



PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 59136

Title: Extracellular histones stimulate collagen expression in vitro and promote liver fibrogenesis in a mouse model via the TLR4-MyD88 signaling pathway

Reviewer's code: 02441070

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Doctor, Professor

Reviewer's Country/Territory: China

Author's Country/Territory: United Kingdom

Manuscript submission date: 2020-08-27

Reviewer chosen by: Jia-Ping Yan

Reviewer accepted review: 2020-09-24 15:07

Reviewer performed review: 2020-09-24 15:13

Review time: 1 Hour

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input checked="" type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input type="checkbox"/> Grade B: Minor language polishing <input checked="" type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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SPECIFIC COMMENTS TO AUTHORS

this paper report Extracellular histones stimulate collagen expression and potentially promote liver fibrogenesis via TLR4-MyD88 signaling pathway. it would bring some new information in this area.



PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 59136

Title: Extracellular histones stimulate collagen expression in vitro and promote liver fibrogenesis in a mouse model via the TLR4-MyD88 signaling pathway

Reviewer's code: 00505755

Position: Editorial Board

Academic degree: PhD

Professional title: Senior Research Fellow

Reviewer's Country/Territory: Japan

Author's Country/Territory: United Kingdom

Manuscript submission date: 2020-08-27

Reviewer chosen by: Jia-Ping Yan

Reviewer accepted review: 2020-09-24 05:23

Reviewer performed review: 2020-09-28 07:52

Review time: 4 Days and 2 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input checked="" type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input type="checkbox"/> Yes <input checked="" type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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SPECIFIC COMMENTS TO AUTHORS

This study demonstrates the important role of histones in liver fibrosis via TLR4-MyD88 signaling pathway. Figure 1 may be revised to add the results of Western blots in 4 weeks after CCl4 injection. The labels of CCl4 and CCl4+NAHP may be added in Fig1 A and B. The results of Student Newman-Keuls test may be added in figures. Please carefully proofread the manuscript.



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Name of journal: World Journal of Gastroenterology

Manuscript NO: 59136

Title: Extracellular histones stimulate collagen expression in vitro and promote liver fibrogenesis in a mouse model via the TLR4-MyD88 signaling pathway

Reviewer's code: 03476715

Position: Editorial Board

Academic degree: MD, PhD

Professional title: Professor

Reviewer's Country/Territory: China

Author's Country/Territory: United Kingdom

Manuscript submission date: 2020-08-27

Reviewer chosen by: Jia-Ping Yan

Reviewer accepted review: 2020-09-13 14:17

Reviewer performed review: 2020-10-03 13:09

Review time: 19 Days and 22 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input checked="" type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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SPECIFIC COMMENTS TO AUTHORS

This research is interesting through exploring the importance of histones in liver fibrosis via TLR4/MyD88. In vivo, how does the downstream signal pathway express through TLR4 or MyD88 knockout. Please check Fig 1 carefully.



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Name of journal: World Journal of Gastroenterology

Manuscript NO: 59136

Title: Extracellular histones stimulate collagen expression in vitro and promote liver fibrogenesis in a mouse model via the TLR4-MyD88 signaling pathway

Reviewer's code: 00646519

Position: Editorial Board

Academic degree: PhD

Professional title: Doctor

Reviewer's Country/Territory: Mexico

Author's Country/Territory: United Kingdom

Manuscript submission date: 2020-08-27

Reviewer chosen by: Jia-Ping Yan

Reviewer accepted review: 2020-09-24 14:22

Reviewer performed review: 2020-10-05 18:58

Review time: 11 Days and 4 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input checked="" type="checkbox"/> Grade B: Very good <input type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input checked="" type="checkbox"/> Minor revision <input type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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SPECIFIC COMMENTS TO AUTHORS

In the manuscript the authors present the effect of histones in liver fibrogenesis, these are some of my comments. Title should include "in vitro and in mouse" and exclude "and potentially promote liver fibrogenesis". Please, be more precise with the P value. Although there are differences in 5 and 10 Ug/ml (in figure 2), the standar deviatios are large to give a solid interpretation. Authors should be cautions aboout this. This is an interesting paper with its limitations. As the same authors point out, the fibrinogenesis process is more complex than only the synthesis of collagen. They must indicate it in their manuscript



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Name of journal: World Journal of Gastroenterology

Manuscript NO: 59136

Title: Extracellular histones stimulate collagen expression in vitro and promote liver fibrogenesis in a mouse model via the TLR4-MyD88 signaling pathway

Reviewer's code: 00255973

Position: Peer Reviewer

Academic degree: PhD

Professional title: Associate Professor, Professor

Reviewer's Country/Territory: Canada

Author's Country/Territory: United Kingdom

Manuscript submission date: 2020-08-27

Reviewer chosen by: Jia-Ping Yan

Reviewer accepted review: 2020-09-09 15:35

Reviewer performed review: 2020-10-06 03:41

Review time: 26 Days and 12 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
Peer-reviewer statements	Peer-Review: <input checked="" type="checkbox"/> Anonymous <input type="checkbox"/> Onymous Conflicts-of-Interest: <input type="checkbox"/> Yes <input checked="" type="checkbox"/> No



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SPECIFIC COMMENTS TO AUTHORS

In this manuscript, the authors provide evidence for histones released during hepatocyte injury as an important contributor to the pathogenesis of liver fibrosis and that neutralizing the circulating histones may have a beneficial effect. The idea has novelty and the data are supportive of this notion. However, the data shown are minimal and additional experiments and data could make it an interesting paper. The protocol for eliciting liver fibrosis and treatment with NAHP must give adequate details. Specifically, on page 11, for how long did the mice receive CCl₄ for liver fibrosis induction? In mice receiving CCl₄, for how long NAHP was administered? If it is every 12h, 4 weeks of CCl₄ treatment (8 injections) was also accompanied by 56 injections of NAHP? (which is quite a lot of manipulation!) A schematic diagram representing CCl₄ and NAHP administration is required to understand Fig 3C. I presume ICR mice were used in all experiments other than Fig. 4. Please indicate that in figure legends. The authors note that histones can activate TLR2, TLR4 and TLR9. They use only TLR2 neutralizing Ab (PAb-hTLR-2), and there is an error in the 'heading' of the methods section as well as in Fig. 4A, which indicate 'neutralization of TLR4'. Do LX-2 cells express all three TLRs? If yes, what is the relative contribution of each to histone stimulation? Can it be blocked by a drug targeting a common downstream effector molecule/pathway? The LX2 activation by histones is related only to increased levels of collagen 1 on cells and in media (Fig. 2A, 3A, 4A). It is possible that the added histones may just interfere with the degradation of collagen. To determine if histones indeed elicit a pro-fibrogenic response in LX-2 cells, it is necessary to evaluate the expression of fibrogenic genes such as Acta1, col1a1, etc. by RT-PCR. The change in morphology of LX-2 after histone exposure (Fig. 2B) does not indicate strong activation. Therefore, it is necessary to use some other ligands (e.g., TGF β) for comparison. The authors show reduced fibrosis in TLR4^{-/-},



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MyD88^{-/-} mice (already known) as evidence for impaired histone mediated fibrogenesis. It is important to show if these mice present comparable amounts of histones in circulation following CCl₄ treatment. Besides, it is important to show activation of downstream signaling molecules of TLR4 in CCl₄ and CCl₄+NAHP - treated mouse livers.