



ESPS PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 17160

Title: Overexpression of pim-3 and its role in activation of rat hepatic stellate cell stimulated by LPS

Reviewer's code: 02822399

Reviewer's country: United States

Science editor: Ya-Juan Ma

Date sent for review: 2015-02-23 10:03

Date reviewed: 2015-03-09 19:10

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input checked="" type="checkbox"/> Grade C: Good	<input type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Duplicate publication	<input type="checkbox"/> Rejection
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input checked="" type="checkbox"/> Minor revision
<input type="checkbox"/> Grade E: Poor		BPG Search:	<input type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

Liu and colleagues tried to investigate pim-3 expression in HSC-T6 stimulated by LPS as well as its protective effect on apoptosis of HSC-T6. They performed several techniques to suggest that LPS could play a protective role through up-regulation of pim-3 expression in HSC-T6. I have some comments: 1- The calculation of OD is strange. Is it 52.33? Or it is percentage of results calculated in relation to control? Why the control in table 1 and 2 has values other than 100%. 2- In the discussion: "Normal cells induced by special stimulus can also up-regulate expression of pim-3 kinase, such as anoxia/reoxygenation injury or ischem/reperfusion injury or cytokine or LPS treatment[9,10]". It have been reported previously that pim3 is overexpressed by LPS? What is the novelty of your manuscript. 3- Why did you starved your cells in the culture protocol. This starvation will reduce viability and increase apoptosis, while your results illustrated the opposite. 4- Regarding the significance signs in the figures: - In figure 2, you used: *p<0.05 vs 0h and 3h. **p<0.01 vs 0h. You should use different signs for different time points. In addition, it appears that in figure 2D there is a significant difference between 0h and 3h. - In figure 3, you did not measure the significance against



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LPS group. - In figure 4b, you did not measure the significance of LPS against control group. 5- There are some typos and grammatical errors.



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Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 17160

Title: Overexpression of pim-3 and its role in activation of rat hepatic stellate cell stimulated by LPS

Reviewer's code: 02439020

Reviewer's country: China

Science editor: Ya-Juan Ma

Date sent for review: 2015-02-23 10:03

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Table with 4 columns: CLASSIFICATION, LANGUAGE EVALUATION, SCIENTIFIC MISCONDUCT, CONCLUSION. It contains checkboxes for various evaluation criteria like 'Grade A: Excellent', 'Duplicate publication', 'Plagiarism', etc.

COMMENTS TO AUTHORS

Dear Dr. Lin-hua Liu, Thanks for submitting your manuscript to WJG. I have read it with interest, the study is meaningful. However, the present manuscript lacks comprehensiveness and some information are not clearly presented. I would suggest the authors to carry out a revision on the present manuscript before it can be accepted for publication in WJG. My specific comments are as follows: 1. The "Abstract" is written too simple and not well managed. Please write the "abstract" section properly and include all the important information in your study. 2. In the "Introduction" section, please pay attention to the standardization of abbreviation in scientific writing. 3. A reference is needed regarding the rat hepatic stellate cell lines HSC-T6 gifted from Scott L. Friedman' 4. Please give manufacturer information: company name, town, state (if applicable) and country. 5. The "Material and methods" section is too simple, no mention of many methods, such as flow cytometry analysis. 6. The "Results" section is very confusing and hard to understand, and needs to be reorganized and properly written to enable a better understanding of the data. In the third paragraph of "Results" section, the title is misleading, please check it and correct it. 7. More fresh references



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within 5 years are more persuasive. 8. The figure 4 is in poor quality. Please provide better quality figure. 9. The English of the manuscript is pretty ordinary, and not very scientific. Thanks for submitting your manuscript to World Journal of Gastroenterology.

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Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 17160

Title: Overexpression of pim-3 and its role in activation of rat hepatic stellate cell stimulated by LPS

Reviewer's code: 02447901

Reviewer's country: Taiwan

Science editor: Ya-Juan Ma

Date sent for review: 2015-02-23 10:03

Date reviewed: 2015-02-26 11:31

CLASSIFICATION	LANGUAGE EVALUATION	SCIENTIFIC MISCONDUCT	CONCLUSION
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	Google Search:	<input type="checkbox"/> Accept
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language polishing	<input type="checkbox"/> The same title	<input type="checkbox"/> High priority for publication
<input type="checkbox"/> Grade C: Good		<input type="checkbox"/> Duplicate publication	
<input checked="" type="checkbox"/> Grade D: Fair	<input checked="" type="checkbox"/> Grade C: A great deal of language polishing	<input type="checkbox"/> Plagiarism	<input checked="" type="checkbox"/> Rejection
<input type="checkbox"/> Grade E: Poor	<input type="checkbox"/> Grade D: Rejected	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Minor revision
		BPG Search:	<input type="checkbox"/> Major revision
		<input type="checkbox"/> The same title	
		<input type="checkbox"/> Duplicate publication	
		<input type="checkbox"/> Plagiarism	
		<input checked="" type="checkbox"/> No	

COMMENTS TO AUTHORS

This study tested the expression and role of pim-3 in rat hepatic stellate cell line, HSC-T6, stimulated by LPS. The authors found that LPS stimulated HSC-T6 cell proliferation and inhibited spontaneous apoptosis. LPS stimulation caused a delayed expression in pim-3 mRNA and protein. The silence of pim-3 expression decreased HSC-T6 cell proliferation and caused apoptosis as well as attenuated LPS-induced alterations. The authors concluded that LPS could play a protective role through up-regulation of pim-3 expression in HSC-T6. Pim-3 belongs to a serine/threonine kinase family and plays a role in promoting cell proliferation and antagonizing apoptosis. Its involvement in malignancy is reported in several cases. Evidence also showed that the elevated expression of pim-3 protected liver against fulminant hepatitis. The findings that pim-3 might involve in LPS-stimulated hepatic stellate cells are interesting. However, there still remain several criticisms for improvement. Specific comments were appended as follow. 1. The English should ask careful editing to improve its readable. 2. The spontaneous apoptosis of HSC-T6 cells over 16% was strange. Generally, cell lines grew well. As said in Materials and Methods "For experiment, HSC-T6 cells were seeded into 96-well



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plates at 1×10^4 cells/well. After being cultured in complete medium for 12 h, HSC-T6 cells were starved for 24 h in DMEM supplemented with 0.75% fetal bovine serum, then the culture medium was replaced with DMEM complete medium." Why the cells should be starved prior to experiments? How about the outcomes of regular cells culturing growth media? 3. In Table 1 and 2, the value in control was 50.33±2.3%. How to get this value? 4. The figure legends should state the experimental conditions. 5. The labels of statistical significance should be checked. 6. Normally, LPS shows mitogenic and fibrogenic effects on hepatic stellate cells. In addition to proliferation and apoptosis, the changes in fibrogenic genes should be investigated. 7. In figure 2, paired control should be included in time course study. 8. According to Table 1, HSC-T6 cells grew well at 24 and 48 h after seeding. If the cells kept growing, the role of spontaneous apoptosis was bare. 9. In Table 2, the silence of pim-3 attenuated LPS-stimulated proliferation. However, si-pim3 plus LPS-treated cells still showed better viability than si-pim3-treated cells. These findings suggest that LPS also showed proliferative potential in si-pim3-treated cells. Then, prosurvival mediators other than pim-3 might present. 10. As said in Discussion "However, about the proliferation of HSC in vitro stimulated by LPS, different cells and different detection assays have different outcomes. The proliferation from primary HSC assessed by [³H] Thymidine incorporation is unchanged[5], while that from HSC lines assessed by MTT is increased[16]." If so, what is the conclusion after this study?