To:

Ze-Mao Gong

Director, Editorial Office of **WJGO**

April 8, 2019

Dear Dr. Gong,

Thank you for your response and for the reviewers' further comments concerning our manuscript entitled **"MicroRNA-331 inhibits development of gastric cancer through targeting MSI1" (ID: 47504).** We are willing to publish our manuscript in WJGO and we have addressed the comments meticulously, and the amendments are made in the revised manuscript. The major revised parts in the text have been marked in "manuscript with tracks.doc". And point by point response to the reviewers' comments is listed below. We hope that the revised version of this document is now acceptable for publication in your journal.

Reviewer #1: The present paper by Leiying Yang, described the Article, "MicroRNA-331 inhibits development of gastric cancer through targeting MSI1". In this report, they demonstrated that MiR-331 inhibited development (metastasis and tumor growth) of GC through targeting MSI1. This is a potentially interesting report, but there are some issues to be discussed more. The authors should consider the following questions and answer them adequately.

1) The cell lines used for experiments are only 1 cell line (except for Fig1B). Other cell lines of GC should be done the experiments and show the result similarly.

Response: Thank you for your advice. In this study, MKN-45 cell line was selected to explore the function of miR-331 in gastric cancer because of the significant differences in expression of miR-331. And because of the small number of cells we collected in SGC-7901 and BGC-803 cell lines, we are unable to complete the repetition of other cell line experiments in a short time.

2) In Figure 1, the authors showed the patients data and correlation of miR-331 expression. Also, the authors performed migration and invasion assay to investigate how miR-331 regulates metastasis. So the authors should analysis clinicopathological data (including stage, etc.) and its correlations to miR-331 expression.

Response: Thank you for your suggestion. The correlation between miR-331 and clinicopathological data has been added. And new Table 2 and information have been provided.

3) In Figure 2, the authors demonstrated that miR-331 regulated the tumor growth of MKN-45. The immunohistochemistry examination, for example Ki-67, is strongly recommended.

Response: Thank you for your advice. According to your suggestion, the immunohistochemistry examination of Ki-67 has been added in Figure 2. And new Figure 2 has been provided.

Reviewer #2: The manuscript "MicroRNA-331 inhibits development of gastric cancer through targeting MSI1" submitted by Leiying Yang et al. is a very good study that demonstrates that MiR-331 might inhibits the development of GC through targeting MSI1. However, some modifications must be done, such as:

1- Change the phrase "The effect of miR-331 on cell metastasis and tumor growth was illuminated in GC" that describes the aim of the study in abstract by a infinitive verbal form, such as "To illuminate the effect of miR-331 on cell metastasis and tumor growth in GC";

Response: Thank you for your suggestion. The phrase "The effect of miR-331 on cell metastasis and tumor growth was illuminated in GC" has been revised in **Abstract**.

2- On the phrase "And miR-331 inversely regulated MSI1 expression in GC tissues" in the abstract, I suggest you exchange "And" for "In addition" or "Moreover";

Response: Thank you very much. The word "And" has been exchange using "In addition".

3- It is necessary to write correctly the name of the animal care and use committee in the topic "Xenograft tumor formation assay".

Response: We sincerely apologize to you for the trouble caused by this mistake. We have added the the animal care and use committee in the topic "Xenograft tumor formation assay".

Reviewer #3: In the manuscript “MicroRNA-331 inhibits development of gastric

cancer through targeting MSI1" submitted by Leiying Yang et al., authors show that in gastric cancer tissue there is a low expression of miR-331, and an increased expression of the MSI1 gene, both related to poor survival. The authors performed a series of functional studies demonstrating that miR-331 inhibits cell proliferation, migration, and invasion in vitro, and tumor growth in vivo. Very interesting is the part that demonstrates a direct relation between miR-331 and MSI1, with miR-331 expression negatively regulating MSI1 expression. The study is very good, with a lot of methods and interesting results, however there are several improvements that need to be considered before publication (included in the Comments for authors): the methods section should be completed with absolutely necessary data, some figures and legends needs correction, and additional tests are required for demonstrating the effect if miR-331 on cell adhesion. Also, English spelling should be thoroughly checked. In the following, the points to be revised:

1. Abstract: "qTR-PCR" – to be corrected with qRT-PCR; "CONLUSION" – misspelled word

Response: We sincerely apologize for the troubles caused by our carelessness. The words "qTR-PCR" and "CONLUSION" have been corrected in the manuscript.

2. Page 4- Drosophila must be written with capital letter

Response: Thank you for your suggestion. The word "Drosophila" has been written with capital letter.

3. Methods: Cell transfection – what kind of mimics and inhibitors were used for miR-331 and MSI1? The nucleotide sequence must be provided.

Response: The nucleotide sequences of miR-331mimics and inhibitors have been added in the text. The nucleotide sequence of MSI1 plasmid cannot be obtained, because it was purchased directly.

4. Quantitative real-time polymerase chain reaction – primer sequence for miR-331 and MSI1 must be provided

Response: primer sequence for miR-331 and MSI1 are given in Table 1.

5. Xenograft tumor formation assay – “The xenograft study was approved by the Animal Care and Use Committee of ××”. – incomplete phrase

Response: We sincerely apologize for the troubles caused by our carelessness. The information has been added.

6. Cell migration and invasion assay – the MKN-45 cells were starved prior to invasion assay?

Response: The MKN-45 cells were starved prior to invasion assay. The information has been added in the manuscript.

7. The luciferase reporter assay - information about the MSI1 gene: wild type (Ensembl number) and mutated sequence should be provided.

Response: The information about the MSI1 gene has been provided in the text.

8. Western blot analysis – the antibodies producers should be mentioned

Response: The antibodies producers have been provided in the text.

Results Fig. 1C, 1D – the cut-off value after which the samples were divided into low vs high miR-331 should be mentioned

Response: Thank you for your suggestion. Based on the expression of miR-331, these cases were divided into a high miR-331 expression group and a low expression group based on its median value in GC patients (cutoff point =0.75).

9. Results: Fig 2C, 2D – the use of miR-331 mimics for in vivo tests should be mention also in the legend and 2C, 2D figures

Response: The use of miR-331 mimics has been added in the legend and 2C, 2D figures. And new Figure 2 has been provided.

11. Results Fig 3D – The adhesion test is not convincing. How do the authors explain that although the ability to migrate and invade decreased when using miR-331 mimics, the same (miR-331 mimics) induce a decrease in cell adherence as well, when normally is the opposite: a decrease in cell adherence is linked to an increased cell invasion? The same question for Fig 5F. Testing the E-cadherin and vimentin proteins could be more useful in determining the adhesion level and metastatic potential (PMID: 21684617)

Response: Thank you for your advice. According to your suggestion, we have examined the E-cadherin, E-cadherin and vimentin proteins to determine the adhesion level and metastatic potential. The information and new Figure 3 and Figure 5 have been provided.

Thank you again for your patient editing of our paper. We are pleased to see the paper is in a better shape now.

I look forward to hearing from you soon.

Yours sincerely,

Mingshun Zhou

E-mail: zhoumingshun790@163.com

To:

Dr Ze-Mao Gong,

Science Editor of **World Journal of Gastrointestinal Oncology**,

May 23, 2019

Dear Dr. Gong,

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "MicroRNA-331 inhibits development of gastric cancer through targeting musashi1" (Manuscript No. 47504). We have studied comments carefully and made corrections on our manuscript that we hope meet with approval. The specific responses to the points are listed below.

Special comments from the editor:

1 Please check the number of animals in each group, and at least provide three images of animal tumors in Figure 2C.

Answer: Thank you for your comments. The number of animals in each group has been given in figure legend of Figure 2, and animal tumors in Figure 2C have been replaced.

2 Please provide us the ARRIVE Guidelines Checklist.

Answer: The ARRIVE Guidelines Checklist and Institutional Animal Care and Use Committee Approval Form have been uploaded.

Thank you again for your patient editing of our paper. We are pleased to see the paper is in a better shape now.

Sincerely

Mingshun Zhou