PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 85885

Title: Angiotensin-converting enzyme 2 improves liver fibrosis in mice by regulating autophagy of hepatic stellate cells

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05359269

Position: Editorial Board

Academic degree: MSc, PhD

Professional title: Associate Professor

Reviewer's Country/Territory: India

Author's Country/Territory: China

Manuscript submission date: 2023-05-25

Reviewer chosen by: AI Technique

Reviewer accepted review: 2023-06-01 06:19

Reviewer performed review: 2023-06-14 17:20

Review time: 13 Days and 11 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No novelty
Creativity or innovation of this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No creativity or innovation

Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The research paper presents a study investigating the effects of ACE2 overexpression on liver fibrosis and hepatic sinusoidal remodeling using a mouse model induced by CCl4. The authors explore various aspects, including autophagy, the AMPK/mTOR signaling pathway, HSC activation and apoptosis, intrahepatic angiogenesis, and LSEC capillarization. Overall, the study provides valuable insights into the potential mechanisms underlying the beneficial effects of ACE2 in liver fibrosis. However, there are some areas that need improvement before considering publication. Clarity and Structure: 1. The introduction provides a general overview of liver fibrosis and its significance but lacks a clear research objective. Please revise the introduction to state the aim and objectives of the study clearly. 2. The methods section requires more detailed information. Specify the number of animals used, the specific protocols and techniques employed, and the statistical analyses performed. This will enhance the re-productivity of the study. 3. The results section presents findings in a concise manner but lacks interpretation and discussion of the results. Provide a more in-depth analysis and relate the findings back to the research objectives. 4. The discussion section briefly touches on

the results but lacks a comprehensive analysis and comparison with existing literature. Include a more extensive discussion of the implications of the findings and their relevance to the field and cite this article https://www.mdpi.com/1999-4915/15/6/1231 Scientific and technical improvements 5. Long-term follow-up studies to assess the sustained effects of ACE2 overexpression on liver fibrosis regression and potential side effects. 6. Investigation of the interplay between ACE2 and other signaling pathways or molecules involved in liver fibrosis to provide a more comprehensive understanding of the underlying mechanisms. 7. Inclusion of human samples or clinical data to validate the findings in a translational context and increase the relevance to human liver fibrosis. 8. Exploration of the impact of ACE2 overexpression on liver function, systemic effects, and potential interactions with existing therapies or interventions for liver fibrosis. 9. Consideration of additional techniques, such as gene expression profiling or proteomics, to provide more detailed insights into the molecular changes associated with ACE2 overexpression in liver fibrosis. 10. Discuss the potential adverse effects or safety concerns associated with ACE2 overexpression. This is particularly important if considering the clinical translation of ACE2-based therapies. Statistical Analysis: 11. Specify the statistical tests used and provide appropriate p-values for all comparisons made in the results section. This information is crucial for assessing the significance of the findings. Strengths and Limitations: 12. Emphasize the strengths of the study, such as the comprehensive exploration of various aspects of liver fibrosis and the use of multiple techniques to support the findings. 13. Clearly outline the limitations of the study, including the use of a mouse model, the need for further validation in human studies, and potential unexplored factors or mechanisms. Grammar and Language: 14. The manuscript contains several grammar and punctuation errors throughout. Carefully proofread the entire paper to rectify these errors. 15. The writing style is somewhat convoluted in

certain sections. Simplify the language to improve readability and comprehension for readers. Sentence Structure: Example: "After the activation of quiescent HSCs due to liver injury, their retinoid droplets are lost, α-SMA expression is increased, and large amounts of ECM are released, ultimately leading to liver fibrosis [28]." Revision: "Upon activation due to liver injury, quiescent HSCs lose their retinoid droplets, exhibit increased α-SMA expression, and release large amounts of ECM, ultimately resulting in liver fibrosis [28]." Subject-Verb Agreement: Example: "The results of pathological staining showed that a mouse model of liver fibrosis was successfully established after 8 weeks of intraperitoneal injection of CCl4." Revision: "The results of pathological staining showed the successful establishment of a mouse model of liver fibrosis after 8 weeks of intraperitoneal injection of CCl4." Word Choice and Sentence Clarity: Example: "The regulation of autophagy is closely related to autophagy-related signaling pathways such as the AMPK/mTOR pathway." Revision: "Autophagy regulation is intricately associated with signaling pathways such as the AMPK/mTOR pathway." Use of Articles (a, an, the): Example: "Expression levels of p-AMPK, AMPK, and p-mTOR in HSCs in different groups were detected by western blot." Revision: "The expression levels of p-AMPK, AMPK, and p-mTOR in HSCs in different groups were detected by western blot." Verb Tense Consistency: Example: "rAAV-ACE2 administration increased HSC apoptosis." Revision: "rAAV-ACE2 administration increases HSC apoptosis." Use of Abbreviations: Example: "α-SMA is well established as an important indicator for evaluating HSC activation and proliferation." Revision: "Alpha-smooth muscle actin (α-SMA) is well established as an important indicator for evaluating HSC activation and proliferation." Sentence Clarity and Wordiness: Example: "The present results showed that CCl4 injection increased Beclin-1 and LC3II protein levels, while rAAV2/8-ACE2 injection notably decreased them, indicating that ACE2 overexpression can effectively ameliorate autophagy in liver fibrosis in mice." Revision: "The results indicated that

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CCl4 injection increased the levels of Beclin-1 and LC3II proteins, whereas rAAV2/8-ACE2 injection notably decreased them, suggesting that ACE2 overexpression effectively ameliorates autophagy in mouse liver fibrosis." Overall, with substantial revisions addressing the mentioned concerns, this research paper has the potential for publication. The suggested improvements will enhance the clarity, rigor, and applicability of the study, strengthening its contribution to the field of liver fibrosis research.

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Creativity or innovation of this manuscript	[Y] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No creativity or innovation

Scientific significance of the conclusion in this manuscript	[Y] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[Y] Grade A: Priority publishing [ ] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [Y] Accept (General priority) [ ] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The present manuscript describes data which strongly suggest that ACE-2 plays a crucial role in HSC activation, thus modulating ECM formation, sinusoidal capillarization, and sinusoidal fibrosis. The authors present several lines of evidence: ACE-2 overexpression was achieved in an animal model by injection of a viral vector containg the ACE-2 gene. Fibrosis was induced by CCl4. ACE-2 overexpression alleviated CCl4-induced fibrosis, as shown by immunohistochemistry, e.g. Fibronectin and alpha-SMA, reduced serum markers of fibrosis, e.g. PDGF-BB and VEGF. TEM and TUNEL staining demonstrated reduced apoptotic bodies in HSC. The role of the AMPK/mTOR pathway was investigated using the mTOR inhibitor rapamycin. Western blot analysis whowed characteristic overexpression or dowregulation of constitutents of the pathway and the effect of rapamycin. Generally, the manuscript presents evidence to prove a hypothesis established earlier about the role of HSCs in development of hepatic fibrosis. In addition, it opens a novel possibility to mitigate or even reverse liver firbosis or even cirrhosis in the clinical context. ACE-2 may a novel target for pharmacological interventions

RE-REVIEW REPORT OF REVISED MANUSCRIPT

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Academic degree: MSc, PhD

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Author's Country/Territory: China

Manuscript submission date: 2023-05-25

Reviewer chosen by: Li Li

Reviewer accepted review: 2023-07-28 03:02

Reviewer performed review: 2023-07-28 03:13

Review time: 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
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| **statements** | Conflicts-of-Interest: [   ] Yes   [Y] No |

SPECIFIC COMMENTS TO AUTHORS

The authors have addressed all my comments in a positive way