Author's response to reviews

Title: MiR-199a-5p is up-regulated in gastric cancer and functions as an oncogene by targeting klotho

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Author's response to reviews:

Dear editor,

Thank you very much for forwarding the referee's reports on our manuscript, and we also appreciate your advice and encouragement. Following your suggestions, we have revised our manuscript according to the referee's comments point-by-point.

Reviewer: An-Mei Deng

Question 1: Are the methods appropriate and well described?

Partially. In statistical analysis section, the authors stated that “miR-199a-5p level obtained by In Situ Hybridization and klotho expression level obtained by Immunohistochemical Staining (categorical data) were described by their frequency and analyzed by Chi-square test or Fisher’s exact test. Spearman’s rank correlation coefficient was used to assess the relationship between miR-199a-5p and klotho expression levels”. The statement is generally correct, however, TMN stage and differentiation in Table 1 and 2 are graded data, Mann-Whitney U test should be employed instead of Chi-square test or Fisher’s exact test (The Journal of pathology, 2013, 231(3): 335-341). In table 3, I am rather confused how the investigators calculate the coefficient of miR-199-5p and klotho expression levels? Based on Table 3, it is clearly that either miR-199-5p or klotho expression level is a binary, rather than a continuous or graded variable. Under that condition, it seems Chi-square test is appropriate. Please explain.

Answer: As TMN stage and differentiation in Table 1 and 2 are graded data, we used nonparametric test (Kruskal-Wallihis H test) to detect the relationship between clinicopathological features and expression levels of miR-199a-5p or klotho. The results were added in the tables colored in red. In table 3, we
detected correlation between miR-199-5p and klotho expression levels using Spearman correlation analysis, which was a more conservative statistical analysis.

Question 2: Are the data sound?
Yes. The investigators explored the effect of miR-199a-5p on the migration activity and invasion ability of gastric cell line by a gain and loss of function strategy. The transfection efficiency should be examined first.
Answer: The data of transfection efficiency was added and described in result. Please see Figure 6.

Question 3: Are the discussion and conclusions well balanced and adequately supported by the data?
Yes. The last sentence of discussion section should be revised. The major clinical implication of present study findings is that miR-199-5p is a potential therapy target, rather than discovering a diagnostic and prognostic biomarker, for gastric cancer.
Answer: We have revised the last sentence of discussion section.

Question 4: Do the title and abstract accurately convey what has been found?
The title should be revised. The authors found that miR-199-5p could affect the migration activity and invasion ability of gastric cell line, and it also target klotho, however, it remains unknown whether klotho can affect the migration activity and invasion ability of gastric cell line, although previous study has partially support their hypothesis. Therefore, they should not concluded that miR-199a-5p functions as an oncogene by targeting klotho. “Up-regulated miR-199a-5p in gastric cancer functions as an oncogene and targets klotho” may be an appropriate title for their work.
In fact, the work would be better if the investigators explored the effects of klotho on the migration activity and invasion ability of gastric cell lines.
Answer: Thank you for your advice. We have changed the title to what you recommend, and we will do the work of exploring the effects of klotho on the migration activity and invasion ability of gastric cell lines in the future.

Question 5: Is the writing acceptable?
Partially. Some sentences were started with Arabic numbers and should be revised.
Answer: We have revised the Arabic numbers in the manuscript.
MiR-199a was an oncogene in gastric cancer, which was reported by Zhang et al (Zhang et al, Nucleic Acids Res, 2012). The aim of our study was just to verify its role in gastric cancer.

Question 2: In order to identify klotho as a target of miR-199a-5p, it is insufficient only using luciferase reporter assay. It is essential to determine the expression levels of klotho at both the mRNA and protein after the transfection of gastric cancer cells with miR-199a-5p inhibitor or mimic.

Answer: We had added the expression levels of klotho at both the mRNA and protein after the transfection of gastric cancer cells with miR-199a-5p inhibitor or mimic in Figure 6.

Question 3: #Materials and methods, miR-199a-5p transfection, ........ MKN-28 and MKN-45, which had high expression level of miR-199a-5p; .......... AGS and BGC-823, which had low expression level of miR-199a-5p. #Results, MiR-199a-5p is up-regulated in gastric cancer tissues and cell lines , ....As shown in Figure 1B, gastric cancer cell lines expressed higher level of miR-199a-5p than GES-1. Which one is right?

Answer: In Figure 1B, GES-1 is a gastric normal epithelial mucosa cell line. Compared with GES-1, gastric cancer cell lines (AGS, BGC-823, MKN-28, MKN-45, SGC-7901, and HGC-27) expressed higher level of miR-199a-5p. Among the gastric cancer cells, MKN-28 and MKN-45 have a relatively high expression of miR-199a-5p and AGS, BGC-823 have a relatively low expression of miR-199a-5p. In “#Materials and methods, miR-199a-5p transfection” and “#Results, MiR-199a-5p is up-regulated in gastric cancer tissues and cell lines”, we have changed the description of it.

Question 4: The gastric cancer cell line transfected with miR-199a-5p inhibitor or mimic should not be the different cells.

Answer: As described above, due to the relatively high expression of miR-199a-5p in MKN-28 and MKN-45, we select those two cells to transfect the miR-199a-5p inhibitor to study the function of corresponding influence of miR-199a-5p. In the same way, due to the relatively low expression of miR-199a-5p in AGS and BGC-823, we up-regulate the expression level of miR-199a-5p by transfecting with miR-199a-5p mimic. Compared with the corresponding negative control group, the migration and invasion ability change should be able to reflect the role of miR-199a-5p in migration and invasion of gastric cancer cell.

Thank you very much for your attention and consideration.

Sincerely yours,

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