Author’s response to reviews

Title: HIF-1α contributes to Ang II-induced inflammatory cytokine production in podocytes

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Version: 2 Date: 01 Jun 2019

Author’s response to reviews:

Dear reviewers and editors,

Thanks for your constructive comments, I have learned a lot from your pertinent advices for my manuscript, "HIF-1α contributes to Ang II-induced inflammatory cytokine production in podocytes" (PHAT-D-19-00021R1). To further improve my article as well to strengthen the conclusion of this paper, I made many modifications according to your opinion. Thanks again for your valuable comments, we have studied comments carefully and have made correction which we hope meet with approval. Revised words are marked in red in the paper. The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

Reviewer #1:
1. Response to comments 1-2.
Previous studies have found that HIF-1α expression changes under Ang II stimulation in podocytes and endothelial cells (PMID 22555025 and 25987665), thus the hypothesis could be made that HIF-1α may participate in the occurrence and the progression of CKD. However, the specific mechanism is not
clear. Based on the detection of inflammatory factors and knockdown the expression of HIF-1α, our findings linked HIF-1α to inflammatory injury in podocytes under Ang II stimulation, thus providing a new potential therapeutic target for Ang II-induced inflammatory injury in podocytes. To measure HIF-1α expression in several time-points. In the Results section, page 8, line 149-150, additional experiments were done to detect the expression of HIF-1α in podocytes under different Ang II stimulation time-points, so “At the same time, up-regulation of HIF-1α expression was detected in different Ang II stimulation duration (Fig. 3b)” and In the Figure legends section, page 17, line 336-339, “(b) The protein expression of HIF-1α in podocytes under Ang II (10−7 M) stimulation in different time-points. β-Tubulin was used as an equal loading marker. (*p < 0.05 in t-test, n=3; ns, not significant)” were added in the revised manuscript.

2. Response to comments 3.
In fact, Ang II-induced changes in blood pressure in rats were detected and recorded in our previous studies (PMID: 28676854) and that systolic blood pressure and urinary albumin level were increased in Ang II-infused rats. In this study, we focused on the mechanism of Ang II-induced inflammatory stress in podocytes and the potential role of HIF-1α in the pathological process, so the renal structural and functional damage caused by hypertension was ignored, which might be the main limitation of this research and will be further explored in our future research.

Corrected the typo (MPC-1) in Figure 1.

3. Response to comments 5.
The immunohistochemistry positive area was indicated with arrowheads. In the Figure legends section, page 16, line 319-326, described as “Arrows pointing to the positive area of immunochemical staining”.

We are very sorry for our negligence of the mark of the statistical significance (*) in Figure 2, 3, 4, all were added in the revised manuscript.

3. Response to comments 7.
Podocytes stimulated with 10−7 M Ang II for 12 h to detect the changes of TNF-a and MCP-1. In the Figure legends section, page 17, line 349-350, described as “Podocytes were transfected with siRNA 934 and then stimulated with 10−7 M Ang II for 12 h”.

Reviewer #2:
Reviewer 2 has no special requests in this manuscript.

Reviewer #3:
1. The names of internal controls (Beta actin and GAPDH) in western analysis as well as in the figure legends were added in the revised manuscript. In the Methods section, page 6, line 103-104, “GAPDH rabbit polyclonal antibody, 1:1000, Proteintech; beta actin rabbit polyclonal antibody, 1:1000, Proteintech”. In the Figure legends section, page 16, line 328-329, “β-actin was used as an equal loading marker”. In the Figure legends section, page 16, line 335-336, “β-
Tubulin was used as an equal loading marker. (*p < 0.05 in t-test, n=3”). In the Figure legends section, page 16, line 338-339, “β-Tubulin was used as an equal loading marker. (*p < 0.05 in t-test, n=3; ns, not significant)”. In the Figure legends section, page 16, line 345-352, “β-Tubulin was used as an equal loading marker. (*p < 0.05 in one-way ANOVA, n=3)”.

2. The first sentence in the result section (It is well known …) was deleted in the revised manuscript.

3. The SNC lane in Figure 4 was explained, In the Figure legends section, page 16, line 344, described as “(scramble negative control, SNC)”.

4. According to the requirement: “Discussion: The last part of the second paragraph (line 45: An increasing number of reports …) needs citation. Please check it.” In the References section, In Page 14, line 296-306, references 14-16 were added in the revised manuscript.

5. The conclusion part was improved by emphasizing on clinical implication of the results. In the Conclusions section, page 10, line 209-213, described as “The present study shows that Ang II induced overexpression of HIF-1α may be related to the increased of inflammatory factors in podocytes. Therefore, HIF-1α is expected to be a new target for the diagnosis and treatment of CKD. However, a lot of research is needed to clarify the mechanism of HIF-1α in the occurrence and progress of CKD”.

Other changes:
We have accepted the language polish service provided by AJE to improve the description of the structure and content of the article. Revised words are marked in red in the paper.

Thanks again to the reviewers and editors for their help. If there is any part that needs to be changed, I will make further improvements.

Sincerely,
Hao Huang and Guohua Ding.