflammation in doxorubicin-induced heart failure through the TLR4/NF-κB pathway, which aimed to probe whether FTO manipulated cardiomyocyte pyroptosis, oxidative stress, and inflammation in Dox-induced HF by affecting the m6A modification of TLR4 and then the TLR4/NF-κB pathway. It is well-written with extensive laboratory methodology applied.

However, I have some issues which I would like the authors to address before I proceed reviewing the whole paper.

1. Please describe the demographics of the 20 patients with heart failure, on whom the blood samples were collected, as to age, gender, duration of heart failure , whether the cardiomyopathy is doxorubicin-induced. This is vital for the impact on the clinical application of their paper. Please also describe demographics of the other 20 healthy patients.

Reply 1: Thanks for your professional suggestion. In this revision, we provided clinicopathologic features of the patients and healthy individuals in table 2 (changes in the table file). In addition, we added the description about the clinicopathologic features in result 1 (changes in the text: Page 10, line 232 ~ 236).

2. Was the selection of the study and control patients case-matched, or random?

Reply 2: Thanks for your question. We randomly selected patients with heart failure and healthy individuals.

3. I would like to see in the Results the comparison of the findings in the HF and control patients. A table or graphical presentation would suffice.

Reply 3: Thanks for your useful advice. We have examined the expression of relevant genes in the serum of patients with heart failure and healthy individuals in result 1, and have also supplemented the results of relevant clinicopathologic features.

Changes in the text: Page 10, line 232 ~ 236.

4. Likewise, please include Limitations of the Study.

Reply 4: Your suggestion is well received. We have added the limitation of this study in the discussion part.

Changes in the text: Page 17, line 408 ~ 410.

Reviewer D

1) Please provide more detail the role of YTHDF1 and its relation of TLR4 in the introduction section and discuss it further in the discussion section.

Reply 1: Greatly appreciate your suggestion. We have added relevant content in the introduction and discussion of this manuscript.

2) Figure legends need more detail. For example, how were the western blots analysis? What statistical test was used? What is the n for these studies?

Reply 2: Thanks for your questions. We would like to explain that, first, we have elaborated on western blot in detail in the method part (see in line 162 ~ 180). Next, we have described that differences were analyzed between groups with the unpaired *t*-test and among multiple groups with one-way analysis of variance, with Tukey's multiple comparisons test as the post hoc analysis and n = 3 in the method part (see in line 222 ~ 227) and the legend part.

3) All abbreviations should be defined before the first use.

Reply 3: Thanks for your precious suggestion. We have defined the abbreviations used for the first time in the manuscript. Moreover, we have summarized the abbreviations and uploaded them as a separate file.