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OPEN PEER REVIEW

The Expression of miR-34c-5p Induces G0/G1 Cell Cycle Arrest and Apoptosis in SW480 Colon Cancer Cell

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1 Original submission

2 comments · 10 Feb 2023 – 27 Feb 2023

Referee 1 Reviewer

MAJOR REVISION 10 Feb 2023

Thanks to the authors. Some points need further clarification:
1- The miR-34c category has two members, miR-34c-5p and miR-34c-3p. If you have used the miR-34c-5p mimic in your study, then you should specify this precisely throughout the manuscript, including the title.
2- The rationale for choosing miR-34c-5p over miR-34c-3p is not clear.
3- Anti-proliferative and apoptotic effects of miR-34c-5p have been studied in various previous investigations. Some are listed below:
a) Methylation detection of circulating tumor cell miR-486-5p/miR-34c-5p in the progression of colorectal cancer
b) Downregulation of SIRT6 by miR-34c-5p is associated with poor prognosis and promotes colon cancer proliferation through inhibiting apoptosis via the JAK2/STAT3 signaling pathway
c) Pien Tze Huang inhibits the proliferation of colorectal cancer cells by increasing the expression of miR 34c 5p
d) LINC01123 facilitates proliferation, invasion and chemoresistance of colon cancer cells
How do you think your study augments and expands on the previous studies?

Associate Editor

MAJOR REVISION 27 Feb 2023

Major revision

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Dear Editor in Chief,

We would like to take this opportunity to thank you, the editor and reviewers for the useful and insightful comments on our manuscript “The expression of miR-34c-5p induces G0/G1 cell cycle arrest and apoptosis in SW480 colon cancer cell”. We answered all comments relating to the revision of the manuscript, in a point-by-point fashion. All corrections and changes are highlighted within the manuscript. All page and line numbers refer to the revised manuscript file with tracked changes.

Yours sincerely,

Corresponding author name, MSc, PhD

Department of …….

Faculty of……..

University name

Reviewer 1

Thanks to the authors, some points need more clarifications:

Response: Thank you for your thoughtful and constructive comments on our manuscript.

1. miR-34c category has two members, miR-34c-5p and miR-34c-3p. If you have used miR-34c-5p mimic in your study, then all over the manuscript you should mention it precisely; including the title.

Response: Thanks for pointing this out. As suggested by the reviewer, all “miR-34c” throughout the manuscript were revised accordingly.

2. The rationale for choosing miR-34c-5p over miR-34c-3p is not clear.

Response: We agree that this is a potential limitation of the study. Thus, we have added limitations of our study on page 15, lines 276-280.

3. Anti-proliferative and apoptotic effects of miR-34c-5p have been studied in various investigations before. Some are as below:

a) Methylation detection of circulating tumor cell miR-486-5p/miR-34c-5p in the progression of colorectal cancer
b) Downregulation of SIRT6 by miR-34c-5p is associated with poor prognosis and promotes colon cancer proliferation through inhibiting apoptosis via the JAK2/STAT3 signaling pathway
c) Pien Tze Huang inhibits the proliferation of colorectal cancer cells by increasing the expression of miR34c5p
d) LINC01123 facilitates proliferation, invasion and chemoresistance of colon cancer cells
What do you think are the augmentation and expansion of your study comparing to the previous ones?

Response: We agree that the anti-proliferative role of miR-34c-5p has been previously studied in the colorectal cancer. However, as we noted in the last paragraph of “introduction” section, we

aimed to understand the exact mechanism of miR-34c-5p inhibitory effects on colorectal cancer cell viability and proliferation. It is also important to note that the above mentioned studies are, to some etent, different from ours as they have different objectives and outcomes, which are as follows:

a) Methylation detection of circulating tumor cell miR-486-5p/miR-34c-5p in the progression of colorectal cancer: The objective of this study was to determine expression and methylation level of miR-486-5p/miR-34c-5p and their mode of action in colorectal cancer, while our study mainly focused on the effect of miR-34c-5p on cell cycle, viability and the rate of apoptosis and necrosis in the SW480 colorectal cancer cell line.

b) Downregulation of SIRT6 by miR-34c-5p is associated with poor prognosis and promotes colon cancer proliferation through inhibiting apoptosis via the JAK2/STAT3 signaling pathway: This study reported results opposite to our findings. This one shows that miR-34c-5p is capable of inducing colorectal cancer cell proliferation. However, we reported that miR-34c-5p could potently induce colorectal cancer cell apoptosis and reduce its proliferation and viability.

c) Pien Tze Huang inhibits the proliferation of colorectal cancer cells by increasing the expression of miR34c5p: This study has determined the impact of miR-34c-5p transfection and Pien Tze Huang (PZH) treatment on the viability and proliferation of human colon carcinoma (HCT)-8 cell lines, while we attempted to study the effect of miR-34c-5p transfection on the survival rate of SW480 cell line.

d) LINC01123 facilitates proliferation, invasion and chemoresistance of colon cancer cells: This study introduces a new perspective into colorectal cancer by proposing LINC01123/miR-34c-5p/VEGFA axis, which leads to colon cancer malignancy and causes chemoresistance. This is different from our study, which has proposed cellular mechanisms of miR-34c-5p against colorectal cancer cell proliferation.

Reviewer 2

Please reply to each question completely and separately. Please change or add different parts in the manuscript using track-change or with different colors to prevent ambiguity.

Response: We would like to thank the reviewer for careful and thorough reading of this manuscript and for the constructive comments.

1. Please describe abstract section according to the journal instruction.

Response: Thank you for pointing this out. The abstract section has been revised accordingly.

2. Please describe about the targets of miR-34 in introduction section.

Response: Thank you for this suggestion. We have added a recently published review demonstrating numerous target genes of miR-34, the most important of which are related to cancer progression (page 2, lines 43-44).

3. Please insert the country of all companies.

Response: Thanks for the reminder. The country of origin for all companies has been added in the revised manuscript.

4. Please change all units according to the SI system (eg. Change µl to µL and etc.).

Response: Thank you for pointing this out. We have corrected all units based on SI system.

5. Please report the data of MTT assay according to “viability” in figure 1 rather than OD.

Response: We appreciate the reviewer’s comment. Accordingly, MTT data has been corrected and reported as viability rate.

6. Please revise the data presentation in line 127 and (148-149).

Response: These two parts were revised (page 6, lines 124-126 & page 8, lines 151-152).

7. It should be described in figure legend 1 and 2D that in comparison to which group the statistical analysis was performed.

Response: We have corrected the figure legends based on the reviewer’s suggestion.

8. Please revise Figure legend 2D.

Response: This part was revised accordingly.

9. It is highly suggested to perform Western blot to analyze the effect of miR-34c transfection on expression of different genes.

Response: We thank the reviewer for this comment. It would have been interesting to assess protein expression in parallel with analysis of gene expression. However, we are experiencing a harsh economic condition, whereupon we are not able to obtain more funding to perform Western blotting.

2 Revision 1

1 comment · 14 Apr 2023

Hamed Montazeri Reviewer

ACCEPT 14 Apr 2023

Thanks for your replies and corrections.