**Author’s response to reviews**

**Title:** miR-302b inhibits tumorigenesis by targeting EphA2 via Wnt/β-catenin/EMT signaling cascade in gastric cancer

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**Author’s response to reviews:**

Dear editors,

Thank you for your encouraging and thorough review of our submitted manuscript, “miR-302b inhibits tumorigenesis by targeting EphA2 via Wnt/β-catenin/EMT signaling cascade in gastric cancer”.
cancer". I feel that the reviewers offered helpful and productive suggestions, and I appreciate the reviewer comments. All authors have read and approved the revised manuscript.

Below, I have restated each esteemed reviewer’s comments and have included my responses: Please see the attached revised manuscript and the replies for reviewer comments. We believe the revised manuscript has benefitted from the peer review process and we look forward to hearing from you regarding the status of our submission.

Kwang-Huei Lin (Reviewer 1): The authors aimed to study the function of miR-302b in GC cells. They show that miR-302b is a suppressor of GC cell growth and metastasis both in vitro and in vivo, to inhibit its downstream pathways by directly targeting EphA2.

1. The authors mentioned that "the role of miR-302b in GC has not been fully documented, we thus chose miR-302b for further investigation". However, there have at least 4 articles (PMID: 28743112; 27465546; 25904219; 23508453) focused on the study of miR-302b in gastric cancer.

Reply: Thank you for all of your advices. Because there are just only 4 papers focused on the study of miR-302b in gastric cancer, and the role of miR-302b in GC has not been fully investigated. And except for the 3 articles that had been included in our article, we also included Guo et al (PMID 28743112) as our references (Discussion section, line 19, page 14).

2. Overexpression of EphA2 in gastric cancer has been reported. Also, Huang et al (the same group of the current study) reported that EphA2 promotes epithelial-mesenchymal transition through the Wnt/β-catenin pathway in gastric cancer cells. Based on the above two points, the current study is short of novelty.

Reply: Based on our previous study, we proceed our research for what kinds of micRNAs and how to modulate EphA2 in gastric cancer.

3. The authors reported that miR-302b is a suppressor of GC cell growth and metastasis. Its function is mediated by directly targeting EphA2. Since the target genes of miR-302b are several, can authors design experiments to validate the EphA2 but not others is the most critical gene to exert its inhibitory effects.
Reply: We are sorry for our experiments not involved in denying others target by miR-302b. We will design experiments about this in our further research.

4. Khodayari et al (PMID: 27725905) reported that Ephrin-A1 treatment induced miR-302b expression. I wonder whether EphA2 can induce miR-302b expression in GC also? If yes, the authors may need to consider the reciprocal effects of two genes? Whether Ephrin-A1 is also the target of miR-302b?

Reply: Ephrin-A1/EphA2 bidirectional mechanism is involved in tumor metastasis. EphA2 activation and Ephrin-A1 inactivation promote metastasis of cancers including gastric cancer. In our experiments, we didn’t check express level of miR-302 induced by EphA2.

5. The study is based on the EphA2 overexpression and miR-302b down-expression in GC. However, the authors should provide the data to validate the same results in the clinical specimens.

Reply: In our further study, we will check the EphA2 overexpression and miR-302b down-expression in the clinical specimens of GC including the OS data. So this study we didn’t show these results.

Aditi Chatterjee (Reviewer 2): This manuscript by Jin Huang et. al. titled 'miR-302b inhibits tumorigenesis by targeting EphA2 via Wnt/β-catenin/EMT signaling cascade in gastric cancer' studies the role of miR-302b and its molecular target EphA2 in the tumorigenesis and metastatic capability of gastric cancer.

Major

1. In Figure 1 (B, C, D and E), the western blot bands of GAPDH used to depict loading control by the author appears unclear and burnt out due to prolonged exposure on the autoradiograph. This could result in an imprecise interpretation of the expression of EphA2 in these experiments by the reader, despite the authors' claims. It is recommended that the author repeats the western blotting experiment for the aforementioned sets and presents clear and distinguishable GAPDH bands.
Reply: Thank you for all of your advices. We have exchanged the WB of GAPDH in Figure 1(B,C,D,E) by reproducing the experiments.

2. In Figure 4 A, the images of the cells (all sets of AGS and SGC7901) appear to have been captured at a high confluency, making it difficult for the reader to interpret the authors’ claims of cellular morphology mentioned in 'Results' section (Page 13, lines 15-17). Hence the author is recommended to re-capture the images of the cells (treated with miR-NC, miR-302b, si-NC and si-EphA2 conditions) at a lower confluency.

Reply: We have exchanged the Figure 4A by reproducing the experiments.

3. Figure 5 is of poor quality and an inadequate portrayal of the work carried out in this manuscript. The author is suggested to add more details regarding the pathway focused in this work, highlighting targeted molecules, or the image can be removed altogether.

Reply: We also exchanged the Figure 5 by repainting the mechanism diagram.

Minor

1. If the protocol for experiments such as cell cycle analysis and scratch wound healing assay have been adopted from a previously published literature, the author is suggested to reference them in the 'Methodology' section.

Reply: Thank you for all of your advices. We added reference for these two experiments for in the methods section (methods section, line 42 and 54, page 7).

2. In the 'Results' section (Page 10, line 18-20), the author has mentioned how 'only miR-302b led to the decreased EphA2 expression'. However, in the western blot and qPCR images (Figure 1 B and 1 C) it is obvious that miR-143 also confers similar effects on EphA2 expression as miR-302. Hence the author is suggested to re-write the aforementioned sentence including the fact about miR-143. Additionally, the author may also include the reasons, if any, for not choosing miR-143 as a candidate for further analysis along with miR-302b.

Reply: Thank you for your suggestion, we’ve rephrased that sentence (Results section, Page 10, line 18-20). At first, we checked the miR-302 and miR-143 at the same time. But we found that the results of miR-302 are very solid while those of miR-143 are very volatile with the research proceeding. We choose the miR-302b as our study object.
3. In results section (Page 10, line 48), the notation of miR-302b has been mentioned incorrectly. Correction suggested.

Reply: We have corrected.

4. In the 'Discussion' section (Page 15, line 41-47), the sentence 'Translocation of ... epithelial differentiation' needs to be referenced.

Reply: We have profoundly referenced it.