

DDP-resistant ovarian cancer cells-derived exosomal microRNA-30a-5p reduces the resistance of ovarian cancer cells to DDP

Ronghua Liu, Yucan Zhang, Peiwen Sun and Changxiu Wang

Article citation details

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Review timeline

Original submission: 31 July 2019
Revised submission: 2 December 2019
Final acceptance: 9 January 2020

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

Review History

RSOB-19-0173.R0 (Original submission)

Review form: Reviewer 1 (Jingshi Shen)

Recommendation

Major revision is needed (please make suggestions in comments)

Do you have any ethical concerns with this paper?

Yes

Comments to the Author

This manuscript presents some original and interesting findings and will likely be of interest to many people in the field. Besides the scientific comments listed below, I'd like to point out that the manuscript contains a large number of typos and grammatical errors. Both new experiments and substantial editorial revisions are needed to improve the quality of the manuscript.

1. While the authors showed a correlation between Sox9/miR 30a-5p and susceptibility to drug treatment, they did not definitively show the involvement of sox9 in the pathway. They acknowledged that this miRNA is known to affect expression of other proteins in various types of

cancer. The authors should create an siRNA that specifically targets Sox9 or use an overexpression vector to show that overexpression of Sox9 is sufficient to cause resistance.

2. In figure 2, the binding site shown is labeled as being for miR 23a-5p, not miR 30a-5p. Is this an error? If not, please explain how the two molecules are related. Exosomes were labelled with pkh67, a dye that may form nanoparticles similar to exosomes in size. Ultracentrifugation used to isolate exosomes would not separate PKH67 nanoparticles from exosomes. Sucrose gradient separation is a feasible way to remove dye aggregates.

3. In Figure 5, the microscopy was performed using PKH26 while exosomes were stained with PKH67. While biochemically similar, these two dyes have different fluorescent characteristics with PKH67 visualized in the GFP channel and PKH26 in the red.

4. I also recommend substantial editing as there are many typos and grammar issues, examples:

- Fig 5b Y-axis label numbei instead of number
- Sentence beginning with "And". " On the contrary, the expression of miR-30a-5p prominently decreased in exosomes derived from SKOV3 cells that had been transfected with miR30a-5p inhibitor. And the expression of . . . "
- Fig7 description scar bar vs scale bar.

Review form: Reviewer 2

Recommendation

Accept with minor revision (please list in comments)

Do you have any ethical concerns with this paper?

No

Comments to the Author

Liu et al. have presented a significant body of work describing the relationship between miR-30a-59/Sox9 and cisplatin resistance in ovarian cancer. Through the use of a miR-mimic, the authors demonstrate that miR-30a-5p targets Sox9 mRNA, which in turn decreases Sox9 protein levels. Increased Sox9 was observed in cisplatin resistant cells and decreasing Sox9 returned sensitivity to drug. Finally, the authors report that exosomes containing miR-30a-5p reduced both in vitro cell line proliferation and in vivo tumor growth.

Overall the report is well written and the data is presented well. To strengthen the relationship between Sox9 and cisplatin sensitivity I would suggest silencing Sox9 by RNAi and/or over-expressing (Sox9) in OC cells. This will confirm whether sensitivity is due to regulation of Sox9 or other miR-30a-5p target genes.

Minor:

1. Define DDP in the Abstract and manuscript body. Likewise, minimize switching between DDP and cisplatin (e.g. Abstract vs Introduction).
2. Define DEGs in the first results section.
3. The first paragraph of the introduction (references 3-6) could be condensed as it currently repetitive.
4. Figure 7A and B. Order the tumor images the same as the adjacent graph key (Fig. 7B).

Decision letter (RSOB-19-0173.R0)

17-Sep-2019

Dear Dr Wang,

We are writing to inform you that the Editor has reached a decision on your manuscript RSOB-19-0173 entitled "Exosomal transfer of cisplatin-resistant ovarian cancer cells-derived microRNA-30a-5p reduces resistance of ovarian cells to cisplatin", 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)

This manuscript presents some original and interesting findings and will likely be of interest to many people in the field. Besides the scientific comments listed below, I'd like to point out that the manuscript contains a large number of typos and grammatical errors. Both new experiments and substantial editorial revisions are needed to improve the quality of the manuscript.

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 - Fig7 description scar bar vs scale bar.

Referee: 2

Comments to the Author(s)

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Minor:

1. Define DDP in the Abstract and manuscript body. Likewise, minimize switching between DDP and cisplatin (e.g. Abstract vs Introduction).
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3. The first paragraph of the introduction (references 3-6) could be condensed as it currently repetitive.
4. Figure 7A and B. Order the tumor images the same as the adjacent graph key (Fig. 7B).

Author's Response to Decision Letter for (RSOB-19-0173.R0)

See Appendix A.

RSOB-19-0173.R1 (Revision)

Review form: Reviewer 1

Recommendation

Accept as is

Do you have any ethical concerns with this paper?

No

Comments to the Author

The authors addressed my concerns. I do not have any additional scientific comments but I'd like to point out that the clarity and accuracy of the text could be further improved, either by the production editor or an independent text-editing service.

Review form: Reviewer 2

Recommendation

Accept as is

Do you have any ethical concerns with this paper?

No

Comments to the Author

The authors have thoroughly addressed all of my comments and concerns. Thank you.

Decision letter (RSOB-19-0173.R1)

09-Jan-2020

Dear Dr Wang

We are pleased to inform you that your manuscript entitled "DDP-resistant ovarian cancer cells-derived exosomal microRNA-30a-5p reduces resistance of ovarian cancer cells to DDP" 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.

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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 have thoroughly addressed all of my comments and concerns. Thank you.

Referee: 1

Comments to the Author(s)

The authors addressed my concerns. I do not have any additional scientific comments but I'd like to point out that the clarity and accuracy of the text could be further improved, either by the production editor or an independent text-editing service.

Appendix A

Response to Reviewers

Dear Reviewers,

Thank you very much for your letter and the comments from the referees about our paper entitled “*DDP-resistant ovarian cancer cells-derived exosomal microRNA-30a-5p reduces resistance of ovarian cancer cells to DDP*” submitted to “**Open Biology**”. We have checked the manuscript and revised it according to the comments. We submit here the revised manuscript as well as a list of changes. If you have any question about this paper, please don’t hesitate to let me know.

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

Referee: 1

Comments to the Author(s)

This manuscript presents some original and interesting findings and will likely be of interest to many people in the field. Besides the scientific comments listed below, I’d like to point out that the manuscript contains a large number of typos and grammatical errors. Both new experiments and substantial editorial revisions are needed to improve the quality of the manuscript.

1. While the authors showed a correlation between Sox9/miR 30a-5p and susceptibility to drug treatment, they did not definitively show the involvement of sox9 in the pathway. They acknowledged that this miRNA is known to affect expression of other proteins in various types of cancer. The authors should create an siRNA that specifically targets Sox9 or use an overexpression vector to show that overexpression of Sox9 is sufficient to cause resistance.

Response: Following your suggestion, we have supplemented “*In order to directly prove the role of SOX9 in this regulation, we delivered si-SOX9 into SKOV3/DDP cells and found that si-SOX9 significantly decreased DDP IC₅₀, blocked cell cycle in G1 phase, and enhanced the apoptosis rate significantly (Figure 4f). In addition, we noted that the inhibitory effects of miR-30a-5p*

inhibitor on DDP sensitivity could be reversed by the inhibition of SOX9 (Figure 4g), while si-SOX9 counteracted the inhibitory effect of miR-30a-5p mimic on OC resistance to DDP (Figure 4h)."

2. In figure 2, the binding site shown is labeled as being for miR 23a-5p, not miR 30a-5p. Is this an error? If not, please explain how the two molecules are related. Exosomes were labelled with pkh67, a dye that may form nanoparticles similar to exosomes in size. Ultracentrifugation used to isolate exosomes would not separate PKH67 nanoparticles from exosomes. Sucrose gradient separation is a feasible way to remove dye aggregates.

Response: Sorry for the negligence, we have revised the figure 2. Moreover, we have revised the centrifugation method into sucrose gradient separation as "*The mixture was centrifuged at 100000 g at 4 °C for 2 h so that the exosomes in the sample were enriched in the sucrose density range of 1.13-1.19g/mL.*" in the manuscript.

3. In Figure 5, the microscopy was performed using PKH26 while exosomes were stained with PKH67. While biochemically similar, these two dyes have different fluorescent characteristics with PKH67 visualized in the GFP channel and PKH26 in the red.

Response: Thank you for your kindly reminding. We found that PKH26 was more stable in the pre-experiment, and we ended up using PKH26, which has been corrected in the revised manuscript.

4. I also recommend substantial editing as there are many typos and grammar issues, examples:

- Fig 5b Y-axis label numbei instead of number

- Sentence beginning with “And”. “ On the contrary, the expression of miR-30a-5p prominently decreased in exosomes derived from SKOV3 cells that had been transfected with miR30a-5p inhibitor. And the expression of . . . “

- Fig7 description scar bar vs scale bar.

Response: Thank you for your comment. We have already checked and proofread the whole text thoroughly to avoid typos and grammar issues. Besides, we asked a native English speaker to help edit the manuscript. We hope the language of this manuscript can meet your expectation.

Referee: 2

Comments to the Author(s)

Liu et al. have presented a significant body of work describing the relationship between miR-30a-5p/Sox9 and cisplatin resistance in ovarian cancer. Through the use of a miR-mimic, the authors demonstrate that miR-30a-5p targets Sox9 mRNA, which in turn decreases Sox9 protein levels. Increased Sox9 was observed in cisplatin resistant cells and decreasing Sox9 returned sensitivity to drug. Finally, the authors report that exosomes containing miR-30a-5p reduced both in vitro cell line proliferation and in vivo tumor growth.

Overall the report is well written and the data is presented well. To strengthen the relationship between Sox9 and cisplatin sensitivity I would suggest silencing Sox9 by RNAi and/or over-expressing (Sox9) in OC cells. This will confirm whether sensitivity is due to regulation of Sox9 or other miR-30a-5p target genes.

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rate significantly (Figure 4f). In addition, we noted that the inhibitory effects of miR-30a-5p inhibitor on DDP sensitivity could be reversed by the inhibition of SOX9 (Figure 4g), while si-SOX9 counteracted the inhibitory effect of miR-30a-5p mimic on OC resistance to DDP (Figure 4h).”

Minor:

1. Define DDP in the Abstract and manuscript body. Likewise, minimize switching between DDP and cisplatin (e.g. Abstract vs Introduction).

Response: Considering your suggestion, we have defined DDP as cis-diamminedichloroplatinum(II) in the Abstract and in the Introduction in the first mentioned, and used DDP throughout the revised manuscript.

2. Define DEGs in the first results section.

Response: We have defined DEGs as differentially expressed genes in the first results section according to your comment.

3. The first paragraph of the introduction (references 3-6) could be condensed as it currently repetitive.

Response: Following your suggestion, we have removed reference 4 and unnecessary repetitive descriptions.

4. Figure 7A and B. Order the tumor images the same as the adjacent graph key

Response: Thank you for your kindly reminding. We have ordered the tumor images the same as

the adjacent graphs.

We regularly screen submitted papers for originality and we have some queries. We would be grateful if you could provide the raw (uncut and unprocessed) blots for the SOX9 and GAPDH panels for Figure 7F. Please include these in your response for the revised version of the manuscript.

Response: On the basis of your suggestion, we have provided the raw blots for the SOX9 and GAPDH panels for Figure 7F in the response.

These changes will not influence the central content and framework of the paper. Here we did not list all the specific changes but they are marked in red in the revised paper. We greatly appreciate your review and hope that the corrections meet your expectations.

Once again, thank you very much for your comments and suggestions.

Thank you and best regards.

Yours sincerely,

Dr. Changxiu Wang

E-mail: wangcx1981@yeah.net