Long non-coding RNA LINC00467 regulate hepatocellular carcinoma progression by modulating miR-9-5p/PPARA expression

Kerui Cai, Tieling Li, Ling Guo, Haifeng Guo, Wei Zhu, Lei Yan and Fujuan Li

Review History

RSOB-19-0074.R0 (Original submission)

Review form: Reviewer 1

Recommendation
Major revision is needed (please make suggestions in comments)

Are each of the following suitable for general readers?

a) Title
Yes

b) Summary
Yes

c) Introduction
Yes
Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Is it clear how to make all supporting data available?
Yes

Is the supplementary material necessary; and if so is it adequate and clear?
Not Applicable

Do you have any ethical concerns with this paper?
No

Comments to the Author
I thank the editor for the assignment of the review task of this manuscript, and I thank the authors for their contribution.

This paper describes the involvement of long noncoding RNA Linc00467 in hepatocellular carcinoma progression and the underlying molecular mechanisms. They provided clinical and in vitro data to support the importance of Linc00467 in the hepatocellular carcinoma. Overall, the data looks nice and convincing. Some revisions are preferred before publishing these results. The comments are specified below:

Major concerns:
1. The number of samples for normal and cancer is not equal in Figure 1A. Please explain. Also, what did you mean "normal"? Did you mean healthy participants?
2. The idea to employ two cell lines is good. However, it would be nice to include some discussion of limitations about the findings in other cancer cells.

Minor concerns:
3. The "Hepatocellular Carcinoma" in the title should not be capitalized.
4. The first sentence in the abstract is not complete. Please use complete sentence here.
5. Some abbreviations, including in the title and other sections, are not defined. Please note that there is no need to abbreviate them if used only once.
6. Do you justify the cells before use?
7. Some more experimental details are required, such as the PCR primer sequence.
8. MTT is usually employed for cell viability assessment, not proliferation. Please check.
9. S.D is wrongly defined in the Statistical analysis.
10. It seems that some set of the data is not appropriate to be analyzed with ANOVA analysis. Please check.
11. Some typos should be corrected throughout the manuscript.

Review form: Reviewer 2

Recommendation
Accept with minor revision (please list in comments)
Are each of the following suitable for general readers?

a) Title
   Yes

b) Summary
   Yes

c) Introduction
   Yes

Is the length of the paper justified?
   Yes

Should the paper be seen by a specialist statistical reviewer?
   No

Is it clear how to make all supporting data available?
   Yes

Is the supplementary material necessary; and if so is it adequate and clear?
   Not Applicable

Do you have any ethical concerns with this paper?
   No

Comments to the Author
In this MS entitled "Long noncoding RNA LINC00467 regulate Hepatocellular Carcinoma progression by modulating miR-9-5p/PPARA expression", the authors did a nice work to elucidate the role of LINC00467 in the hepatocellular carcinoma. It is found that LINC00467 is aberrantly decreased in hepatocellular carcinoma, while ectopic expression of LINC00467 significantly suppresses cell proliferation, migration and invasion in two cell lines SMMC-7721 and HepG2. Furthermore, LINC00467 is found to function as sponge of miR-9-5a and negatively regulates miR-9-5p expression, which target is PPAR. These results are interesting and add new knowledge to the understanding of long noncoding RNAs in hepatocellular carcinoma. Although the findings deserve publication, some clarifications and modifications are needed. They are outlined as follows.

1--Abstract: Please unify the verb tense here. Sometimes it is passive tense, sometimes it is active tense, especially in presenting the results.
2--Introduction: In the last several sentences, please list your hypothesis or study design instead of results or findings.
3--Methods and materials: It seems that the data for figure 1 come from the clinical patients, but I can not find any information in the methods.
4--Results: The unit for Figure 2B Y axis is not correct. It will be better to change it.
5--Discussion: Some more in-depth discussion is needed.
6--Figure legends: In some figure legends, the statistical method is introduced, while there is not in other figures.
Decision letter (RSOB-19-0074.R0)

10-May-2019

Dear Dr Li,

We are writing to inform you that the Editor has reached a decision on your manuscript RSOB-19-0074 entitled "Long noncoding RNA LINC00467 regulate Hepatocellular Carcinoma progression by modulating miR-9-5p/PPARA expression", submitted to Open Biology.

As you will see from the reviewers’ comments below, there are a number of criticisms that prevent us from accepting your manuscript at this stage. The reviewers suggest, however, that a revised version could be acceptable, if you are able to address their concerns. If you think that you can deal satisfactorily with the reviewer’s suggestions, we would be pleased to consider a revised manuscript.

The revision will be re-reviewed, where possible, by the original referees. As such, please submit the revised version of your manuscript within four weeks. If you do not think you will be able to meet this date please let us know immediately.

To revise your manuscript, log into https://mc.manuscriptcentral.com/rsob and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.

You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, please revise your manuscript and upload a new version through your Author Centre.

When submitting your revised manuscript, please respond to the comments made by the referee(s) and upload a file "Response to Referees" in "Section 6 - File Upload". You can use this to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please be as specific as possible in your response to the referee(s).

Please see our detailed instructions for revision requirements https://royalsociety.org/journals/authors/author-guidelines/

Once again, thank you for submitting your manuscript to Open Biology, we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,

The Open Biology Team
mailto: openbiology@royalsociety.org

Reviewer(s)' Comments to Author(s):

Referee: 1

Comments to the Author(s)
I thank the editor for the assignment of the review task of this manuscript, and I thank the authors for their contribution.
This paper describes the involvement of long noncoding RNA Linc00467 in hepatocellular carcinoma progression and the underlying molecular mechanisms. They provided clinical and in vitro data to support the importance of Linc00467 in the hepatocellular carcinoma. Overall, the data looks nice and convincing. Some revisions are preferred before publishing these results. The comments are specified below:

Major concerns:
1. The number of samples for normal and cancer is not equal in Figure 1A. Please explain. Also, what did you mean "normal"? Did you mean healthy participants?
2. The idea to employ two cell lines is good. However, it would be nice to include some discussion of limitations about the findings in other cancer cells.

Minor concerns:
3. The "Hepatocellular Carcinoma" in the title should not be capitalized.
4. The first sentence in the abstract is not complete. Please use a complete sentence here.
5. Some abbreviations, including in the title and other sections, are not defined. Please note that there is no need to abbreviate them if used only once.
6. Do you justify the cells before use?
7. Some more experimental details are required, such as the PCR primer sequence.
8. MTT is usually employed for cell viability assessment, not proliferation. Please check.
9. S.D is wrongly defined in the Statistical analysis.
10. It seems that some set of the data is not appropriate to be analyzed with ANOVA analysis. Please check.
11. Some typos should be corrected throughout the manuscript.

Referee: 2

Comments to the Author(s)
In this MS entitled "Long noncoding RNA LINC00467 regulate Hepatocellular Carcinoma progression by modulating miR-9-5p/PPARA expression", the authors did a nice work to elucidate the role of LINC00467 in the hepatocellular carcinoma. It is found that LINC00467 is aberrantly decreased in hepatocellular carcinoma, while ectopic expression of LINC00467 significantly suppresses cell proliferation, migration and invasion in two cell lines SMMC-7721 and HepG2. Furthermore, LINC00467 is found to function as sponge of miR-9-5a and negatively regulates miR-9-5p expression, which target is PPAR. These results are interesting and add new knowledge to the understanding of long noncoding RNAs in hepatocellular carcinoma.

Although the findings deserve publication, some clarifications and modifications are needed. They are outlined as follows.
1–Abstract: Please unify the verb tense here. Sometimes it is passive tense, sometimes it is active tense, especially in presenting the results.
2–Introduction: In the last several sentences, please list your hypothesis or study design instead of results or findings.
3–Methods and materials: It seems that the data for figure 1 come from the clinical patients, but I can not find any information in the methods.
4–Results: The unit for Figure 2B Y axis is not correct. It will be better to change it.
5–Discussion: Some more in-depth discussion is needed.
6–Figure legends: In some figure legends, the statistical method is introduced, while there is not in other figures.

Author’s Response to Decision Letter for (RSOB-19-0074.R0)

See Appendix A.
RSOB-19-0074.R1 (Revision)

Review form: Reviewer 1

Recommendation
Accept as is

Are each of the following suitable for general readers?

a) Title
Yes

b) Summary
Yes

c) Introduction
Yes

Is the length of the paper justified?
Yes

Should the paper be seen by a specialist statistical reviewer?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
Good revisions. No further comments.

Review form: Reviewer 2

Recommendation
Accept as is

Are each of the following suitable for general readers?

a) Title
Yes

b) Summary
Yes

c) Introduction
Yes

Is the length of the paper justified?
Yes
Decision letter (RSOB-19-0074.R1)

20-Jun-2019

Dear Dr Li

We are pleased to inform you that your manuscript RSB-19-0074.R1 entitled "Long noncoding RNA LINC00467 regulate hepatocellular carcinoma progression by modulating miR-9-5p/PPARA expression" has been re-reviewed and no further changes have been recommended by the referees.

The Editor is ready to accept the manuscript however, there is one remaining query regarding the Western blot films in Figures 5D and 5E that we would be grateful if you could address. When comparing the bands in the figures 5D and 5E with the raw files provided, the bands do not appear to match. We would be grateful if you could re-supply the raw blots with weight markers. The two PPARA panels also appear to have vertical splice lines as attached; please can you review this and provide the uncut files or an explanation for this.

Please address the queries within 14 days. If you do not think you will be able to meet this date please let us know immediately and we can extend this deadline for you.

Please see our detailed instructions for revision requirements. It is essential these instructions are followed carefully to minimise any delay to publication: https://royalsocietypublishing.org/rsob/for-authors

Before uploading your revised files please make sure that you have:

1) A text file of the manuscript (doc, txt, rtf or tex), including the references, tables (including captions) and figure captions. Please remove any tracked changes from the text before submission. PDF files are not an accepted format for the "Main Document".

2) A separate electronic file of each figure (tiff, EPS or print-quality PDF preferred). The format should be produced directly from original creation package, or original software format. Please note that PowerPoint files are not accepted.
3) Electronic supplementary material: this should be contained in a separate file from the main text and meet our ESM criteria (see http://rsob.royalsocietypublishing.org/site/misc/styleandpolicy.xhtml#question11). Please note that ESM files are NOT edited by the Royal Society so should be submitted as the authors intend readers to view them with accompanying title/s and caption/s. Where possible we request that authors combine multiple ESM files into one file (for example, where ESM files are in Word or PDF format). In addition, the number of references included in the ESM should be kept to an absolute minimum as these are not recognised by many indexing services.

4) A media summary: a short non-technical summary (up to 100 words) of the key findings/importance of your manuscript. Please try to write in simple English, avoid jargon, explain the importance of the topic, outline the main implications and describe why this topic is newsworthy.

Images
We require suitable relevant images to appear alongside published articles. Do you have an image we could use? Images should be approximately 200 mm x 300 mm, be in a four-colour format and have a resolution of at least 300 dpi.

Once again, thank you for submitting your manuscript to Open Biology, we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Sincerely,

The Open Biology Team
mailto:openbiology@royalsociety.org

Reviewer(s)' Comments to Author:

Referee: 2
Comments to the Author(s)
No comments.

Referee: 1
Comments to the Author(s)
Good revisions. No further comments.

Author’s Response to Decision Letter for (RSOB-19-0074.R1)

See Appendix B.
Decision letter (RSOB-19-0074.R2)

24-Jul-2019

Dear Dr Li

We are pleased to inform you that your manuscript entitled "Long noncoding RNA LINC00467 regulate hepatocellular carcinoma progression by modulating miR-9-5p/PPARA expression" has been accepted by the Editor for publication in Open Biology.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it within the next 10 working days. Please let us know if you are likely to be away from e-mail contact during this time.

Article processing charge
Please note that the article processing charge is immediately payable. A separate email will be sent out shortly to confirm the charge due. The preferred payment method is by credit card; however, other payment options are available.

Thank you for your fine contribution. On behalf of the Editors of Open Biology, we look forward to your continued contributions to the journal.

Sincerely,

The Open Biology Team
mailto: openbiology@royalsociety.org
I thank the editor for the assignment of the review task of this manuscript, and I thank the authors for their contribution. This paper describes the involvement of long noncoding RNA Linc00467 in hepatocellular carcinoma progression and the underlying molecular mechanisms. They provided clinical and in vitro data to support the importance of Linc00467 in the hepatocellular carcinoma. Overall, the data looks nice and convincing. Some revisions are preferred before publishing these results. The comments are specified below:

Major concerns:
1. The number of samples for normal and cancer is not equal in Figure 1A. Please explain. Also, what did you mean "normal"? Did you mean healthy participants?

   Reply:
   We thank this reviewer for the question. Normal means adjacent non-cancerous tissue, we have changed this in the figure 1 and the legend. Yes, indeed, the number is not equal, as some of the adjacent non-cancerous tissues were contaminated during experiments, and we have no way to go back to collect these tissues.

2. The idea to employ two cell lines is good. However, it would be nice to include some discussion of limitations about the findings in other cancer cells.

   Reply:
   We thank for the comment. More discussion of limitations was included in this revision as requested by the reviewer. Please see the below for your reference.
   “It is worth noting that there are several limitations in the current study. First, although two human hepatocellular carcinoma cell lines were employed, it would be more convincing to see similar regulation of LINC00467 in other tumor cells. Second, only in vitro studies were conducted in this research, evidences from in vivo animals would strengthen the conclusions. Last, a larger number of clinical samples should be further verified for the correlation between the expression of LINC00467 and hepatocellular carcinoma.”

Minor concerns:
3. The "Hepatocellular Carcinoma" in the title should not be capitalized.

   Reply:
   It was corrected.

4. The first sentence in the abstract is not complete. Please use complete sentence here.

   Reply:
   We re-phased this sentence as requested.

5. Some abbreviations, including in the title and other sections, are not defined. Please note that there is no need to abbreviate them if used only once.

   Reply:
   We carefully checked the entire manuscript and made necessary corrections.
6. Do you justify the cells before use?  
Reply:  
Yes, the cells were verified by STR analysis before use. This information was added in the Methods.

7. Some more experimental details are required, such as the PCR primer sequence.  
Reply:  
The PCR primer sequences had been showed in method.

8. MTT is usually employed for cell viability assessment, not proliferation. Please check.  
Reply:  
Yes, we agree with the reviewer on this point. We have modified the relevant writing.

9. S.D is wrongly defined in the Statistical analysis.  
Reply:  
We corrected this typo now.

10. It seems that some set of the data is not appropriate to be analyzed with ANOVA analysis. Please check.  
Reply:  
We agree with this reviewer on this point. Data for Figure 2C, D, Figure 6B, C were re-analyzed with two-way ANOVA. We have modified the relevant writing.

11. Some typos should be corrected throughout the manuscript.  
Reply:  
We proofread the entire manuscript and made necessary modifications.

Responses to Referee: 2  
Comments to the Author(s)  
In this MS entitled "Long noncoding RNA LINC00467 regulate Hepatocellular Carcinoma progression by modulating miR-9-5p/PPARA expression", the authors did a nice work to elucidate the role of LINC00467 in the hepatocellular carcinoma. It is found that LINC00467 is aberrantly decreased in hepatocellular carcinoma, while ectopic expression of LINC00467 significantly suppresses cell proliferation, migration and invasion in two cell lines SMMC-7721 and HepG2. Furthermore, LINC00467 is found to function as sponge of miR-9-5a and negatively regulates miR-9-5p expression, which target is PPAR. These results are interesting and add new knowledge to the understanding of long noncoding RNAs in hepatocellular carcinoma. Although the findings deserve publication, some clarifications and modifications are needed. They are outlined as follows.
1--Abstract: Please unify the verb tense here. Sometimes it is passive tense, sometimes it is active tense, especially in presenting the results.

Reply: We proofread this section, and made necessary modifications as suggested by this reviewer.

2--Introduction: In the last several sentences, please list your hypothesis or study design instead of results or findings.

Reply: We agree with this reviewer on the point. We modified these several sentences as suggested.

3--Methods and materials: It seems that the data for figure 1 come from the clinical patients, but I can not find any information in the methods.

Reply: We apologize for the missing information. We added the patient's information to the method section.

4--Results: The unit for Figure 2B Y axis is not correct. It will be better to change it.

Reply: We have corrected Figure 2B Y axis.

5--Discussion: Some more in-depth discussion is needed.

Reply: More discussion of limitations was included in this revision as requested by the reviewer. Please see the below for your reference.

“IT is worth noting that there are several limitations in the current study. First, although two human hepatocellular carcinoma cell lines were employed, it would be more convincing to see similar regulation of LINC00467 in other tumor cells. Second, only in vitro studies were conducted in this research, evidences from in vivo animals would strengthen the conclusions. Last, a larger number of clinical samples should be further verified for the correlation between the expression of LINC00467 and hepatocellular carcinoma.”

6--Figure legends: In some figure legends, the statistical method is introduced, while there is not in other figures.

Reply: We deleted the statistical method description in the figure legends now.
Appendix B

The Editor is ready to accept the manuscript however, there is one remaining query regarding the Western blot films in Figures 5D and 5E that we would be grateful if you could address.

When comparing the bands in the figures 5D and 5E with the raw files provided, the bands do not appear to match. We would be grateful if you could re-supply the raw blots with weight markers. The two PPARA panels also appear to have vertical splice lines as attached; please can you review this and provide the uncut files or an explanation for this.

Response to editors:

We would like to point out that, in previous response to revision, we have submitted a revised version of Figure 5, with replaced Fig 5D and E panels along with the raw files. However, the editor was comparing the old Figure 5 with the new raw files.

Next, please allow us to explain the reason for changing Fig 5D and E in previous revision:

1) In our previous revision, we received the query from the editor, that “The blots (in Fig 5D and E) are over-contrasted to the point where the background has ‘disappeared’”.
2) We went back to re-scan the original film, only to find that it has been improperly cut, therefore unable to be scanned as uncropped raw file.
3) In order to ensure the reproducibility of our results, we then repeated the experiments described in Fig 5D and E, with the intention to use the newly acquired Western blot films to replace the over-contrasted ones in Fig 5D and E.
4) The experiments were successfully reproduced, and the results were exactly the same as previous findings. These new films were then used as raw files.
5) We then submitted the revised Figure 5, with Fig 5D and E panels matching the newly scanned raw film submitted the new raw files.

Second, those “vertical splice lines” were in fact caused by a defect in our scanner, which always gives a line across scanned files, like this demonstrated one below (the line is now roughly horizontal in the middle, due to orientation of scanning).
As this small scanner imperfection merely appears in extreme contrast setting, it has little effect, if any, on our data analysis, so we never replaced the scanner (for funding restrains as well).