

October 22, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 13730-review.doc).

Title: MiR-19a promotes epithelial-mesenchymal transition through PI3K/AKT pathway in gastric cancer

Author: Wei-Dong Lu, Zhen Xu, Min Zhang, Yun Zuo

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 13730

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

Reviewer #1:

1. The paper should be further edited and all the abbreviation (such as AKT, Akt), upper and lower case as well as the citations should be in uniform.

Answer: Revision has been made according to the suggestions of the reviewer

2. In the section of Introduction, the authors mentioned: "Recent reports have highlighted the importance of microRNA as a powerful regulator of EMT in cancer cells", these references are very important to cite, the authors should list these studies in the end of the paper.

Answer: Thank you for your suggestion. Two references (Braun J, Hoang-Vu C, Dralle H, Hüttelmaier S. Downregulation of microRNAs directs the EMT and invasive potential of anaplastic thyroid carcinomas. *Oncogene*, 2010; 29: 4237-4244. Paterson EL, Kazenwadel J, Bert AG, Khew-Goodall Y, Ruzskiewicz A, Goodall GJ. Down-regulation of the miRNA-200 family at the invasive front of colorectal cancers with degraded basement membrane indicates EMT is involved in cancer progression. *Neoplasia*, 2013; 15: 180-191.) have been added in the end of the paper.

3. In section 2.1, the authors mentioned: "Non-tumor samples from the macroscopic tumor margin were isolated at the same time...". I wonder how the authors define these "Non-tumor samples". I recommend the following reference: Liu et al. Decreased expression of IGFBP7 was a poor prognosis predictor for gastric cancer patients. *Tumor Biol*. 2014 Jun 4. [Epub ahead of print].

Answer: Thank you for your suggestion. The following reference: Liu et al. Decreased expression of

IGFBP7 was a poor prognosis predictor for gastric cancer patients. Tumor Biol. 2014 Jun 4. was added into our manuscript to define these “Non-tumor samples”

3.The authors described that they used the cell lines SGC-7901 and NUGC-3, however, in section 2.4, MKN28 was used.

Answer: Revision has been made according to the suggestions of the reviewer.

4.In section 2.5, again, the authors said: “Migration and invasion assays were performed as described previous”, but no references were cited.

Answer: One reference (Cao W, Yang W, Fan R, Li H, Jiang J, Geng M, Jin Y and Wu Y. miR-34a regulates cisplatin-induce gastric cancer cell death by modulating PI3K/AKT/survivin pathway. Tumor Biol 2014; 35: 1287-1295.) was added into the manuscript.

5.As one of the important results addressed in the Abstract, miR-19a could promote cell proliferation; the authors should display the data of MTT assay.

Answer: Thank you for your suggestion. The results of MTT assay was performed in **figure 2C**

6.Figure 2, the figure legends was not corresponding to the figure.

Answer: Revision has been made according to the suggestions of the reviewer.

7.In the paper, the cell line NUGC-3 often miswritten into SUGC-3.

Answer: Revision has been made according to the suggestions of the reviewer.

8.In the section of Discussion, the authors should highlight the main results in the first paragraph.

Answer: Thank you for your suggestion. We have optimize the Discussion and highlight the main results in the first paragraph.

9.The quality of the figures was extremely poor, which makes it very hard to understand the results.

In section 3.2, the hint of the figure is wrong.

Answer: We have promoted the ppi and quality of our picture which may meet the standard of this journal. Further more, the hint of the figure in section 3.2 was corrected accordingly.

10. For figure 4, the authors should mark the concentration of the Ly294002, not only state -, +.

Answer: Thank you for your suggestion. Revision has been made according to the suggestions of the reviewer.

The marker for EMT is very important in this paper, although the authors performed the western blotting to check these markers, but more directly results should come from the IHC study, I recommend the authors display some representative figures for EMT.

Answer: Thank you for your suggestion. I agree that IHC study may be more convincing for our study,

but we didn't perform it instead of the evaluation of the morphological changes.

Reviewer #2:

1. The first paragraph of the Discussion can be moved to the Introduction with some modifications.

Answer: Thank you for your suggestion. We have optimize the Introduction and Discussion.

2. Page 9, section 3.2: Because the cell lines already express excessive miR-19a, I think the knockdown, rather than the over expression is more meaningful. Thus, it would be better to emphasize the knockdown.

Answer: Thank you for your suggestion. Revision has been made according to the suggestions of the reviewer.

3. Fig 3a: Morphological changes regarding EMT by miR-19a over expression should be shown (general, immunostaining etc.) - this is important.

Answer: Thank you for your suggestion, we have added morphological changes of SGC7901 cells in the paper.

4. Similarly, Fig 4c needs a photo to show morphological changes.

Answer: Thank you for your suggestion, we agreed that a photo of morphological changes of Fig 4c may be more better. Here we offered a figure which is clear to clarify the trend.

The authors investigate the expression level of miR-19a in gastric cancer and the function of miR-19a on gastric cancer progression. The study is well designed and there some minor issues need to be addressed.

Reviewer #3:

1. The authors missed many references and should examine the article carefully.

Answer: We have added the missed references and examined the article carefully according to the suggestions of the reviewer.

2. There are missing words or grammar mistakes need to be corrected.

Answer: Revision has been made according to the suggestions of the reviewer.

3. In part 2.4, the authors claimed that they used MTT assay to measure cell proliferation, however the kit provided in the article (cell counting kit-8) does not contain MTT at all.

Answer: Revision has been made according to the suggestions of the reviewer.

4. Please clarify how they define "normal" tissue. The normal tissue used to compare miR-19a level should be normal gastric mucosa, not other layers of stomach.

Answer: The following reference: Liu L, Yang Z , Zhang W, Yan B, Gu Q, Jiao J, Yue X. Decreased expression of IGFBP7 was a poor prognosis predictor for gastric cancer patients. Tumor Biol 2014; 35:

8875-8881. was added into our manuscript to define these “Non-tumor samples”

5. Please explain why they used lentiviral infection for cell proliferation and transient transfection for other experiments.

Answer: All cell proliferation and other experiments were performed by lentiviral infection.

6. The picture quality is very poor. The authors also need to provide pictures for EMT phenotype.

Answer: Thank you for your suggestion. We have promoted the ppi and quality of our picture which may meet the standard of this journal. Furthermore, we have added morphological changes of SGC7901 cells in the paper.

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

Sincerely yours,



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