MALAT1 promotes gastric adenocarcinoma through MALAT1-miR-181a-5p-AKT3 axis

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Original submission: 25 April 2019
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Final acceptance: 8 August 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History
RSOB-19-0095.R0 (Original submission)

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

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
This paper is well written and I suggest acceptance.

Review form: Reviewer 2

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?
Yes

Do you have any ethical concerns with this paper?
No
Comments to the Author

This is an interesting study with novel findings. The regulatory role of MALAT1 in gastric adenocarcinoma was well elucidated. First, the authors found that MALAT1 was highly expressed in the serum of gastric adenocarcinoma patients and cell lines. Second, down-regulating MALAT1 was able to inhibit the proliferation and promoted apoptosis in MGC-803 cells. Third, MALAT1 was found to directly target miR-181a-5p and decreased the expression of miR-181a-5p, therefore upregulate the expression of AKT3. Last, overexpressing miR-181a-5p or directly inhibiting the AKT pathway with an inhibitor Ipatasertib exhibited similar effects as MALAT1 knockdown. Based on these findings, the authors conclude that MALAT1 competes with AKT3 for miR-181a binding, thereby up-regulating AKT3 protein level and ultimately promoting the growth of gastric adenocarcinoma. These results deserve publication, giving my following concerns could be addressed.

1. The rational to study MALAT1 is not clearly explained. Need more background information, especially in the tumor field.
2. It is better not to include the results and the significance in the introduction section (the last para. in this section).
3. Any informed consents from the participants? This needs to be clarified.
4. The time point to conduct the CCK-8 assay needs to be indicated.
5. Figure legends should be carefully revised. There are some unidentified characters, and the presentation is poor.
6. Why I can not see any error bars in some set of the data in figure 3 and 6?
7. It is preferred to re-arrange the subfigures in Figure 5, especially for the WB image. It looks too small in this figure.

Review form: Reviewer 3

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?
No

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

Major comments:
1. In the results section, the authors mentioned that MALAT1 is highly expressed in gastric adenocarcinoma patients. Yet they only showed that serum MALAT1 level is higher in gastric adenocarcinoma patients compared to healthy control. Why didn't they compare the expression in tumor and adjacent normal tissue as well?

2. The authors should also show the clinical parameter between the two groups of samples, such as age, gender, and use statistical analysis to determine if the serum level of MALAT1 was affected by these parameters.

3. The authors should include more cell-lines of gastric normal and adenocarcinoma cell-line for comparing their MALAT1 levels.

4. More details are needed for the following parts:
What bioinformatic methods did the authors use to predict the possible binding targets between MALAT1 and miR-181a-5p/miR-181a-5p and AKT3?
How were MALAT1 and miR-181a-5p level knocked down?
Did the authors use GAPDH as normalization for lncRNA and miRNA? Why?

5. The expression of miR-181a-5p was negatively correlated with the expression of MALAT1, hence the R2 value should be negative.

6. No evidence showing direct interaction between MALAT1 and miR-181a-5p/miR-181a-5p and AKT3. Why was luciferase reporter assay not performed? I think the luciferase reporter assay is important to draw the conclusion "Our data further indicates that MALAT1 competitively binds to miR-181a, making miR-181a unable to bind to AKT3 mRNA, thereby up-regulating AKT3 protein levels and ultimately promoting tumor growth".

7. The authors had used miR-181, miR-181a and miR-181a-5p in the manuscript. If they are talking about miR-181a-5p in all occasions, miR-181a-5p should be used consistently.

8. Ipatasertib is an inhibitor of Akt pathway, but not specific for Akt3. I think siRNA against Akt3 should be used instead of Ipatasertib.

9. To show that MALAT1 promoted gastric adenocarcinoma a through MALAT1-miR-181a-AKT3 axis, the functional effect of MALAT1 overexpression with or without transfection of miR-181a-5p or Akt3 siRNA will be a more direct evidence.

10. Some words in figure legend are not displayed appropriately. Figure legend of figure 6 should be A and B.

11. There are some minor grammatical errors.
Decision letter (RSOB-19-0095.R0)

27-Jun-2019

Dear Dr Xue,

We are writing to inform you that the Editor has reached a decision on your manuscript RSOB-19-0095 entitled "MALAT1 promotes gastric adenocarcinoma through MALAT1-miR-181a-AKT3 axis", 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 six 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)
This paper is well written and I suggest acceptance.
Referee: 2

Comments to the Author(s)

This is an interesting study with novel findings. The regulatory role of MALAT1 in gastric adenocarcinoma was well elucidated. First, the authors found that MALAT1 was highly expressed in the serum of gastric adenocarcinoma patients and cell lines. Second, down-regulating MALAT1 was able to inhibit the proliferation and promoted apoptosis in MGC-803 cells. Third, MALAT1 was found to directly target miR-181a-5p and decreased the expression of miR-181a-5p, therefore upregulate the expression of AKT3. Last, overexpressing miR-181a-5p or directly inhibiting the AKT pathway with an inhibitor Ipatasertib exhibited similar effects as MALAT1 knockdown. Based on these findings, the authors conclude that MALAT1 competes with AKT3 for miR-181a binding, thereby up-regulating AKT3 protein level and ultimately promoting the growth of gastric adenocarcinoma. These results deserve publication, giving my following concerns could be addressed.

1. The rational to study MALAT1 is not clearly explained. Need more background information, especially in the tumor field.
2. It is better not to include the results and the significance in the introduction section (the last para. in this section).
3. Any informed consents from the participants? This needs to be clarified.
4. The time point to conduct the CCK-8 assay needs to be indicated.
5. Figure legends should be carefully revised. There are some unidentified characters, and the presentation is poor.
6. Why I can not see any error bars in some set of the data in figure 3 and 6?
7. It is preferred to re-arrange the subfigures in Figure 5, especially for the WB image. It looks too small in this figure.

Referee: 3

Comments to the Author(s)

Major comments:

1. In the results section, the authors mentioned that MALAT1 is highly expressed in gastric adenocarcinoma patients. Yet they only showed that serum MALAT1 level is higher in gastric adenocarcinoma patients compared to healthy control. Why didn't they compare the expression in tumor and adjacent normal tissue as well?

2. The authors should also show the clinical parameter between the two groups of samples, such as age, gender, and use statistical analysis to determine if the serum level of MALAT1 was affected by these parameters.

3. The authors should include more cell-lines of gastric normal and adenocarcinoma cell-line for comparing their MALAT1 levels.

4. More details are needed for the following parts:
   What bioinformatic methods did the authors use to predict the possible binding targets between MALAT1 and miR-181a-5p/miR-181a-5p and AKT3?
   How were MALAT1 and miR-181a-5p level knocked down?
   Did the authors use GAPDH as normalization for lncRNA and miRNA? Why?

5. The expression of miR-181a-5p was negatively correlated with the expression of MALAT1, hence the R2 value should be negative.
6. No evidence showing direct interaction between MALAT1 and miR-181a-5p/miR-181a-5p and AKT3. Why was luciferase reporter assay not performed? I think the luciferase reporter assay is important to draw the conclusion "Our data further indicates that MALAT1 competitively binds to miR-181a, making miR-181a unable to bind to AKT3 mRNA, thereby up-regulating AKT3 protein levels and ultimately promoting tumor growth".

7. The authors had used miR-181, miR-181a and miR-181a-5p in the manuscript. If they are talking about miR-181a-5p in all occasions, miR-181a-5p should be used consistently.

8. Ipatasertib is an inhibitor of Akt pathway, but not specific for Akt3. I think siRNA against Akt3 should be used instead of Ipatasertib.

9. To show that MALAT1 promoted gastric adenocarcinoma a through MALAT1-miR-181a-AKT3 axis, the functional effect of MALAT1 overexpression with or without transfection of miR-181a-5p or Akt3 siRNA will be a more direct evidence.

10. Some words in figure legend are not displayed appropriately. Figure legend of figure 6 should be A and B.

11. There are some minor grammatical errors.

Author's Response to Decision Letter for (RSOB-19-0095.R0)

See Appendix A.

RSOB-19-0095.R1 (Revision)

Review form: Reviewer 2

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good
It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author
The authors had addressed the comments raised from previous review process, I suggest accept the study in current version.

Review form: Reviewer 3 (Lui Ng)

Recommendation
Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?
Good

General interest: Is the paper of sufficient general interest?
Good

Quality of the paper: Is the overall quality of the paper suitable?
Good

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?
Yes

Is it clear?
Yes

Is it adequate?
Yes

Do you have any ethical concerns with this paper?
No
Comments to the Author
All my concerns have been addressed.

Decision letter (RSOB-19-0095.R1)

08-Aug-2019

Dear Dr Xue

We are pleased to inform you that your manuscript entitled "MALAT1 promotes gastric adenocarcinoma through MALAT1-miR-181a-5p-AKT3 axis" has been accepted by the Editor for publication in Open Biology.

If applicable, please find the referee comments below. No further changes are recommended.

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.

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

Reviewer(s)' Comments to Author:

Referee: 2
Comments to the Author(s)
The authors had addressed the comments raised from previous review process, I suggest accept the study in current version.

Referee: 3
Comments to the Author(s)
All my concerns have been addressed.
Appendix A

Referee: 1
Comments to the Author(s)
This paper is well written and I suggest acceptance.
Response:
We thank the reviewer for the favorable comments.

Referee: 2
Comments to the Author(s)
This is an interesting study with novel findings. The regulatory role of MALAT1 in gastric adenocarcinoma was well elucidated. First, the authors found that MALAT1 was highly expressed in the serum of gastric adenocarcinoma patients and cell lines. Second, down-regulating MALAT1 was able to inhibit the proliferation and promoted apoptosis in MGC-803 cells. Third, MALAT1 was found to directly target miR-181a-5p and decreased the expression of miR-181a-5p, therefore upregulate the expression of AKT3. Last, overexpressing miR-181a-5p or directly inhibiting the AKT pathway with an inhibitor Ipatasertib exhibited similar effects as MALAT1 knockdown. Based on these findings, the authors conclude that MALAT1 competes with AKT3 for miR-181a binding, thereby up-regulating AKT3 protein level and ultimately promoting the growth of gastric adenocarcinoma. These results deserve publication, giving my following concerns could be addressed.

1. The rational to study MALAT1 is not clearly explained. Need more background information, especially in the tumor field.
Response:
Thanks for the comments. We have added more background information of the function of MALAT1 in the tumor field.

2. It is better not to include the results and the significance in the introduction section (the last para. in this section).
Response:
We have removed results and significance from the introduction part.

3. Any informed consents from the participants? This needs to be clarified.
Response:
Yes, informed consent was derived from each participant, which was specified in the original paper (patients and samples section).

4. The time point to conduct the CCK-8 assay needs to be indicated.
Response:
We have added this information in the figure legends.

5. Figure legends should be carefully revised. There are some unidentified characters, and the presentation is poor.
Response:
We have revised the figure legends and made the correction.

6. Why I can not see any error bars in some set of the data in figure 3 and 6?
Response:
In fact all points have an error bar, but the error bar for some points is too small to show up proportionally in the figure.

7. It is preferred to re-arrange the subfigures in Figure 5, especially for the WB image. It looks too small in this figure.
Response:
We have re-arranged the subfigures in Figure 5 according to your suggestion.

Referee: 3
Comments to the Author(s)
Major comments:
1. In the results section, the authors mentioned that MALAT1 is highly expressed in gastric adenocarcinoma patients. Yet they only showed that serum MALAT1 level is higher in gastric adenocarcinoma patients compared to healthy control. Why didn't they compare the expression in tumor and adjacent normal tissue as well?
Response:
It is indeed a better choice to use cancerous tissue and adjacent tissues. But we don't have these samples. We only found serum samples in the sample library. At that time, these samples did not have corresponding tissue samples, so we can only compare the expression levels of MALAT1 in the serum of patients and normal healthy controls.

2. The authors should also show the clinical parameter between the two groups of samples, such as age, gender, and use statistical analysis to determine if the serum level of MALAT1 was affected by these parameters.
Response:
We have provided the clinical parameters in FigureS1 and the results showed the expression of MALAT1 was not affected by age or gender.

3. The authors should include more cell-lines of gastric normal and adenocarcinoma cell-line for comparing their MALAT1 levels.
Response:
We have added two more cell lines in FigureS2. The results showed that the expression level of MALAT1 was significantly higher in adenocarcinoma cell-lines than that in gastric normal cell lines.

4. More details are needed for the following parts:
What bioinformatic methods did the authors use to predict the possible binding targets between MALAT1 and miR-181a-5p/miR-181a-5p and AKT3?
How were MALAT1 and miR-181a-5p level knocked down?
Did the authors use GAPDH as normalization for lncRNA and miRNA? Why?
Response:
The interaction between lncRNA and miRNA was predicted by online tool http://starbase.sysu.edu.cn/index.php, and the miRNA target prediction was performed by the online tool http://www.targetscan.org/vert_72/.
Details of how we knocked down MALAT1 and overexpressed miR-181a-5p was added to the method part.
We used U6 snRNA as the internal control for lncRNA and miRNA, since this is a common choice and it works well in our hands. This information has been updated in the revised “QRT-PCR analysis” section.

5. The expression of miR-181a-5p was negatively correlated with the expression of MALAT1, hence the R2 value should be negative.
Response:
The expression of miR-181a-5p was negatively correlated with the expression of MALAT1, therefore, \( r \) should be negative. However, \( R^2 \) (square of \( R \)) should always be positive.

6. No evidence showing direct interaction between MALAT1 and miR-181a-5p/miR-181a-5p and AKT3. Why was luciferase reporter assay not performed? I think the luciferase reporter assay is important to draw the conclusion "Our data further indicates that MALAT1 competitively binds to miR-181a, making miR-181a unable to bind to AKT3 mRNA, thereby up-regulating AKT3 protein levels and ultimately promoting tumor growth".
Response:
You are absolutely right. We performed the luciferase reporter assay as you suggested and presented the results in Figure S3.

7. The authors had used miR-181, miR-181a and miR-181a-5p in the manuscript. If they are talking about miR-181a-5p in all occasions, miR-181a-5p should be used consistently.
Response:
We unified this term as miR-181a-5p in all the figures, figure legends and the text.

8. Ipatasertib is an inhibitor of Akt pathway, but not specific for Akt3. I think siRNA against Akt3 should be used instead of Ipatasertib.
Response:
That is a good point. First, we actually did try the siRNA against Akt3, but the knock down efficiency was very poor. Second, the usage of inhibitors and direct knockdown is a completely different effect. The inhibitor only affects its function and does not affect its expression, although there will be some non-specific conditions. However, knockdown directly changes the amount of protein expressed, which may have a more serious impact.
9. To show that MALAT1 promoted gastric adenocarcinoma a through MALAT1-miR-181a-AKT3 axis, the functional effect of MALAT1 overexpression with or without transfection of miR-181a-5p or Akt3 siRNA will be a more direct evidence.

**Response:**
It is a good point. We initially tried to over express MALAT1, which is frustrating. If a small amount of overexpression does not significantly increase the expression level of MALAT1, if the expression level is increased, the state of the cells will be affected, and the cells will tend to apoptosis, so we are still trying with different experimental conditions

10. Some words in figure legend are not displayed appropriately. Figure legend of figure 6 should be A and B.

**Response:**
We have revised the figure legends and made the correction, and also re-arranged the subfigures in Figure 5 according to your suggestion.

11. There are some minor grammatical errors.

**Response:**
Thanks for the comments. We have revised the manuscript for grammatical errors.