

Dear Professor Ma:

Manuscript NO: 80219

Title: **miR-627-5p inhibits colorectal cancer cell proliferation, migration and invasion by targeting Wnt2**

Thank you for your letter and the reviewer's comments concerning our manuscript. These comments are all valuable and very helpful for revising and improving our paper. According to the suggestions of the science editor, we have added the "article highlights" section at the end of the main text. Besides, we have studied the reviewers' comments very carefully and have tried our best to improve the manuscript. The followings are our point-by-point responses to the original reviewers' remarks underneath each comment. Revised portions are marked in yellow in the revised version of the manuscript. I hope that the adjustments made to the manuscript are satisfactory, and I am looking forward to your correspondence!

All the best.

Yours Sincerely,

Shu-Kun Yao

Department of Gastroenterology, China-Japan Friendship Hospital, 2nd

Yinghua East Road, Chaoyang District, Beijing 100029, China.

Email: shukunyao@126.com

Answer reviewer 05573866:

Thank you very much for your kind response to this manuscript. We should like to express our appreciation to you. We have made revisions or explanations point by point. Revised portions are marked in yellow in the paper.

1) Introduction is too long and needs to be summarized.

Answer: Thank you for your careful review. We have focused our content, and tightened our language in the *introduction* section. More details have been modified in the *introduction* section of the revised manuscript.

2) In page 5: authors said “Individuals with negative colonoscopy results were selected” selected for what?

Answer: We are deeply sorry for the ambiguous statement. Individuals with negative colonoscopy results were selected as healthy control group. We have replaced the statement “Individuals with negative colonoscopy results were selected” with “Individuals with negative colonoscopy results were selected as healthy control group”. Corresponding statements have been modified in the *methods* section of the revised, highlighted in yellow.

3) Section materials and methods needs more structuring, it should be subtitled. Authors need to give detailed information about the grouping, culturing, detailed information about transfection, western blot analysis, and quantitative real-time polymerase chain reaction (qRT-PCR).

Answer: Thank you very much for your kind comments and valuable suggestions. According to your suggestion, we described the methods used in the study in as much detail as we can and readjusted the structure of this section. Please see the detail in the revised manuscript.

4) Why authors combined a normal colonic mucosal epithelial cell line (FHC), human colon carcinoma cell lines (HCT116, RKO, and SW480), and a human embryonic kidney cell line (HEK-293T) during culturing?

Answer: We are sorry for our unclear statements. Dual luciferase reporter

assays were performed using a human embryonic kidney cell line (HEK-293T) because of its high transfection efficiency^[1]. A normal colonic mucosal epithelial cell line (FHC) and human colon carcinoma cell lines (HCT116, RKO, and SW480) were used to detect the mRNA expressions of Wnt2. Besides, two human colon carcinoma cell lines (HCT116 and SW480) were used for gain of function experiments. More details were added in the *methods* section of the revised manuscript, highlighted in yellow.

Answer reviewer 03767650:

Thank you for giving us the precious opportunity for a revision of our manuscript. We appreciate very much the comments and suggestions from you. Those comments are all valuable and very helpful for revising and improving our paper. We have studied the comments very carefully and tried our best to improve the manuscript. The followings are our point-by-point responses to the remarks underneath each comment.

1. Since the subtitles of the Results are in sentence form, they should be changed to noun form. For example, miR-627 was highly decreased in CRC tissues To miR-627 in CRC tissues

Answer: Thank you very much for your kind comments. We have changed the subtitles of the results to noun form. More details were modified in the *results* section of the revised manuscript, highlighted in yellow.

2. The Discussion is redundant. I think the first paragraph of the Discussion is unnecessary.

Answer: Thank you for your careful review. We have deleted the first paragraph according to your suggestion.

3. The Discussion is redundant. Despite many explanations of the previous reports, there are few interpretations of the results of this study in the Discussion. Please improve this point throughout the Discussion.

Answer: We appreciate your valuable comments. We totally agree with you

that there are few interpretations of the results of this study in the *discussion* section. We have focused our content, tightened our language, and clarified our opinion through the *discussion* section. More details have been modified in the revised manuscript, highlighted in yellow.

4. Figure legends 2, 3, and 4 contain explanations of the results. Please only mention these in the main text.

Answer: Thank you for your careful review. We have modified the explanations of results in the *figure legends* section.

5. The following sentences in the Results should be included in the Discussion. Our previous study investigated the expression of miR-627-5p in the same clinical tissues and showed significantly decreased expression in CRC and AA tissues compared to HC tissues[19]. Besides, miR-627-5p was found to be decreased in CRC cell lines compared with those in FHC cells[19].

Answer: Thank you for your kind suggestion. In the present study, we analyzed the correlation between the expressions of Wnt2 and miR-627-5p in CRC and AA tissues. However, the expression of miR-627-5p in the same clinical tissues were published in our previous study^[2]. Therefore, we added this description in the *results* section to avoid misunderstanding. According to your suggestion, we have modified the description in the *results* section and added it in the *discussion* section. Thank your again for your valuable suggestion.

References

- 1 Backliwal G, Hildinger M, Hasija V, Wurm FM. High-density transfection with HEK-293 cells allows doubling of transient titers and removes need for a priori DNA complex formation with PEI. *Biotechnol Bioeng* 2008; 99(3): 721-727 [PMID: 17680657 DOI: 10.1002/bit.21596]
- 2 Zhao DY, Zhou L, Yin TF, Zhou YC, Zhou GY, Wang QQ, Yao SK. Circulating miR-627-5p and miR-199a-5p are promising diagnostic biomarkers of colorectal neoplasia. *World J Clin Cases* 2022; 10(16): 5165-5184

Round 2:

We are very pleased to receive your revised manuscript (No.80219). However, there are some questions that need to be addressed. -----1. Specific Comments To Authors: The authors have improved the paper. But, there are some issues to be resolved. Please change the subtitles of the Results as the following: From The inverse relationship between miR-627-5p and Wnt2 expression in colorectal neoplasm tissues To The relationship between miR-627-5p and Wnt2 expression in colorectal neoplasm tissues From The tumor suppressive role of miR-627-5p in CRC cells To The role of miR-627-5p in CRC cells From The regulatory role of miR-627-5p in the Wnt/ β -catenin signalling pathway To The role of miR-627-5p in the Wnt/ β -catenin signalling pathway. -----2. Please provide the decomposable figure of figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as "Manuscript No. -Figures.ppt" on the system, we need to edit the words in the figures. All submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes. -----3. Your manuscript has been checked by CrossCheck. Please read the attached CrossCheck report for details. Similar sentences with other articles (highlighted in the CrossCheck Report), please rephrase these sentences. Our editorial policy states the overall similarity should be less than 30%, the overlapped section should be less than 5% in single papers, including author's own work. Please modify on the basis of the attached manuscript.-----Please revise in the attached file "80219 Auto_edited" and reply within seven days, thank you! Only one file is available in F6publishing system, please upload all files with zip format, or send to me by email (y.l.chen@wjgnet.com).

Answer:

Dear Professor Chen: Thank you for your letter and the reviewer's comments concerning our manuscript. These comments are all valuable and very helpful for revising and improving our paper. According to the suggestions of the science editor and reviewers, we have changed the subtitles of the Results, rephrased the overlapped sections and modified the manuscript. Revised portions are marked in red in the revised version of the manuscript. The decomposable figure of figures were attached. I hope that the adjustments made to the manuscript are satisfactory, and I am looking

forward to your correspondence! If you have any questions, please do not hesitate to contact us.

All the best. Yours Sincerely, Shu-Kun Yao