THE ROYAL SOCIETY PUBLISHING

OPEN BIOLOGY

HOXD-AS1 confers cisplatin resistance in gastric cancer through epigenetically silencing PDCD4 via recruiting EZH2

Yafei Ye, Shengnan Yang, Yanping Han, Jingjing Sun, Lijuan Xv, Lina Wu and Liang Ming

Article citation details
Open Biol. **9**: 190068.
http://dx.doi.org/10.1098/rsob.190068

Review timeline

Original submission:	8 April 2019
1st revised submission:	16 June 2019
2nd revised submission:	15 July 2019
Final acceptance:	28 August 2019

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

Review History

RSOB-19-0068.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?
Not Applicable

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 the present study, authors explore epigenetic mechanisms leading to chemo-resistance in Gastric Cancer. Hence, they hypothesized if LncRNA HOXD-AS1 could be involved in resistance of gastric cancer to cisplatin. To achieve their objectives, Yafei Ye and co-workers analyzed 42 tumor samples and their normal adjacent tissues. After obtaining tumor tissues, HOXD-AS1 was over-expressed in tumor samples respecting to their normal counterparts and in gastric cancer cell lines in comparison to normal GES-1 cells. A positive correlation between HOXD-AS1 expression level and poor survival of analyzed patients was demonstrated.

Then, HOXD-AS1 was knocked-down in gastric cancer cells. Suppression of HOXD-AS1 expression was associated with chemo-sensitivity. To further explore the role of HOXD-AS1 in the regulation of chemo-resistance. It was analyzed whether HOXD-AS1 epigenetically inhibits PDC4 by means of EZH2 recruitment to PDC4 promoter. Moreover, they showed an H3K27me3 occupancy mediated by HOXD-AS1.

Finally HOXD-AS1 knockdown overcame DDP resistance both in vitro and in vivo models. In general terms the manuscript is well written and results are properly presented.the conclusions are well argued and based on the results presented.

Major concerns
1. siRNAs employed showed a slightly inhibition of PDCD4 and EZH2. It is strongly recommended when using siRNAs to knockdown a gene, to design at least 3, due to the formation of secondary structures and binding to regulatory proteins
2. Figure 3B, authors state that HOXD-AS1 knockdown pointedly elevated PDCD4 expression in BGC823/DDP cells. However, the result is completely different, there is a slight decrease in the expression of PDCD4.

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?
No

Do you have any ethical concerns with this paper?
No

Comments to the Author
In this study, the authors investigated the role and mechanism of HOXD-AS1 in cisplatin resistance of gastric cancer cells. The experimental design was clear and reasonable. However, there are some minor issues which should be addressed.

1. The primer sequences of HOXD-AS1 and PDCD4 for qRT-PCR were not showed. What are the the sequence of primers used in the qRT-PCR assay?
2. The vendor of MTT should be indicated.
3. How DDP-resistant and -sensitive gastric cancer tissue was defined is not mentioned.
4.What is the cut off of HOXD-AS1 “high” or “low” expression?
5. There is no table about the association of HOXD-AS1 expression with the clinical characteristics of gastric cancer patients.

Decision letter (RSOB-19-0068.R0)

31-May-2019

Dear Dr Ming,

We are writing to inform you that the Editor has reached a decision on your manuscript RSOB-19-0068 entitled "HOXD-AS1 confers cisplatin resistance in gastric cancer through epigenetically silencing PDCD4 via recruiting EZH2", 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

Editor's Comments to Author(s):Please address all comments of the referees

Board Member's Comments to Author(s):
The manuscript needs major revisions in order to be considered for publication at the Open Biology. A major weakness is use of a single siRNA construct to demonstrate function of HoxD-AS1, at least one other siRNA should be added. Some minor comments include mistakes in text and methodological clarifications as pointed out by the reviewers.

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

Referee: 1

Comments to the Author(s)
In the present study, authors explore epigenetic mechanisms leading to chemo-resistance in Gastric Cancer. Hence, they hypothesized if LncRNA HOXD-AS1 could be involved in resistance of gastric cancer to cisplatin. To achieve their objectives, Yafei Ye and co-workers analyzed 42 tumor samples and their normal adjacent tissues. After obtaining tumor tissues, HOXD-AS1 was over-expressed in tumor samples respecting to their normal counterparts and in gastric cancer cell lines in comparison to normal GES-1 cells. A positive correlation between HOXD-AS1 expression level and poor survival of analyzed patients was demonstrated.

Then, HOXD-AS1 was knocked-down in gastric cancer cells. Suppression of HOXD-AS1

expression was associated with chemo-sensitivity. To further explore the role of HOXD-AS1 in the regulation of chemo-resistance. It was analyzed whether HOXD-AS1 epigenetically inhibits PDC4 by means of EZH2 recruitment to PDC4 promoter. Moreover, they showed an H3K27me3 occupancy mediated by HOXD-AS1.

Finally HOXD-AS1 knockdown overcame DDP resistance both in vitro and in vivo models. In general terms the manuscript is well written and results are properly presented.the conclusions are well argued and based on the results presented.

Major concerns
1. siRNAs employed showed a slightly inhibition of PDCD4 and EZH2. It is strongly recommended when using siRNAs to knockdown a gene, to design at least 3, due to the formation of secondary structures and binding to regulatory proteins
2. Figure 3B, authors state that HOXD-AS1 knockdown pointedly elevated PDCD4 expression in BGC823/DDP cells. However, the result is completely different, there is a slight decrease in the expression of PDCD4.

Referee: 2

Comments to the Author(s)
In this study, the authors investigated the role and mechanism of HOXD-AS1 in cisplatin resistance of gastric cancer cells. The experimental design was clear and reasonable. However, there are some minor issues which should be addressed.

1. The primer sequences of HOXD-AS1 and PDCD4 for qRT-PCR were not showed. What are the the sequence of primers used in the qRT-PCR assay?
2. The vendor of MTT should be indicated.
3. How DDP-resistant and -sensitive gastric cancer tissue was defined is not mentioned.
4.What is the cut off of HOXD-AS1 “high” or “low” expression?
5. There is no table about the association of HOXD-AS1 expression with the clinical characteristics of gastric cancer patients.

Author's Response to Decision Letter for (RSOB-19-0068.R0)

See Appendix A.

RSOB-19-0068.R1 (Revision)

Review form: Reviewer 1

Recommendation
Accept with minor revision (please list in comments)

Do you have any ethical concerns with this paper?
No

Comments to the Author
Primers for HOXD-AS1 and PDCD4 does not correspond to gene sequences, please add a supplementary file for the alignment results to each gene for corresponding primers.

Review form: Reviewer 2

Recommendation
Accept as is

Do you have any ethical concerns with this paper?
No

Comments to the Author
Authors carefully addressed all concerns raised by the reviewers.

Decision letter (RSOB-19-0068.R1)

11-Jul-2019

Dear Dr Ming

We are pleased to inform you that your manuscript RSOB-19-0068.R1 entitled "HOXD-AS1 confers cisplatin resistance in gastric cancer through epigenetically silencing PDCD4 via recruiting EZH2" has been accepted by the Editor for publication in Open Biology. The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, we invite you to respond to the reviewer(s)' comments and revise your manuscript.

Please submit the revised version of your manuscript 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.

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, you will be able to 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/.

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://royalsocietypublishing.org/instructions-authors#question5). All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rsob.2016[last 4 digits of e.g. 10.1098/rsob.20160049].

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 have a resolution of at least 300 dpi, if possible.

Data-Sharing
It is a condition of publication that data supporting your paper are made available. Data should be made available either in the electronic supplementary material or through an appropriate repository. Details of how to access data should be included in your paper. Please see http://royalsocietypublishing.org/site/authors/policy.xhtml#question6 for more details.

Data accessibility section
To ensure archived data are available to readers, authors should include a ‘data accessibility’ section immediately after the acknowledgements section. This should list the database and accession number for all data from the article that has been made publicly available, for instance:
- DNA sequences: Genbank accessions F234391-F234402
- Phylogenetic data: TreeBASE accession number S9123
- Final DNA sequence assembly uploaded as online supplemental material
- Climate data and MaxEnt input files: Dryad doi:10.5521/dryad.12311

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)
Authors carefully addressed all concerns raised by the reviewers.

Referee: 1

Comments to the Author(s)
Primers for HOXD-AS1 and PDCD4 does not correspond to gene sequences, please add a supplementary file for the alignment results to each gene for corresponding primers.

Author's Response to Decision Letter for (RSOB-19-0068.R1)

See Appendix B.

Decision letter (RSOB-19-0068.R2)

28-Aug-2019

Dear Dr Ming

We are pleased to inform you that your manuscript entitled "HOXD-AS1 confers cisplatin resistance in gastric cancer through epigenetically silencing PDCD4 via recruiting EZH2" 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

Appendix A

Dear Editor,

Thank you very much for your letter and advice. We have revised the paper and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the revision is highlighted in red in the revised manuscript.

We hope that the revision is acceptable, and I look forward to hearing from you soon.

With best wishes,

Yours sincerely,

Liang Ming

Editor's Comments to Author(s): Please address all comments of the referees

Board Member's Comments to Author(s):

The manuscript needs major revisions in order to be considered for publication at the Open Biology. A major weakness is use of a single siRNA construct to demonstrate function of HoxD-AS1, at least one other siRNA should be added. Some minor comments include mistakes in text and methodological clarifications as pointed out by the reviewers.

Re: Thanks for your comments. The function of HOXD-AS1 after one other siRNA (HOXD-AS1 #1) has been added in Supplement Figure 1. All minor comments include mistakes in text pointed out by the reviewers has been revised.

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

Referee: 1

Comments to the Author(s)

In the present study, authors explore epigenetic mechanisms leading to chemo-resistance in Gastric Cancer. Hence, they hypothesized if LncRNA HOXD-AS1 could be involved in resistance of gastric cancer to cisplatin. To achieve their objectives, Yafei Ye and co-workers analyzed 42 tumor samples and their normal adjacent tissues. After obtaining tumor tissues, HOXD-AS1 was over-expressed in tumor samples respecting to their normal counterparts and in gastric cancer cell lines in comparison to normal GES-1 cells. A positive correlation between HOXD-AS1 expression level and poor survival of analyzed patients was demonstrated.

Then, HOXD-AS1 was knocked-down in gastric cancer cells. Suppression of HOXD-AS1 expression was associated with chemo-sensitivity. To further explore the role of HOXD-AS1 in the regulation of chemo-resistance. It was analyzed whether HOXD-AS1 epigenetically inhibits PDC4 by means of EZH2 recruitment to PDC4 promoter. Moreover, they showed an H3K27me3 occupancy mediated by

HOXD-AS1.

Finally, HOXD-AS1 knockdown overcame DDP resistance both in vitro and in vivo models. In general terms the manuscript is well written and results are properly presented the conclusions are well argued and based on the results presented.

Major concerns

1. siRNAs employed showed a slightly inhibition of PDCD4 and EZH2. It is strongly recommended when using siRNAs to knockdown a gene, to design at least 3, due to the formation of secondary structures and binding to regulatory proteins

Re: Thank you for your valuable comments. We are sorry for our less rigorous design. We have added the function of HOXD-AS1 after another siRNA of HOXD-AS1 transfection in Supplement Figure 1.

2. Figure 3B, authors state that HOXD-AS1 knockdown pointedly elevated PDCD4 expression in BGC823/DDP cells. However, the result is completely different, there is a slight decrease in the expression of PDCD4.

Re: We are sorry for our mistake. The correct protein image has been placed in Figure 3B.

Referee: 2

Comments to the Author(s)

In this study, the authors investigated the role and mechanism of HOXD-AS1 in cisplatin resistance of gastric cancer cells. The experimental design was clear and reasonable. However, there are some minor issues which should be addressed.

1. The primer sequences of HOXD-AS1 and PDCD4 for qRT-PCR were not showed. What is the sequence of primers used in the qRT-PCR assay?

Re: The sequence of primers used in the qRT-PCR assay has been added in the “2.3.

Quantitative real-time PCR (qRT-PCR)" section.

2. The vendor of MTT should be indicated.

Re: The vendor of MTT has been added in the "2.4. Drug sensitivity assay" section.

3. How DDP-resistant and -sensitive gastric cancer tissue was defined is not mentioned.

Re: The definition of DDP-resistant and -sensitive gastric cancer tissues have been added in the "2.1. Sample collection and cell culture" section.

4.What is the cut off of HOXD-AS1 "high" or "low" expression?

Re: The cut off of HOXD-AS1 "high" or "low" expression is the median of HOXD-AS1 levels.

5. There is no table about the association of HOXD-AS1 expression with the clinical characteristics of gastric cancer patients.

Re: The association of HOXD-AS1 expression with the clinical characteristics of gastric cancer patients has been shown in Table 1.

Appendix B

Dear Editor,

Thank you very much for your letter and advice. We have revised the paper and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the revision is highlighted in red in the revised manuscript.

We hope that the revision is acceptable, and I look forward to hearing from you soon.

With best wishes,

Yours sincerely,

Liang Ming

Reviewer(s)' Comments to Author:

Referee: 2

Comments to the Author(s)

Authors carefully addressed all concerns raised by the reviewers.

Re: Thank you for your comments.

Referee: 1

Comments to the Author(s)

Primers for HOXD-AS1 and PDCD4 does not correspond to gene sequences, please add a supplementary file for the alignment results to each gene for corresponding primers.

Re: Thank you for your comments. The primers for HOXD-AS1 and PDCD4 we used came from the publishing papers. For HOXD-AS1, the primers were obtained from the paper “HOXD-AS1 functions as an oncogenic ceRNA to promote NSCLC cell progression by sequestering miR-147a”. (Wang Q, Jiang S, Song A, et al. HOXD-AS1 functions as an oncogenic ceRNA to promote NSCLC cell progression by sequestering miR-147a[J]. OncoTargets and therapy, 2017, 10: 4753.) For PDCD4, the primers were obtained from the papers “MicroRNA-182 modulates chemosensitivity of human non-small cell lung cancer to cisplatin by targeting PDCD4” and “miR-21 modulates chemosensitivity of tongue squamous cell carcinoma cells to cisplatin by targeting PDCD4”. (Ning F, Wang F, Li M, et al. MicroRNA-182 modulates chemosensitivity of human non-small cell lung cancer to cisplatin by targeting PDCD4[J]. Diagnostic pathology, 2014, 9(1): 143; Ren W, Wang X, Gao L, et al. MiR-21 modulates chemosensitivity of tongue squamous cell carcinoma cells to cisplatin by targeting PDCD4[J]. Molecular and cellular biochemistry, 2014, 390(1-2): 253-262.)