

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5102-review.doc).

Title: Anti-microRNA-221 sensitizes human colorectal carcinoma cells to radiation by up-regulating PTEN

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 5102

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer 1

- (1) The weakness of this study is that it has been conducted in this single cell line only. It remains to be shown whether regulation of radiosensitivity by miR-221 and PTEN applies for other CRC cell lines too and whether it might apply in an in vivo situation. This limitation might be mentioned in the Discussion.

In this study, we explore the expression values of miR-221 in HT-29, Lovo, SW-480 and Caco2 cells firstly. The Caco2 cell line was chosen for both pre-miR-221 and anti-miR-221 transfection in the subsequent experiment because of its intermediate miR-221 expression level among the four tested cell lines. It is not evident to transfect pre-miR-221 into cell line which shows highly primary miR-221 expression pattern. On the other hand, it is also not suitable to transfect anti-miR-221 into cell line which shows lowly primary miR-221 expression pattern. Thus, we think it would be better to choose Caco2 as target cell line. According to the reviewers advice, we mentioned this limitation in the Discussion section.

- (2) Method. Regarding real-time PCR for micro RNAs and transcripts, the authors referred to a previous publication by them, ref#10. As this article is in Chinese, it is not suitable as a reference, as it can't be read/checked by the whole community. The authors must describe the technique again within this manuscript in the respective paragraph.

As a matter of fact, the reference 10 (Sun K, Zeng JJ, Wang W, Wu CT, Lei ST, Li GX. MicroRNA-221 inhibits CDKN1C/p57 expression in human colorectal carcinoma. *Acta Pharmacol Sin* 2011; 32: 375-384), which was reported by us, was written and published in totally English. Additionally, the published journal, *Acta Pharmacol Sin*, is a SCI journal (IF 2.354). Thus, we think it is suitable to refer our own original article as a reference to describe the real-time PCR method.

- (3) Title. Phrasing of the title could be improved to my view. The term 'targeting' might be misleading in the context. Please try to substitute by 'up-regulating' or 'boosting' or something in that sense.

According to the reviewers advice, we have modified the present title and please review again.

- (4) Citations in Introduction. (At least) refs #1 and #2 might mention the statements given in the sentences they refer to but they do not document them. They are secondary sources in this

context. The authors have to cite the primary sources!

According to the reviewers advice, we have changed some references and please review again.

- (5) Language. Language is above-average, no doubt. The manuscript would benefit from a language editing though.

According to the reviewers advice, we have sent our manuscript to professional English language editing company in this revision and achieve the language certificate. Please review again.

Reviewer 2

- (1) Language. The manuscript would benefit from a language editing though.

According to the reviewers advice, we have sent our manuscript to professional English language editing company in this revision and achieve the language certificate. Please review again.

- (2) Figure 4. "Anti-miR-221" ---- "As-miR-221", etc. Carefully check the similar mistake.

According to the reviewers advice, we have modified these errors in the manuscript and please review again.

- (3) What is the basis of radiation dose selection? What is the choice on the basis of the measurement point after radiation exposure? References are given and experimental data.

As a matter of fact, we just refer the original article of Zhang C (reference #14 Zhang C, Kang C, Wang P, Cao Y, Lv Z, Yu S, Wang G, Zhang A, Jia Z, Han L, Yang C, Ishiyama H, Teh BS, Xu B, Pu P. MicroRNA-221 and -222 regulate radiation sensitivity by targeting the PTEN pathway. *Int J Radiat Oncol Biol Phys* 2011; 80: 240-248 [PMID: 21481725 DOI: 10.1016/j.ijrobp.2010.12.049]) as the experimental design. We have strengthened this reference in the Methods section.

Reviewer 3

- (1) Please provide the densitometry data for all of the western blots.

According to the reviewers advice, we have provide the densitometry data for some of the western blots. Please review again.

- (2) In figure 5, the authors presented only the representative graphical data. Please provide the statistical data from multiple repeats.

According to the reviewers advice, we have provide the statistical data of the FCM. Please review again.

- (3) About figure 6D, the authors described that "When pre-miR-221 was transfected into Caco2 cells previously treated with anti-PTEN-siRNA, we observed that anti-PTEN-siRNA and miR-221 seemed to co-operate to enhance the survival rate (Figure 6D)". However, figure 6D does not seem to be cooperative when anti-PTEN siRNA and pre-miR-221 was combined. Provide the statistical analysis for this result.

As a matter of fact, in this study, we just described the trend that anti-PTEN-siRNA and pre-miR-221 seemed to co-operate to enhance the survival rate. However, this effect cannot reach the statistical significance compared with other groups.

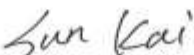
- (4) The authors also described as "These results indicated that the inhibitory effect of anti-miR-221 on CRC cell survival following irradiation was largely, but not completely, mediated by PTEN, suggesting that anti-miR-221 could also activate some PTEN-independent signaling pathway to repress CRC cell growth in addition to the up-regulation of PTEN." In this sentence, "largely" may not be appropriate since the effect looks like just partially.

According to the reviewers advice, we have corrected this description and please review again.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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