Involvement of miR-4262 in paclitaxel resistance through the regulation of PTEN in non-small cell lung cancer

Hongwen Sun, Xiaoting Zhou, Yanan Bao, Guosheng Xiong, Yue Cui and Hua Zhou

Article citation details
Open Biol. 9: 180227.
http://dx.doi.org/10.1098/rsob.180227

Review timeline
Original submission: 16 November 2018
1st revised submission: 15 May 2019
2nd revised submission: 14 June 2019
Final acceptance: 14 June 2019

Review History

RSOB-18-0227.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
   No

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
Sun et al. present intriguing data between the relationship miR-4262 expression and paclitaxel resistance in NSCLC. The authors find that miR-4262 expression in upregulated in NSCLC tumor tissue and cell lines when compared to normal adjacent tissue. They also identified a putative miR-4262 binding site on the 3’ UTR of PTEN mRNA that leads to its suppression. While the presented data is robust, the manuscript as currently written has limitations.

Major concerns:
1. The paper would benefit from grammatical proofreading from a native English-speaking author. There are many grammatical, and formatting issues that make the manuscript difficult to follow at times.
2. What is the sequence of the miR-mimic? Is it the same as endogenous miR-4262?
3. While the miR-4262 mimic suppresses PTEN mRNA, there is no evidence presented that the mimic alone alters PTEN protein levels (modest decrease in combination with PTX shown in Figure 6).
4. PTEN specific siRNAs or shRNAs should be used to confirm the relationship between miR-4262, PTEN, and PTX resistance since miR-4262 has been shown to target other cancer/stemness genes in breast cancer (e.g. PMID 27629257).

Minor issues.
1. Page 4. PMID listed instead of reference.
2. Page 9. The role of miR-4262 in NSCLC development was not investigated.

Review form: Reviewer 2

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

Comments to the Author
This is a nice study of the role of a microRNA in cancer drug resistance. The strengths include the fact that both in vitro and in vivo data are presented and that a potential target of microRNA was identified to be a gene of high relevance, PTEN. And the data presented are of good quality. There are also weaknesses that need to be addressed before going forward. The most serious is the lack of data showing that miR 4262 plays a role in taxol resistance in the parental cancer cell lines used in xenograft studies. Specifically:
Fig. 5 is fine for showing that adding more miR 4262 to already PTX resistant cells provide further protection from the drug. But these data do not address whether miR 4262 is responsible
for the PTX resistant nature of these cells, nor do they address if miR4262 has a role in the parental cancer cells (where PTX induces mir 4262). They should knock down miR 4262 (with the inhibitor or antigomiR) in PTX resistant cells and ask if this restores sensitivity and also in the parental cells, to see if decreasing miR 4264 increases sensitivity to PTX. Because in vivo studies in Fig. 7 are with parental cell lines, it is important to show that inhibition of miR4262 makes the cells more sensitive to PTX in vitro.

Related, Taxol induces miR 4262 expression in cells in Fig. 4 but the opposite happens in the tumors in Fig. 7. Why this difference?

The writing and the grammar need to be improved; it is not at the level acceptable for an international journal.

Minor comments:
CCK8 assay measures cell number. Cell number changes could be due to changes in cell proliferation or cell death or both. In other words, CCK8 does not necessarily measure proliferation as the authors suggest in Fig. 2A. To conclude that miR 4262 affects cell proliferation, the authors need to monitor other markers such as BrdU or EdU incorporation, phospho-H3 or Ki67, or similar. Alternatively, the authors should modify the text to reflect accurately what CCK8 measures. In fact, since miR 4262 inhibition increases apoptosis, this alone can explain the CCK8 data.

Why does miR 4262 mimic increase the level of endogenous miR 4262? Clearly endogenous miR 4262 cannot do this.

The migration assay needs either a citation of a reference or more details. What is in the migration medium? What are the vectors? What are in two chambers and what separates the chambers? Are the chambers commercial?

Are the miR inhibitor and antigomiR the same? What exactly are these?

Decision letter (RSOB-18-0227.R0)

04-Feb-2019

Dear Dr Zhou,

We are writing to inform you that the Editor has reached a decision on your manuscript RSOB-18-0227 entitled "Involvement of miR-4262 in resistance to paclitaxel through regulating PTEN in non-small cell lung cancer", 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)

Sun et al. present intriguing data between the relationship miR-4262 expression and paclitaxel resistance in NSCLC. The authors find that miR-4262 expression is upregulated in NSCLC tumor tissue and cell lines when compared to normal adjacent tissue. They also identified a putative miR-4262 binding site on the 3' UTR of PTEN mRNA that leads to its suppression. While the presented data is robust, the manuscript as currently written has limitations.

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3. While the miR-4262 mimic suppresses PTEN mRNA, there is no evidence presented that the mimic alone alters PTEN protein levels (modest decrease in combination with PTX shown in Figure 6).
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Minor issues.
1. Page 4. PMID listed instead of reference.
2. Page 9. The role of miR-4262 in NSCLC development was not investigated.
Referee 2:

This is a nice study of the role of a microRNA in cancer drug resistance. The strengths include the fact that both in vitro and in vivo data are presented and that a potential target of microRNA was identified to be a gene of high relevance, PTEN. And the data presented are of good quality. There are also weaknesses that need to be addressed before going forward. The most serious is the lack of data showing that miR 4262 plays a role in taxol resistance in the parental cancer cell lines used in xenograft studies. Specifically:

Fig. 5 is fine for showing that adding more miR 4262 to already PTX resistant cells provide further protection from the drug. But these data do not address whether miR 4262 is responsible for the PTX resistant nature of these cells, nor do they address if miR4262 has a role in the parental cancer cells (where PTX induces mir 4262). They should knock down miR 4262 (with the inhibitor or antigomiR) in PTX resistant cells and ask if this restores sensitivity and also in the parental cells, to see if decreasing miR 4264 increases sensitivity to PTX. Because in vivo studies in Fig. 7 are with parental cell lines, it is important to show that inhibition of miR4262 makes the cells more sensitive to PTX in vitro.

Related, Taxol induces miR 4262 expression in cells in Fig. 4 but the opposite happens in the tumors in Fig. 7. Why this difference?

The writing and the grammar need to be improved; it is not at the level acceptable for an international journal.

Minor comments:

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Why does miR 4262 mimic increase the level of endogenous miR 4262? Clearly endogenous miR 4262 cannot do this.

The migration assay needs either a citation of a reference or more details. What is in the migration medium? What are the vectors? What are in two chambers and what separates the chambers? Are the chambers commercial?

Are the miR inhibitor and antigomiR the same? What exactly are these?

Author's Response to Decision Letter for (RSOB-18-0227.R0)

See Appendix A.
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?
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
The authors have addressed my concerns.

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
No
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?
Yes

Do you have any ethical concerns with this paper?
No

Comments to the Author

The authors have responded comprehensively and adequately to my concerns about the science. But the writing still needs to improve. The authors used a professional service to ensure the correctness of the language. The body of the manuscript is in fairly good shape now but there are still mistakes, some of which are listed below. But the worst is the Abstract, which is the most important part because this is what the readers will see first. The Abstract in its current form is a list of experiments along with some results. There is no scientific context provided and no conclusions stated. The results provided are on the role of miR-4262 in apoptosis, proliferation and motility, and on the levels of miR-4262 and PTEN. What about the conclusion that miR-4262 is responsible for paclitaxel resistance? Nothing in the Abstract supports or even addresses this conclusion.

A few examples of writing problems:
The authors need to reconsider word choices; some are sub-optimal. For example, in ‘miR-4262 responded to PTX resistance…’, ‘responded’ may not be the word you want to use.

In the first sentence of the Abstract, do you mean ‘resistant’ instead of ‘resistance’?

The following sentence needs a reference/citation. ‘Previous studies have shown that the PTEN/PI3K-AKT axis is involved in drug resistance in NSCLC.’

Fig. 6A-B label ‘filed’ should be ‘field’.

Decision letter (RSOB-18-0227.R1)

31-May-2019

Dear Dr Zhou

We are pleased to inform you that your manuscript RSOB-18-0227.R1 entitled "The involvement
of miR-4262 in paclitaxel resistance through the regulation of PTEN in non-small cell lung cancer" has been accepted by the Editor for publication in Open Biology.

The referees do not recommend any further changes that need to be made. However, we would like to request that the authors carry out a final language check and proof-read of the manuscript, and upload the final files for publication.

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.

Please see our detailed instructions for revision requirements. It is essential these instructions are followed carefully to minimise any delay to publication:
http://rsob.royalsocietypublishing.org/site/misc/revised.xhtml

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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)
The authors have responded comprehensively and adequately to my concerns about the science. But the writing still needs to improve. The authors used a professional service to ensure the correctness of the language. The body of the manuscript is in fairly good shape now but there are still mistakes, some of which are listed below. But the worst is the Abstract, which is the most important part because this is what the readers will see first. The Abstract in its current form is a list of experiments along with some results. There is no scientific context provided and no conclusions stated. The results provided are on the role of miR-4262 in apoptosis, proliferation and motility, and on the levels of miR-4262 and PTEN. What about the conclusion that miR-4262 is responsible for paclitaxel resistance? Nothing in the Abstract supports or even addresses this conclusion.

A few examples of writing problems:
The authors need to reconsider word choices; some are sub-optimal. For example, in ‘miR-4262 responded to PTX resistance…’, ‘responded’ may not be the word you want to use.

In the first sentence of the Abstract, do you mean ‘resistant’ instead of ‘resistance’?

The following sentence needs a reference/citation. ‘Previous studies have shown that the PTEN/PI3K-AKT axis is involved in drug resistance in NSCLC.’

Fig. 6A-B label ‘filed’ should be ‘field’.

Referee: 1

Comments to the Author(s)
The authors have addressed my concerns.

Author's Response to Decision Letter for (RSOB-18-0227.R1)

See Appendix B.
Decision letter (RSOB-18-0227.R2)

14-Jun-2019

Dear Dr Zhou

We are pleased to inform you that your manuscript entitled "The involvement of miR-4262 in paclitaxel resistance through the regulation of PTEN in non-small cell lung cancer" 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

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

Referee: 1

Comments to the Author(s)

Sun et al. present intriguing data between the relationship miR-4262 expression and paclitaxel resistance in NSCLC. The authors find that miR-4262 expression is upregulated in NSCLC tumor tissue and cell lines when compared to normal adjacent tissue. They also identified a putative miR-4262 binding site on the 3’ UTR of PTEN mRNA that leads to its suppression. While the presented data is robust, the manuscript as currently written has limitations.

Major concerns:

1. The paper would benefit from grammatical proofreading from a native English-speaking author. There are many grammatical, and formatting issues that make the manuscript difficult to follow at times.

Re: Dear reviewer, we’ve carefully revised this manuscript and sent it to a native speaker for help. We hope the current version can meet your satisfaction.

2. What is the sequence of the miR-mimic? Is it the same as endogenous miR-4262?

Re: Dear reviewer, the sequence of the miR-4262 mimics is: 5’-GACAUUCAGACUACCUG-3’. MiRNA mimics could increase the effective concentration of miRNAs within cells and enhance silencing of target genes [1]. Moreover, miR-mimics is one type of synthetic chemicals and has the same sequence
as the naturally occurring miRNA. Therefore, miR-mimics is expected to target the same set of mRNAs that is also regulated by the natural miRNA [2].

References:

1. Matsui M1, Prakash TP, Corey DR. Argonaute 2-dependent Regulation of Gene Expression by Single-stranded miRNA Mimics. Mol Ther. 2016 May;24(5):946-55.

2. Bader AG, Brown D, Winkler M. The promise of microRNA replacement therapy. Cancer Res. 2010 Sep 15;70(18):7027-30.

3. While the miR-4262 mimic suppresses PTEN mRNA, there is no evidence presented that the mimic alone alters PTEN protein levels (modest decrease in combination with PTX shown in Figure 6).

Re: Dear reviewer, we sincerely appreciate your advice. We’ve detected the protein levels of PTEN in A549 and H1299 cells, and the results are shown in Fig 3C-F, which revealed that miR-4262 mimics alone suppress both mRNA and protein expression of PTEN.
4. PTEN specific siRNAs or shRNAs should be used to confirm the relationship between miR-4262, PTEN, and PTX resistance since miR-4262 has been shown to target other cancer/stemness genes in breast cancer (e.g. PMID 27629257).

Re: Dear reviewer, thank you for your suggestion. We’ve confirmed the relationship between miR-4262, PTEN and PTX resistance further by PTEN specific siRNAs. And the results indicated that knocking down of PTEN by specific siRNAs had no effect on miR-4262 expression, but enhanced the resistance of NSCLC cell lines to PTX (Fig S1).

Minor issues.

1. Page 4. PMID listed instead of reference.

Re: Dear reviewer, we are sorry for making such mistake, and we’ve corrected this in our manuscript.

2. Page 9. The role of miR-4262 in NSCLC development was not investigated.

Re: Dear reviewer, we sincerely appreciate for your advice. Actually, we investigated the role of miR-4262 in NSCLC cell viability, proliferation, apoptosis
and migration abilities, which play vital roles in NSCLC progression. The results showed that miR-4262 promoted cell viability, proliferation, migration, and inhibited apoptosis (Fig 2). Therefore, we changed “development” to “progression”.

Referee 2:

This is a nice study of the role of a microRNA in cancer drug resistance. The strengths include the fact that both in vitro and in vivo data are presented and that a potential target of microRNA was identified to be a gene of high relevance, PTEN. And the data presented are of good quality. There are also weaknesses that need to be addressed before going forward. The most serious is the lack of data showing that miR 4262 plays a role in taxol resistance in the parental cancer cell lines used in xenograft studies. Specifically:

Fig. 5 is fine for showing that adding more miR 4262 to already PTX resistant cells provide further protection from the drug. But these data do not address whether miR 4262 is responsible for the PTX resistant nature of these cells, nor do they address if miR4262 has a role in the parental cancer cells (where PTX induces mir 4262). They should knock down miR 4262 (with the inhibitor or antigomiR) in PTX resistant cells and ask if this restores sensitivity and also in the parental cells, to see if decreasing miR 4262 increases sensitivity to PTX. Because in vivo studies in Fig. 7 are with parental cell lines, it is important to show that inhibition of miR4262 makes the cells more sensitive to PTX in vitro.

Re: Dear reviewer, we sincerely appreciate your advices. We’ve reconducted the
expriments about miR-4262 and PTX-resistance with miR-4262 inhibitor treatment group added both in PTX-resistant cells and the parental cells. The results showed that knocking down the expression of miR-4262 can restore the sensitivity of PTX resistant cells to PTX (Fig 5C-D). Meanwhile, knocking down the expression of miR-4262 increases the sensitivity of parental cells to PTX (Fig 5E-F).

Related, Taxol induces miR 4262 expression in cells in Fig. 4 but the opposite happens in the tumors in Fig. 7. Why this difference?

Re: Dear reviewer, thank you for point this. We examined our data and found that paclitaxel induced miR-4262 expression. In order to confirm the accuracy of the data, we verified this result again through experiments. The results showed that paclitaxel induced miR-4262 expression (Fig 7D), and we corrected our mistakes, please check.
Dear reviewer,

we sincerely feel sorry for our terrible writing, and we’ve carefully revised this manuscript and sent it to a native speaker for help. We hope the current version can meet your satisfaction.

Minor comments:

CCK8 assay measures cell number. Cell number changes could be due to changes in cell proliferation or cell death or both. In other words, CCK8 does not necessarily measure proliferation as the authors suggest in Fig. 2A. To conclude that miR 4262 affects cell proliferation, the authors need to monitor other markers such as BrdU or EdU incorporation, phospho-H3 or Ki67, or similar. Alternatively, the authors should modify the text to reflect accurately what CCK8 measures. In fact, since miR 4262 inhibition increases apoptosis, this alone can explain the CCK8 data.

Re: Dear reviewer, we sincerely appreciate your advices. In order to make the effect of miR-4262 on cell proliferation and apoptosis more clearly, we detected the expression
of Ki67, and the results showed that miR-4262 increased the expression of Ki67 and promoted cell proliferation (Fig 2C). Meanwhile, to reflect accurately what CCK8 measures, we’ve modified the description of CCK8 assay in the method section, as follows: Quantitative cell viability was evaluated with a CCK-8 assay. The cells used for the experiments were inoculated into wells of a 96-well plate (1 × 10^4 cells per well) overnight to adhere. After transfection for a certain period of time (24, 48, 72 and 96 h), each well was added 20 μl of CCK-8 solution (Sigma Chemicals, St. Louis, MO) for another 4 hours. Then, the absorbance value (optical density) values were determined at 450 nm with an ELISA reader (MultiskanEX, Lab systems, Helsinki, Finland).

**Why does miR 4262 mimic increase the level of endogenous miR 4262? Clearly endogenous miR 4262 cannot do this.**

Re: Dear reviewer, thank you for pointing this out. We’ve corrected the description about miR-4262 mimics, which has the same sequence but not identical structure with endogenous miR-4262, and clearly clarified that miR-4262 mimics can act as endogenous miR-4262 once it enters the cell and enhance silencing of target genes [1], instead of increase the level of endogenous miR-4262.

**Reference:**

1. Matsui M1, Prakash TP, Corey DR. Argonaute 2-dependent Regulation of Gene
Expression by Single-stranded miRNA Mimics. Mol Ther. 2016 May;24(5):946-55.

The migration assay needs either a citation of a reference or more details. What is in the migration medium? What are the vectors? What are in two chambers and what separates the chambers? Are the chambers commercial?

Re: Dear reviewer, thank you for pointing this. Migration chambers were purchased from Croning. These chambers can be put and suspended in 24-plate. A filter membrane in upper chamber (transwell insert) separates it from the lower chamber [1]. Cells are seeded in the upper chamber and cells migrate through the membrane to the lower chamber of the culture plate that contains chemotactic stimuli (Serum is often utilized) [2]. Thus, cells can migrate to serum-containing side.

References:

1. Justus, C.R., Leffler, N., Ruiz-Echevarria, M., and Yang, L.V. (2014). In vitro cell migration and invasion assays. J Vis Exp.

2. Lu, W., Zhang, H., Niu, Y., Wu, Y., Sun, W., Li, H., Kong, J., Ding, K., Shen, H.M., Wu, H., et al. (2017). Long non-coding RNA linc00673 regulated non-small cell lung cancer proliferation, migration, invasion and epithelial mesenchymal transition by sponging miR-150-5p. Mol Cancer 16, 118.

Are the miR inhibitor and antagomiR the same? What exactly are these?

Re: Dear reviewer, thank you for pointing this. MiR inhibitor and antagomiR are different. Actually, miR inhibitor is one type of synthetic chemicals with complementary sequence of target miRNA, which can reduce the expression of
miRNA. And miR antagonir is another type of inhibitor, which is specifically modified on the base of miR inhibitor. Therefore, antagonir is much more stable than normal inhibitor, and it’s independent of any transfection agent to enter the cell [1, 2].

Reference:

1. Tang L, Chen HY, Hao NB et al.: microRNA inhibitors: Natural and artificial sequestration of microRNA. Cancer Lett. 2017 Oct 28; 407: 139-147.

2. Yoo J, Hajjar RJ, Jeong D.: Generation of Efficient miRNA Inhibitors Using Tough Decoy Constructs. Methods Mol Biol. 2017; 1521: 41-53.
Response to Referees

Referee: 2

Comments to the Author(s)

The authors have responded comprehensively and adequately to my concerns about the science. But the writing still needs to improve. The authors used a professional service to ensure the correctness of the language. The body of the manuscript is in fairly good shape now but there are still mistakes, some of which are listed below. But the worst is the Abstract, which is the most important part because this is what the readers will see first. The Abstract in its current form is a list of experiments along with some results. There is no scientific context provided and no conclusions stated. The results provided are on the role of miR-4262 in apoptosis, proliferation and motility, and on the levels of miR-4262 and PTEN. What about the conclusion that miR-4262 is responsible for paclitaxel resistance? Nothing in the Abstract supports or even addresses this conclusion.

Re: Dear reviewer. Thank you for your advice. We have improved the abstract, as follows:

Non-small cell lung cancer (NSCLC) is considered to be the primary cause of cancer-related mortalities worldwide. Paclitaxel (PTX), either as a monotherapy or in combination with other drugs, is an alternative therapy for advanced NSCLC. However, cancer cell resistance against paclitaxel represents a major clinical problem. This study aimed to investigate the role and underlying mechanism of miR-4262 in paclitaxel (PTX)-resistant NSCLC. The levels of miR-4262 were analyzed by quantitative reverse transcription PCR (qRT-PCR). A luciferase reporter assay and bioinformatics were used to explore the potential target gene of miR-4262. Regulation miR-4262 and PTEN expressions in NSCLC were conducted by transfection. PTX-resistant A549 and H1299 cells were established by stepwise screening through increasing the
PTX concentration in the cultures. *In vivo* tumourigenesis experiments were used to explore the effects of miR-4262 and PTX. Cell proliferation, apoptosis and cell migration were detected using a CCK-8 assay, flow cytometry and Transwell migration assay, respectively. PI3K/Akt pathway-related proteins were detected by western blot. MiR-4262 expression was significantly upregulated in NSCLC tissues and cell lines, and miR-4262 targeted PTEN. Besides, miR-4262 induced PTX chemoresistance by promoting survival and migration in A549/PTX and H1299/PTX cells. Moreover, miR-4262 expression and PI3K/Akt signaling pathway-related proteins was up-regulated and PTEN was down-regulated in A549/PTX and H1299/PTX cells. Our results indicated that miR-4262 enhances PTX resistance in NSCLC cells through targeting PTEN and activating the PI3K/Akt signaling pathway. The inhibition of miR-4262 expression might be an improved treatment to overcome PTX resistance in NSCLC.

A few examples of writing problems:

The authors need to reconsider word choices; some are sub-optimal. For example, in ‘miR-4262 responded to PTX resistance…’, ‘responded’ may not be the word you want to use.

Re: Dear reviewer. Thank you for your advice. ‘We found that miR-4262 responded to PTX resistance by altering the PTEN expression level and the subsequently activated PI3K/AKT signaling pathway’ has been changed to ‘We found that miR-4262 was responsible for PTX resistance by altering the PTEN expression level and the subsequently activated PI3K/AKT signaling pathway’.

In the first sentence of the Abstract, do you mean ‘resistant’ instead of ‘resistance’?

Re: Dear reviewer. Thank you for your advice. We changed ‘resistance’ to ‘resistant’. The
sentence changes consistently from ‘This study aimed to investigate the role and underlying mechanism of miR-4262 in paclitaxel (PTX)-resistance non-small cell lung cancer (NSCLC)’ to ‘This study aimed to investigate the role and underlying mechanism of miR-4262 in paclitaxel (PTX)-resistant non-small cell lung cancer (NSCLC)’.

The following sentence needs a reference/citation. ‘Previous studies have shown that the PTEN/PI3K-AKT axis is involved in drug resistance in NSCLC.’

Re: Dear reviewer. Thank you for your advice. We have supplemented the references as follows:

Previous studies have shown that the PTEN/PI3K-AKT axis is involved in drug resistance in NSCLC [Ref.1].

Ref.1: Lu C, Wang H, Chen S, et al. Baicalein inhibits cell growth and increases cisplatin sensitivity of A549 and H460 cells via miR-424-3p and targeting PTEN/PI3K/Akt pathway. J Cell Mol Med. 2018 Apr;22(4):2478-2487.

Fig. 6A-B label ‘filed’ should be ‘field’.

Re: Dear reviewer. Thank you for your advice. In Fig. 6A-B, the label ‘filed’ has been changed to ‘field’.
Referee: 1

Comments to the Author(s)

The authors have addressed my concerns.