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OPEN PEER REVIEW

MicroRNA-642a-5p Targets DNA Damage-Inducible Transcript 4 to Suppress Hepatitis B Virus Hepatoma Carcinoma Cell

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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Contents

1 Original submission Referee 1 2
2 Revision 1 3
Authors' reply to reviewers 4
Reviewer and editor comments 11

1 Original submission

1 comment · 2 Mar 2024

Referee 1 Reviewer

MINOR REVISION 2 Mar 2024

Dear Editor,
The author of this manuscript evaluated the function of MicroRNA-642a-5p in suppressing hepatitis B virus hepatoma carcinoma cells. The manuscript has a good level of quality. The authors proved their hypothesis using different kinds of experimental techniques. In my view, the manuscript can be accepted for publication after minor revision.
Comments are as follows:
1- Please mention the ethics code in the method section.
2- Please provide a brief explanation of "para-cancer normal liver tissues" as a control group.
3- What does the author mean by the antibacterial rate equation in section 2.4? This equation is usually used for evaluating antibacterial treatment. What is its relation to this study?
4- In section 2.10, please mention that the 3'-UTR of DDIT4 was inserted into a firefly luciferase plasmid that contains a LUC promoter.
5- In section 3.1, what are the author's criteria for HBV positivity? HBsAg or viral DNA? Please make this clear in this part and other parts of the manuscript.
6- Please correct this sentence in section 3.3: "DDIT4 mRNA of HepG2.2.15 HCC transfected with miR-642a-5p mimic was detected"
7- In Figure 5, please correct the figure. Parts A and B should be replaced based on the figure legend. Overall, figures are incompatible with the legends.
8- Please revise the manuscript for alphabetical errors.

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 Sheng Zheng,

Thanks for submitting your valuable manuscript to the journal. As a result of this, we would like to inform you that the review of your manuscript is finished, and based on the editorial decision, you need to do a "**Minor Revision**" on your manuscript. Even though this manuscript has enough quality to enter the review process, it needs some significant (Minor) revisions in some points of view.

How to Submit Revision?

Below you may find those comments. Please read them and answer them one by one.

The **corresponding author** needs to prepare replies as below:

1. In a Word file, reply to all comments one by one (reply to reviewer)
2. In the Word file of your manuscript, reply and apply all comments as marked or highlighted lines/paragraphs.

Instruction: click here

Reviewers' Comments:

Dear Editor,
The author of this manuscript evaluated the function of MicroRNA-642a-5p in suppressing hepatitis B virus hepatoma carcinoma cells. The manuscript has a good level of quality. the authors proved their hypothesis using different kinds of experimental techniques. in my view, the manuscript can be accepted for publication after minor revision. All these comments are very valuable and helpful to us in revising and improving our thesis, as well as important guidance for our research. We have carefully studied these comments and made corresponding revisions in the hope that they will be recognized by you. Our response to the reviewers' comments is as follows.

Reviewer 1:
Comments are as follows:

1- Please mention the ethics code in the method section.

Response: Based on your suggestion, we have added ethical code in the Methods section. See Page 2, lines 80.

2- please a little explanation about "para-cancer normal liver tissues" as a control group

Response: Thank you for your question. Para-cancer normal tissues, also known as non-tumor tissue, is often considered a control group in tumor-related studies. Para-cancer normal tissues is in an intermediate preneoplastic state consisting of morphologically normal but molecularly altered cells that may accumulate oncogenic events. In this study, clinical samples were analyzed mainly for oncogene detection, and the sampling criteria for para-cancer normal liver tissues was a distance of greater than 2 cm from the tumor tissue, and para-cancer tissue within 2 cm may have tumor cell infiltration. The use of para-cancer normal tissue as a control group has several advantages. Current conventional and postoperative treatments for hepatocellular carcinoma include radiotherapy, chemotherapy, antiviral drugs, and traditional Chinese medicine, and the use of para-cancer normal tissues to reflect the microenvironment surrounding the tumor may be helpful in the development of clinical strategies and specific interventions to prevent recurrence. In addition to this, the use of samples from the same individual for comparison may reduce individual-specific and anatomical site-specific effects. See Page 7, lines 279-290.

3- What does the author mean by the antibacterial rate equation in section 2.4? This equation is usually used for Evaluating antibacterial treatment. What is its relation to this study?

Response: Thank you for your question. After we reorganized and checked the formula, the main purpose of the formula is to detect the effect on HBV DNA copy number after transfection treatment and to assess the mechanism of action of up- or down-regulation of MiR-642a-5p and DDIT4 on HBV replication *in vitro*. For clearer presentation as well as better understanding by the readers, we replaced the description of the formula as HBV DNA inhibition rate (%) = [(control DNA copy number - experimental DNA copy number)/control DNA copy number] x 100%. See Page 3, lines 111-113.

4- In section 2.10, Please mention that 3'-UTR of DDIT4 was inserted in firefly luciferase plasmid which contains LUC promoter.

Response: Following your suggestion, we have supplemented our methods with

the insertion of the 3'-UTR of DDIT4 into the luciferase plasmid containing the LUC promoter. See Page 4, lines 163-168.

5- in section 3.1, What are the author's criteria for HBV positivity? HBsAg or viral DNA? Please make it clear in this part and other parts of the manuscript.
Response: Thank you for your suggestion. In our study, clinical samples were collected from 48 cases of HBV-positive HCC tissues, and the criteria for these tissues to patients as study subjects were that they needed to meet the criteria of the relevant diagnostic and treatment protocols, followed by a confirmed diagnosis of hepatocellular carcinoma in combination with pathologic biopsy results, and an HBV DNA level of more than $1x10^3$copies/mL, and serum HBsAg positivity for more than 6 months. See Page 2, lines 69-74.
6- Please correct this sentence in section 3.3: DDIT4 mRNA of HepG2.2.15 HCC transfected with miR-642a-5p mimic was detected.
Response: Thank you for carefully reading our manuscript. We have corrected this sentence in the revised manuscript. See Page 5, lines 217-218.

7- In Figure 5, Please correct the figure. A and B parts should be replaced based on the figure legend. Overall, Figures are incompatible with legends
Response: Thank you very much for reading our paper carefully and we apologize very much for our carelessness. We have corrected the ordering of Figures 5A and B and checked all the images. We confirm that the ordering of all images is now correct.

8- please revise the manuscript for alphabetical errors.
Response: Thank you for your suggestions. We sincerely apologize for the inconvenience caused by the grammatical issues and typos in the reading. These and all similar issues have been revised based on your comments.

Reviewer 2:
Dear Authors:
Strengths:
The study provides important and valuable findings regarding the role of miR-642a-5p and DDIT4 in HBV-induced HCC.
The use of multiple methods, such as the luciferase reporter assay and statistical analysis, adds to the robustness of the study.
The study suggests potential clinical applications, such as using miR-642a-5p as a biomarker for HBV-related HCC detection and treatment.
The study was conducted with ethical considerations in mind and received

ethical approval.
Response: Thank you for your reviewing.

Weaknesses:
1. The methods section could benefit from more detailed information on plasmids, DNA templates, and luciferase activity measurement protocol.
Response: Thank you for your suggestion. Following your suggestion, we have detailed the plasmid, the use of DNA templates and luciferase activity measurements in the Methods section, an improvement that makes it easier for our readers to read and understand the content. See Page 4, lines 168-174.

2. While the study suggests potential clinical applications, further research is needed to fully understand the mechanisms of miR-642a-5p and DDIT4 in HBV-infected HCC.
Response: Thank you for your suggestion. In this study, in order to investigate the specific mechanisms of miR-642a-5p and DDIT4 in HBV-infected HCC, we performed clinical analyses, *in vitro* experiments to detect the proliferation, apoptosis, invasion and migration abilities of HBV-infected HCC cells, and the focus of the article is to provide theoretical foundations for the discovery of new targets in HBV-associated hepatocellular carcinoma. We agree with the reviewers that more research would help to enhance the credibility of the article, but we are currently limited by the lack of the necessary equipment in our laboratory to conduct such in-depth studies. The reviewers' concerns are important for our further research, and we will present the results of our study on the mechanism of action of miR-642a-5p and DDIT4 in HBV-infected HCC in our next paper. See Page 8, lines 339-352.
3. The statistical analysis could benefit from more information on infection analysis and one-way ANOVA.
Response: Your suggestions are very valuable to us. We have added infection analysis and one-way ANOVA. This improvement is intended to make it easier for our readers to read and understand the content and to increase the credibility of the article. See Page 5, lines 182-183.

4- Grammar and wording: The manuscript contains numerous grammatical errors and awkward phrasing that hinder the clarity and readability of the text. For example, in the abstract (line 10), the phrase "to repress Hepatitis B virus (HBV) liver cancer (LC)" is confusing and should be revised for better clarity.
Discover my suggestions and edit your abstract to receive better results for similar publications and journal match.

"Hepatocellular carcinoma (HCC) is a primary liver cancer with high mortality worldwide, mainly caused by hepatitis B virus (HBV) infection. The mechanism of miR-642a-5p in HBV-infected LC is unknown. This study aimed to explore the mechanism of inhibition of HBV HCC by miR-642a-5p targeting DDIT4. The results showed that miR-642a-5p was downregulated in HBV-positive HCC tissues and HepG2.2.15 cells, while DDIT4 was upregulated. Overexpression of miR-642a-5p inhibited HBV DNA replication, cell proliferation, and invasion, and promoted cell apoptosis in HepG2.2.15 cells. Moreover, miR-642a-5p directly targeted DDIT4, and knockdown of DDIT4 inhibited HBV DNA replication, cell proliferation, and invasion, and promoted cell apoptosis in HepG2.2.15 cells. In summary, this study suggests that miR-642a-5p inhibits HBV DNA replication and tumor progression in HBV-infected HCC by targeting DDIT4. These findings provide a potential therapeutic strategy for HBV-infected HCC."

Response: Thank you very much for your valuable comments. We apologize for the errors in the manuscript and the inconvenience caused to your reading. We have completely revised the manuscript, and the language in our revised version has been professionally edited and verified by a native English speaker. See Page 5, lines 182-183. In addition, we have rewritten the abstract section based on your suggestions and hope that it will be up to the standard for final publication. See Page 1, lines 5-26.

5- Weak conclusions: The conclusions drawn from the data are limited and fail to provide significant insights into the broader implications of the study. The authors merely state that DDIT4 is a direct target of miR-642a-5p without discussing the potential clinical relevance or therapeutic applications.

Response: We have done our best to improve the manuscript, and we have rewritten the Discussion section based on the reviewers' comments, adding a comparison of the results of this study with the current latest research results and research progress in HBV-associated hepatocellular carcinoma, highlighting the commonalities and innovations between this study and other studies. At the same time, we have raised questions about the direction and potential limitations of the study in the future, emphasizing that the results of the present study provide a theoretical basis for future research, and discussing the potential clinical significance and application of the miR-642a-5p/DDIT4 axis in HBV-associated hepatocellular carcinoma. See Page 7, lines 270-279 and Page 8, lines339-344.

6- Additionally, it would be valuable to include some discussion on the potential clinical implications of targeting miR-642a-5p and DDIT4 in HBV-related liver cancer.

Response: Thank you for your suggestion. We have added a discussion and analysis of the potential clinical significance of the miR-642a-5p/DDIT4 axis in HBV-associated HCC in the Discussion section based on your suggestion. See Page 8, lines 312-321.

Associate Editor's Comments:
Associate Editor 1:
The manuscript" MicroRNA-642a-5p targets DNA damage-inducible transcript 4 to suppress hepatitis B virus hepatoma carcinoma cell" needs minor revision.
Response: Thank you for your suggestion. Without changing the research content of the original manuscript, we have made significant revisions aimed at increasing the credibility of the manuscript as well as making it easier for our readers to understand. We hope that it will meet the standards for final publication.

EIC Decision:

Thank you for submitting your manuscript to JJM. I have completed the review of your manuscript and a summary is appended below (Pleases find attached files). The reviewers recommend the reconsideration of your paper following major revision. I invite you to resubmit your manuscript after addressing all reviewer comments.
Please see attached reviewer's files.
1. Please answer point by point to the reviewer's comments on a separate sheet under WORD format and write your modifications in the revised version (WORD format) in colored or highlighted letters.
2. Your answers to reviewers' queries are not only for the Editorial Board but for the readers and should be included, when appropriate, in the manuscript for improvement and a better understanding. Moreover, add continuous line numbers for text and put suitable line numbers for each response.
3. When resubmitting your manuscript, please carefully consider all issues mentioned in the reviewers' comments, outline every change made a point by point, and provide suitable rebuttals for any comments not addressed.
4. In addition, JJM INSTRUCTION is attached to the mail, please find it.

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Response: Thank you for your reviewing. We have modified and typeset the article according to the requirements of the journal, please check again, thank you!

2 Revision 1

0 comments

No public comments in this round.