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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 74751

Title: Family with Sequence Similarity 134 Member B-mediated Reticulophagy Ameliorates Hepatocyte Apoptosis Induced by Dithiothreitol

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03560845
Position: Peer Reviewer
Academic degree: DSc, MD, PhD
Professional title: Chairman, Professor, Senior Research Fellow
Reviewer's Country/Territory: Russia
Author's Country/Territory: China

Manuscript submission date: 2022-01-05

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-01-12 05:08

Reviewer performed review: 2022-01-15 08:15

Review time: 3 Days and 3 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] 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

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Peer-reviewer statements	Peer-Review: [ ] Anonymous [Y] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The work presented for review is very interesting and is done using modern research methods. While reading the manuscript, a number of questions arose. 1. How many cultures (biological and technical replicates) were studied for each exposure period? 2. The authors indicate that they used one-way analysis of variance to identify statistically significant differences. However, this criterion assumes the distribution of features corresponding to the normal distribution law. Did the authors check the distributions in the sample and by what criterion? 3. In the case of a Western blot, uncropped membranes with mass markers must be included in the Supplement. 4. For confocal microscopy (Figure 2, 3), it is necessary to provide quantitative data, such as the collocalization coefficient, as well as a statistical analysis of the data obtained.

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Peer-review model: Single blind

Reviewer's code: 05346681
Position: Peer Reviewer
Academic degree: PhD
Professional title: Lecturer
Reviewer's Country/Territory: Thailand
Author's Country/Territory: China

Manuscript submission date: 2022-01-05

Reviewer chosen by: AI Technique

Reviewer accepted review: 2022-01-26 04:22

Reviewer performed review: 2022-01-27 05:30

Review time: 1 Day and 1 Hour

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Authors investigate mechanism underlying protective effect of an ER-phagy mediator FAM134B on the dithiothreitol-induced apoptosis of rat hepatocyte cell line. Understanding in alteration of ER-phagy is valuable target to treat hepatic injury in liver diseases. However, there are concerns regarding utility of in vitro model employed and adequacy of data. Major issues: In experimental design, a rational to use DTT as an inducer of ER stress-caused apoptosis of hepatocytes need to be explained. Major drawback is to use only rat cell line BRL-3A to draw a conclusion. Human hepatocyte cell line might serve as a model physiologically relevant to human more than rat hepatic cell. A. Introduction: some explanations in the result part can move to introduction.

1) GRP78 is a prominent ER molecular chaperone, and CNX is a membrane-bound lectin protein in the ER that can increase the protein folding capacity (Ref). 2) Even though the excessive build-up of misfolded or unfolded proteins can be alleviated via ER stress, previous studies reported that a selective autophagic pathway defined as ER-phagy can also be activated by ER stress to restore ER homeostasis (Ref). B. Methods: 1) To allow others reproduce the data, provide dilutions of all antibodies used in the study. 2) Provide method for measurement of protein concentration. What used as standard? 3) Taking confocal image at each time points is not regarded as liver imaging. Confocal images need nuclear stain to indicate cells. Moreover, higher magnification or zoom-in mode will improve quality of data. Add scale bars. Which program was used to visualize images? Any image modification that will significantly alter quality of images, i.e., contrast adjustment. 4)Provide sequences of siRNA 5)Make sure the appropriate use of statistic. The t-test is a method that determines whether two

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populations are statistically different from each other, whereas ANOVA determines whether three or more populations are statistically different from each other. C. Results 1) DTT-mediated ER stress triggers ER-phagy mediated by FAM134B in BRL-3A cells 1.1 Add references for "GRP78 is a prominent ER molecular chaperone, and CNX is a membrane-bound lectin protein in the ER that can increase the protein folding capacity (Ref)." 1.2 Provide un-cropped images as supplemental data 1.3 Add references for "Even though the excessive build-up of misfolded or unfolded proteins can be alleviated via ER stress, previous studies reported that a selective autophagic pathway defined as ER-phagy can also be activated by ER stress to restore ER homeostasis (Ref)." 1.4 No data show ER-phagy mediated by FAM134B in this section. All data show upregulation of ER stress concomitantly to increased level of ER-phagy-related proteins (FAM134B, ATG12, and LC3). 1.5 Heading of "DTT-mediated ER stress triggers ER-phagy mediated by FAM134B in BRL-3A cells" overwhelms the data. Given a lack of ER-phagy, this heading shall be changed to "DTT-mediated ER stress increase/upregulate ER-phagy-related FAM134B in BRL-3A cells". 1.6 Add reference for "It has been shown that FAM134B may interact with CNX in the cytosol or ER membrane (Ref)." 1.7 It is unclear to me for the sentence of "There is an indirect interaction between FAM134B and lumenal proteins through the lumen-resident segment, which has chaperone activity in CNX." To my understand, CNX is membrane-bound lectin protein. Or do you mean "FAM134AB indirectly interact with luminal proteins via a chaperone CNX". Please clarify. 1.8 As a summary of section, "Collectively, these results suggest that ER-phagy mediated by FAM134B sequesters stressed ER membranes via a CANX-FAM134B-LC3B complex". There was no evidence of ER-phagy here. The ER stress-induced increase of FAM134B and interaction of CANX-FAM134B-LC3B only indicate possibility of ER-phagy. Without the ER-phagy data, please avoid overwhelmed conclusion. 2) Endolysosomal delivery of ER is gradually blocked, but relieved in BRL-3A cells

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treated with DTT long-term 2.1 To avoid confusion, please consistently use ER autolysosome. 2.2 As mentioned above, higher magnification or zoom-in mode of confocal images would allow co-localization. Use arrows or arrow heads to indicate ER-localized lysosomes. Also, nuclear stain is important to define a cell, but not artifacts. 2.3 According to "Notably, the cells treated for 48 h were targeted to the lysosomes at higher rates compared to those treated for 24 h (Fig. 2)", which panels of Fig. 2 indicate higher rate of lysosome targeting? 3) ER-phagy mediated by FAM134B induces mitochondrial calcium uptake at early time points, but mitochondrial calcium uptake is reduced after prolonged DTT treatment 3.1 To emphasize on FAM134B-mediated ER-phagy causing an increase of calcium uptake in mitochondria, knockdown of FAM134B is required. Moreover, level of calcium in Mt should be presented in quantitative manner, i.e., use of ImagJ to analyses fluorescence intensity. 3.2 Use italic for gene names. Also follow gene symbol for species. 4) DTT treatment induces cell cycle arrest and apoptosis of BRL-3A cells, which is relieved at 48 h 4.1 Fig. 4A, provide FSC, SSC and way to gate cell for analysis. Which quadrant represent apoptotic cells? 4.2 Fig. 4C, show gate of S-phase. Gating for G1 and G2 phase is too small. Given three experiment performed, a statistic analysis of cell cycle should be calculated. 5) BRL-3A cells undergo apoptosis upon FAM134B knockdown 5.1 To indicate ER-phagy, ER autolysosome change must be examined 5.2 Fig. 4B show that 24-h DTT induce apoptosis. In Fig. 5E-H, Need untreated control to verify that DTT still able to induce apoptosis. 5.3 There is controversy in Fig. 5Gand Fig. 4A. Fig. 4A, the 24-h DTT-treatment show apoptotic cells. But, here lower percentage of apoptosis was observed in Fig. 5G. Discussion on this controversy might help. 5.3 To draw a conclusion in which FAM134B-mediated ER-phagy in the DTT-treated cells, marker of ER-phagy (ER autolysosome) need to be demonstrated. Bar graph of Fig. 5G-H indicates a synergistic effect of DTT and FAM134B knockdown on apoptosis, implying

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cytoprotective effect of FAM134B likely via ER-phagy if data support.). 5.4 Flow cytometry profile suggest inadequate compensation of the emitted fluorescence of PI and FITC, as suggested by pseudo color dots arranging as a line.

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Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 02440884

Position: Editorial Board

Academic degree: MD

Professional title: Chief Doctor, Full Professor, Professor, Senior Lecturer

Reviewer's Country/Territory: Germany

Author's Country/Territory: China

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Reviewer chosen by: AI Technique

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Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No

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Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In the experimental study the role of FAM134B-mediated ER-phagy on ER stress-induced apoptosis is investigated using BRL-3A rat hepatocytes. The authors demonstrate convincing data that FAM134B-mediated ER-phagy attenuates hepatocyte apoptosis by suppressing the mitochondrial apoptotic pathway. Comments 1. In the abstract, the ER-resident protein called 'family with sequence similarity 134 member B' (FAM134B), that can form a complex with calnexin (CNX) and microtubule-associated protein LC3II should be shortly introduced to the reader as protein which can mediate the selective isolation of ER fragments. 2. Results: in the end of the second paragraph the conclusion should be changed: Thus, our results revealed that expression of FAM134B is induced in response to ER stress. 3. Results: in the begin of the third paragraph a reference should be given: It has been shown that FAM134B may interact with CNX in the cytosol or ER membrane (REFERENCE). 4. In order to form the CANX-FAM134B-LC3B how is the role of the chaperone calnexin? Do you have data argue for calnexin as a core protein or is this role more given by FAM134B? 5. The data could be further validated by the use of another cell line or a functional model. Do you think the molecular findings are characteristic for hepatocytes? Both points should be addressed in the Discussion.

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 74751

Title: Family with Sequence Similarity 134 Member B-mediated Reticulophagy Ameliorates Hepatocyte Apoptosis Induced by Dithiothreitol

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03560845

Position: Peer Reviewer

Academic degree: DSc, MD, PhD

Professional title: Chairman, Professor, Senior Research Fellow

Reviewer's Country/Territory: Russia

Author's Country/Territory: China

Manuscript submission date: 2022-01-05

Reviewer chosen by: Jia-Ru Fan

Reviewer accepted review: 2022-03-25 11:58

Reviewer performed review: 2022-03-26 12:31

Review time: 1 Day

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] 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
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

All comments were answered satisfactorily.