UNIVERSIDAD AUTÓNOMA DE NUEVO LEON
FACULTAD DE MEDICINA
Departamento de Bioquímica y Medicina Molecular

Monterrey, NL México February 10th, 2016

Ze-Mao Gong
Scientific Editor
World Journal of Gastroenterology

Dear Editor,

Thank you for sending us the reviewers' comments on our manuscript entitled **"S-adenosyl-L-methionine modifies antioxidant-enzymes, glutathione-biosynthesis and methionine-adenosyl transferases-1/2 in Hepatitis C Virus-expressing cells"** (ESPS Manuscript NO: 23935) and forwarding your helpful comments. We did a major revision on the manuscript and we wish to submit the revised manuscript for consideration for publication as an invited original article in your prestigious journal. We hope this new version is closer to meet the WJG acceptance criterions.

Attached you will find the new manuscript which contains the changes suggested by the reviewers, they were highlighted in the manuscript with underlined or deleted. In addition you will find a file whit the point –by-point response to the reviewers whit the changes described before. Mainly, the title was changed, material and results section were modified, we included specific highlights, we reviewed the grammar and writing errors as suggested by the reviewers.

All the authors of this manuscript certify that:

a) This manuscript contains original work, has not previously been published and is not being considered for publication elsewhere.
b) All listed authors participated meaningfully in the study and all have seen and approved the final manuscript.

In addition, as you suggested we properly prepared, named and included the new manuscript and all accompanying documents as follow:

1 23935-Revised manuscript---------- Document Anexed
2 23935-Answering reviewers ---------- Document Anexed
3 23935-Copyright assignment -------- Document Anexed
4 23935-Audio core tip ---------- Document Anexed
5 23935-Institutional review board statement ---------- Document Anexed
6 23935-Institutional animal care and use committee statement ----- Do not apply
7 23935-Animal care and use statement ---------- Do not apply
8 23935-Biostatistic statement ---------- Do not apply
9 23935-Conflict-of-interest statement ---------- Document Anexed

Ave. Fco. I. Madero y Dr. Eduardo Aguirre Pequeño, Col. Mitras Centro, C.P. 64460, Monterrey, N.L. (México)
Tel. (52-81) 83-29-41-73 /and (52-81) 83-29-41-74 ext. 2816 Fax. (52-81) 83-33-77-47 e mail: amrivas1@yahoo.ca
arivas@fm.uanl.mx

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10 23935-Data sharing statement ---------- Do not apply
11 23935-Google Scholar ---------- Document Anexed
12 23935-CrossCheck ---------- Document Anexed
13 23935-Grant application form ---------- Document Anexed
14 23935-Language certificate ---------- Document Anexed

Thanks for your invitation and consideration, I am looking forward to hearing from you soon.

Sincerely,

Dr. Ana María Rivas-Estilla PhD
Chief of the Laboratory of Molecular Infectology
Department of Biochemistry and Molecular Medicine

Ave. Fco. I. Madero y Dr. Eduardo Aguirre Pequeño, Col. Mitras Centro, C.P. 64460, Monterrey, N.L. (México)
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POINT- BY- POINT RESPONSE TO REVIEWERS

Name of journal: World Journal of Gastroenterology

ESPS manuscript NO: 23935

Title: S-adenosyl-L-methionine modifies antioxidant-enzymes, glutathione-biosynthesis and methionine-adenosyl transferases-1/2 in Hepatitis C Virus-expressing cells.

Remarks: Date: February 10th, 2016
Reviewers' Comments to Author:

We performed all the suggested modifications to the edited manuscript send by the Editor, and in addition we also performed all modification suggested by the two reviewers as follow:

Reviewer: 1 Reviewer's code: 00032726

COMMENTS TO AUTHORS
In this study, the authors investigated mechanisms by which S-adenosyl-L-methionine (SAM) inhibits the replication of hepatitis C virus (HCV). With a HCV subgenomic replicon cell culture system, the authors evaluated the effects of SAM by quantifying the expression of some antioxidant enzymes and viral proteins on both transcriptional and translational levels. They reported that SAM can inhibit HCV by modulating the expression of antioxidant enzymes, up-regulating glutathione, and switching the ratio of MAT1/MAT2 in HCV infected cells. This study is interesting and can be useful to investigators in related field. However, there are several problems should be addressed before publication.
Major:
1. In the first part of RESULT (line 247), the authors only detected the expression of HCV-NS5A to evaluate the anti-viral effect of SAM. Why chose this viral protein? Is it a conservative protein, a functional protein related with SAM, or the hall marker protein of the HCV replicon that authors used in this study?
Response: The HCV RNA genome is around 9600 nucleotides in length, which encodes a single polyprotein of around 3000 aminoacid residues. This polyprotein is cleaved co- and post-translationally by both viral and host proteases, producing the mature HCV structural (core, E1 and E2) and nonstructural (NS2, NS3, NS4, **NS5A** and NS5B) proteins. NS5A is an HCV non-structural protein which has an important role of favoring conditions for virus propagation, and in a specific way, NS5A interacted with NS5B and formed the HCV replication complex, if this union does not happen the replication rate goes down, then, if NS5A levels decrease, it will reflect on the HCV replication.[1] In addition, by detecting NS5A viral protein we are measuring the overall viral protein yield because all viral protein are synthesized and processed as polyprotein simultaneously.

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arivas@fm.uanl.mx

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1. *Lee, H S, Li, Y S, Park, E M, Baek, S H, Hwang S B. SUMOylation of nonstructural 5A protein regulates hepatitis C virus replication. J Viral Hepat. 2014 Oct;21(10):e108-17. DOI:10.1111/jvh.12241.*

2. The authors investigated the replication of HCV on both transcriptional and translational levels (the second part of RESULT, Fig 2A and Fig 2B). But they used different markers (RPS18 for the mRNA level and N35A for the protein level). Please give the reason.
Response: We investigated the replication of HCV at transcriptional and translational level, as follows:
a) For transcriptional evaluation, we measured by quantitative RT-PCR the viral RNA levels (HCV-RNA) contained into the treated cell cultures under different experimental conditions and, in order to normalize and validate our results we used two different endogenous genes (cell markers) most commonly used for this kind of assays, GAPDH and RPS18 (we chose those genes because its amplification rates are similar to our target).
b) For translational evaluation, we extracted total proteins from cell cultures under different experimental conditions and then we measured levels of a viral protein (NS5A, which is encoded by the HCV-RNA measured before) by western blot assay. In this case we used two cellular protein marker (actin and GAPDH) in order to normalize and validate the western blots performed. These two proteins are the most usually evaluated in this kind of assays.

3. In the third part of RESULT (line305 to line 317, Fig 3A), Why the HCV mRNA level can be significantly increased after blocking the cellular transcription? The reason that authors given in the text (line 310: “not degrade”) seems not convincing enough.
Response: After a cell is infected by virus, numerous cellular mechanisms are triggered to prevent the virus completes its viral cycle. Many of these mechanisms depend on of the activation and increased synthesis of proteins with specific antiviral activity (as PKR, Mx1,OAS), so when cellular translation is inhibited, also decreases the synthesis of these proteins, then the levels of viral RNA could increase during this period, due to the absence of inhibiting replication proteins. We have added this information in the text (please see page 14).

Minor:
1. The English writing of this manuscript should be polished to improve the readability.
Response: As you suggested, we checked the manuscript for grammatical errors to improve the readability. Also we add the language certificate from the reviewer.

2. The structure of the text should be re-edited. Some part describing the result of experiment should not be put into the section of METERIALS AND METHODS (eg. line 147 to line 149).
Response: As the reviewer suggested we moved some information from Materials to Result section (please see page 11)

Abbreviation is not suggested to be used in the ABSTRACT. If must, please give a annotation (eg. SAM, S-adenosyl-L-methionine).

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Response: As the reviewer suggested we revised the total abbreviations contained in whole document and we defined them the first time they are showed.

Conclusion: Minor Revision.
Response: As the reviewer suggested we performed a grammar and content information changes in discussion section (please see page 19).

Reviewer: 2 **Reviewer's code:** 02939835

COMMENTS TO AUTHORS
Well written paper with clear hypothesis. Your explanations of proposed mechanisms were clear and understandable. Figures were well presented. Might it be possible to expand further the statements made from line 473 to 476 (can you explain further the additive effects)?
Response: As the reviewer suggested we expanded the information and discussion of this point in discussion section (please see page 19).

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