Peer Review File

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Reviewer A

The paper titled "Circ_0087378 intensifies the malignancy of non-small cell lung cancer by facilitating DDR1 via sponging miR-199a-5p" is interesting. Circ_0087378 promotes the progression of NSCLC by facilitating DDR1 via sponging miR-199a-5p. Circ_0087378 may be a promising target for the treatment of NSCLC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) There are many circRNA that regulatethe malignancy of NSCLC. Why did the author choose circ_0087378 for research? Please describe the reason.

Response: Thanks for your valuable comment. According to your comment, we have explained why circ_0087378 was selected, as follows: "….Thus, the role of circ_0087378 in cancers remains controversial, and more data about the function of circ_0087378 in other tumors are still not available. At present, there are many circRNAs that have been found to regulate the malignancy of NSCLC. For example…….However, the expression and function of circ_0087378 has never been researched currently. Thus, this paper aimed to elucidate the function of circ_0087378 in NSCLC in order to finding a novel target for NSCLC treatment." Please refer to the second paragraph of the introduction section for details. Your comment is very important to improve this article. Thanks for your valuable comment again.

2) Figures 2-6 are not clear enough. It is recommended to provide clearer figures again.

Response: Thanks for your valuable comment. The quality of Figure 2-6 has been improved, and a clearer Figure 2-6 has been submitted. Thanks for your valuable comment again.

3) In this paper, it is best to supplement the in vivo research. This is more conducive to support the conclusion of this paper.

Response: Thanks for your valuable comment. Your comment is very important to improve this article. However, these studies cannot currently be carried out due to laboratory limitations. We have explained this in the limitation section, as follows: "This study has limitations. Firstly, it should be better to perform the in vivo study by using animals…..However, these studies cannot currently be carried out due to laboratory limitations and this will be the focus of our future research." Your comment has important guiding significance for our future research. Thanks for your valuable comment again.

4) What are the problems and challenges that need to be overcome in the clinical application of circRNA? It is recommended to add relevant content.

Response: Thanks for your valuable comment. According to your comment, we have

added the relevant content in the last paragraph of the discussion section, as follows: "…..Of course, safe and effective drugs to inhibit circ_0087378 expression still need to be discovered. These challenges still need to be overcome in the clinical application of circ_0087378." Please refer to the last paragraph of the discussion section for details. Your comment is very important to improve this article. Thanks for your valuable comment again.

5) What are the correlations between circ_0087378 and NSCLC staging, degree of differentiation, lymphatic metastasis and survival prognosis? It is recommended to add relevant content.

Response: Thanks for your valuable comment. Your comment is very important to improve this article. However, these studies cannot currently be carried out due to laboratory limitations. We have explained this in the limitation section, as follows: "This study has limitations…...Secondly, the correlations between circ_0087378 and NSCLC staging, degree of differentiation, lymphatic metastasis and survival prognosis should be better researched by collecting clinical data. However, these studies cannot currently be carried out due to laboratory limitations and this will be the focus of our future research." Your comment has important guiding significance for our future research. Thanks for your valuable comment again.

6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "CircPTN promotes angiogenesis via the MiR-595/LYRM5 signaling pathway in non-small cell lung cancer, PMID: 35402183", "Noncoding RNAs and modulation of the EGFR/ERK pathway by circRNA C190 in non-small cell lung cancer, PMID: 34753774". It is recommended to quote the articles.

Response: Thanks for your valuable comment. Based on your comment, we have improved the introduction by citing these papers, as follows: "…. At present, there are many circRNAs that have been found to regulate the malignancy of NSCLC. For example, circular pleiotrophin (circPTN) and circRNA C190 have been proven to be oncogenic for NSCLC [13, 14], whereas circ_0002346 and circ_0003176 have been identified to be tumor suppressors for NSCLC……" Please refer to the second paragraph of the introduction section for details. Your comment is very important to improve this article. Thanks for your valuable comment again.

7) What is the impact of this study on the further treatment and prognosis of NSCLC? It is recommended to include relevant content in the discussion.

Response: Thanks for your valuable comments. According to your comment, we have added the relevant content in the last paragraph of the discussion section, as follows: "Thus, circ_0087378 may be a novel target for the clinical treatment of NSCLC in the future, and the suppression of circ_0087378 expression may help improve the prognosis of NSCLC patients….." Please refer to the last paragraph of the discussion section for details. Your comment is very important to improve this article. Thanks for your valuable comment again.

Reviewer B

Ming et al. propose a circular RNA to be involved in the proliferation, migration and invasion of NSCLC cell lines. Furthermore, they link this circular RNA mechanistically to miR-199a-5p and DDR1. The paper contains a decent amount of research results and the data support the conclusions made by the authors. I only have a couple of minor concerns that need to be clarified.

- From the manuscript it is not clear to me why the authors focused on miR-199a-5p. The investigated circular RNA must be able to interact with other microRNAs as well. The authors should explain the rationale of investigating miR-199a-5p regardless if it was based on an experimental screening approach or previous literature.

Reply: Thanks for your valuable comment. Based on your comment, we have explained why miR-199a-5p was selected in this study. Thanks for your valuable comment again.

Changes in the text: In the third part of the result section, we have added the following content: "Through bioinformatics online prediction, this study selected the potential target miRNAs of circ_0087378. Finally, we selected five miRNAs that have been identified to be down-modulated in lung cancer, including miR-1224-3p, miR-197-3p, miR-1298-5p, miR-140-3p and miR-199a-5p. Then we overexpressed circ_0087378 in A549 cells by pcDNA-circ_0087378 vectors, and detected the expression of the five miRNAs. It was found that circ_0087378 overexpression had the most pronounced down-regulation of miR-199a-5p among the five miRNAs (Figure S1). Thus, this study selected miR-199a-5p as the subject. (see page 10 and page 11)". Further, Figure S1 has been submitted. Please refer to the revised section of the article for details. Your comment is very important to improve this article. Thanks for your valuable comment again.

- Likewise, it is not clear why the authors focus on DDR1 as the important miR-199a-5p target. MicroRNAs are molecularly promiscuous players where each microRNA has the ability to target hundreds of mRNAs. The authors need to explain the rationale of selecting DDR1 and not any other targets of miR-199a-5p.

Reply: Thanks for your valuable comment. Based on your comment, we have explained why DDR1was selected in this study. Thanks for your valuable comment again.

Changes in the text: In the fifth part of the result section, we have added the following content: "This paper predicted the potential target mRNAs of miR-199a-5p via online bioinformatics analysis. Among these mRNAs, we selected the five mRNAs that have more binding sites of miR-199a-5p and have been shown to be up-regulated in cancers. As a result, DDR1, SLC25A23, NAA40, ZNF763 and MYRF were selected. This study was then overexpressed miR-199a-5p in A549 cells by miR-199a-5p mimic transfection to detect the effect of miR-199a-5p on the expression of the five target mRNAs. As a result, miR-199a-5p overexpression

showed the stronger inhibitory effect on DDR1 mRNA expression than on the others (Figure S2). Therefore, DDR1 was selected to be the subject in this research. (see page 11 and page 12)". Further, Figure S2 has been submitted. Please refer to the revised section of the article for details. Your comment is very important to improve this article. Thanks for your valuable comment again.

Reviewer C

The manuscript describes the impact of circ_0087378 in relation to NSCLC cell lines. The presented results are new and could have impact together with the numerous other studies recently published, describing the involvement of circ RNA in carcinogenesis,

The study has some limitations as described below.

Major concerns.

The manuscript only address the impact of circ_0087378 in NSCLC cell lines. It would have been beneficial if in vivo data were included. However, if the authors not find it possible to include such in vivo data it is absolutely necessary they rewrite the entire manuscript in a way clearly specifying that their data are in vitro data and only in the discussion briefly address the possible in vivo impact for NSCLC patient treatment and understanding of the NSCLC etiology. Even the title is misinterpreting the actual content of the manuscript and must be modified like the rest of the manuscript to clearly state that this is an in pure in vitro study.

Reply: Thanks for your valuable comment. Based on your comment, we have revised the paper carefully in order to emphasize the in vitro study. Thanks for your valuable comment again.

Changes in the text: The specific amendments in the text are as follows:

1) Title: the original title has been revised into "Circ_0087378 intensifies the malignant behavior of non-small cell lung cancer cells in vitro by facilitating DDR1 via sponging miR-199a-5p". (see page 1)

2) Abstract section: In background section, we revised the description as follows: "….This study revealed the effect of circ_0087378 on the malignant behavior of NSCLC cells in vitro to broaden the options for NSCLC treatment." In conclusion section, we revised the description as follows: "…. Circ_0087378 promotes the malignant behavior of NSCLC cells in vitro by facilitating DDR1 via sponging miR-199a-5p…." (see page 2)

3) Introduction section: In the last paragraph of the introduction section, we have revised as follows: "….Therefore, in the present study, we verified whether circ_0087378 regulated the malignant behavior of NSCLC cells in vitro via targeting miR-199a-5p/DDR1." (see page 4)

4) Discuss section: In Discuss section, we revised as follows: "In the present study, circ_0087378 was identified as an oncogene in NSCLC. It intensified the malignant behavior of NSCLC cells in vitro by facilitating DDR1 via acting as a miR-199a-5p sponge. (see the first paragraph of the discussion in page 11)" "In the present work, circ_0087378 was found to exert cancer-promoting activity in NSCLC; it intensified

the malignant behavior of NSCLC cells in vitro by sponging the miR-199a-5p/DDR1 axis. (see the first paragraph of the discussion in page 12)" "Similarly, the present research revealed the tumor suppressor role of miR-199a-5p in the malignant behavior of NSCLC cells in vitro. Moreover, circ_0087378 exerted its promoting role in the malignant behavior of NSCLC cells in vitro by sponging miR-199a-5p. (see the second paragraph of the discussion in page 12)" "DDR1 up-modulation counteracted the inhibition of miR-199a-5p on the malignancy of NSCLC cells in vitro. Taken together, circ_0087378 might intensify the malignant behavior of NSCLC cells in vitro by facilitating DDR1 via acting as a miR-199a-5p sponge……. Further, this paper provides new insights into the understanding of the etiology of NSCLC. (see the third paragraph of the discussion in page 13)"

5) Limitation section: "This study has limitations. Firstly, in vivo study with animals is preferable, and it will be conductive to the NSCLC treatment with circ_0087378 as the target. (see page 13)"

6) Conclusion section: "This study in vitro experiments and identified circ_0087378 as an oncogene in NSCLC for the first time. circ_0087378 knockdown suppressed the malignancy of NSCLC cells in vitro. Mechanically, circ_0087378 might potentiate the malignant development of NSCLC cells in vitro by facilitating DDR1 via acting as miR-199a-5p sponge. (see page 13)"

The English writing throughout the manuscript needs a professional correction by a native English speaking person. At many places the wording is odd and difficult to understand e.g. lines 93-94, 279-280, 327, 331, and etc.

Reply: Thanks for your valuable comment. We have polished the paper carefully by skilled editor, in order to improve the language. Thanks for your valuable comment again.

Changes in the text: Please refer to the revised section of the article for language touch-ups.

The manuscript would improve with some experiments showing if interfering with the the circ_0087378/mir199/DDR1 pathway resulted in alterations in the EMT profile which could explain the observed cellular phenotypes.

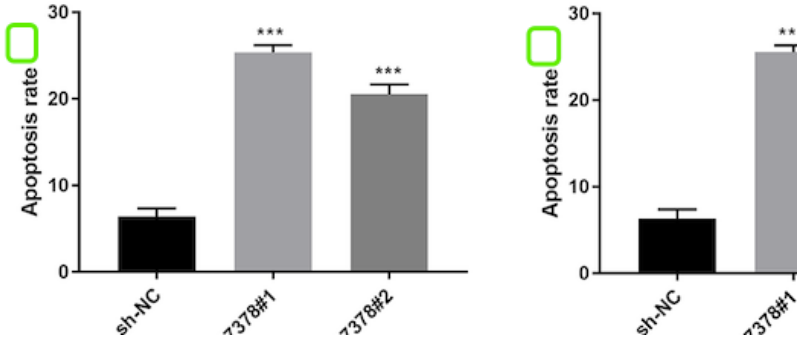
Reply: Thanks for your valuable comment. Your comment is very important to improve this article. However, due to the limitations of the laboratory, these studies cannot currently be carried out. This will be the focus of our future research. We have explained this in the limitation section. Your comment has important guide for our future research. Thanks for your valuable comment again.

Changes in the text: Limitation section: "Further, to show that interfering with the circ_0087378/mir199/DDR1 pathway leads to epithelial mesenchymal transition (EMT) alteration to alter NSCLC cell malignant phenotype, it would be desirable to perform EMT-related experiments. However, these studies cannot currently be carried out due to laboratory limitations and this will be the focus. (see page 13)"

Reviewer D

1. Figure 1:
1) Figure 1 is not clear enough. Please resubmit it in jpg/tiff format to us.
2) Please indicate the full name of "qRT-PCR", "NSCLC" in the legend.
Response: Thank you for your valuable comments.
1) We have resubmit Figure 1 in jpg/tiff format.
2) We have indicated the full name of "qRT-PCR" and "NSCLC" in the legend of Figure 1. Please refer to the revised section of the article for details. Thank you for your valuable comments again.

2. Figure 2:
1) Please indicate the staining method in Figure 2C legend. And indicate the staining method and magnification for cell maps in Figure 2D, E legend.
2) Please indicate the full name in legend for all the abbreviated terms appearing in Figure 2 such as "qRT-PCR", "NSCLC", "CCK-8", "NC", "OD" etc.
3) Please add unit % in the y-axis of Figure 2F for apoptosis rate.



Response: Thank you for your valuable comments.
1) We have indicated the crystal violet (0.1%) staining for Figure 2C, 2D, and 2E. The information of magnification (200 ×) has been provided for Figure 2D, and 2E.
2) We have provided the full name for all the abbreviated terms in the legend of Figure 2.
3) We have added the unit % in the y-axis of Figure 2F for apoptosis rate.
Please refer to the revised section of the article for details. Thank you for your valuable comments again.

3. Figure 3:
Please indicate the full name of "qRT-PCR", "NSCLC", "NC", "WT", "MUT" in the legend.
Response: Thank you for your valuable comments. We have provided the full name of "qRT-PCR", "NSCLC", "NC", "WT", "MUT" in the legend of Figure 3. Please refer to the revised section of the article for details. Thank you for your valuable comments again.

4. Figures 4, 6:

Figures 4, 6 have the same issues with Figure 2. Please revise.

Response: Thank you for your valuable comments. We have revised Figures 4 and Figure 6 according to the issues with Figure 2. Please refer to the revised section of the article for details. Thank you for your valuable comments again.

5. Figure 5:

Please indicate the full name of "DDR1", "NSCLC", “NC”, “WT”, “MUT” in the legend.

Response: Thank you for your valuable comments. We have provided the full name for all the abbreviated terms in the legend of Figure 5. Please refer to the revised section of the article for details. Thank you for your valuable comments again.