

Jan 20, 2014

Dear Editor,

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript, we appreciate your positive and constructive comments and suggestions for our manuscript. Also, we thank very much to the editor to efficiently process our manuscript.

We have carefully studied the comments of reviewers and tried our best to make some revisions which marked in red pen in the paper. Please find enclosed the edited manuscript in Word format (file name: 7465-revised manuscript doc). Furthermore, the manuscript have been edit by the professional English language editing company (Jing-Yun Ma Editorial Office).

Title: Expression of circulating microRNA-218(miR-218) was reduced in gastric cancer and correlated with tumor invasion and prognosis

Author: Shi-Yong Xin, Xiao-Shan Feng, Li-Qing Zhou, Jun-Jun Sun, Xiao-Le Gao, Guo-liang Yao

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO:7465

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

Reviewer1:

This paper is very interesting, with a novel approach to the gastric cancer molecular biology. The assay method is of good quality. The number of patients and controls is adequate. However there is some items to improve.

1. there should be more detailed characteristics of GC patients : N status, resections vs no resection, radicality of surgery, TNM stage.

Reply: We have added the detailed characteristics of GC patients according to the comments of reviewer.(Table2)

2. N status and operability are somewhat commented in discussion, however there is nothing about it in previous sections

Reply: We have revised our manuscript according to the comments of reviewer. In our study, we mainly studied the miR-218 expression in serum of GC patients and its relation with the GC clinico-pathological characteristics and prognosis. We could find that expression of miR-218 in serum was always low in N-metastasis patients compare to the N-non metastasis patients. miR-218 might be considered as a risk factor for GC progression. Therefore, the N status and operability are somewhat commented in discussion.

3.The method of staging is commented only in a legend to the table, not in the main text.

Reply: We have revised our manuscript according to the comments of reviewer.

4.The Cox model in table 3 contains limited and selected set of variables (no TNM stage, no radicality, no T stage) and therefore has limited power. Analysis is rather multivariate than multivariable.

Reply: We have added the variables in table 3 according to the comments of reviewer.

Reviewer 2: no comments.

Reviewer3:

In this study, the authors reported the down-regulation of circulating microRNA-218 (miR-128) in the sera of gastric cancer patients and its correlation with clinical features. Although their findings are interesting, I have several comments for further consideration.

1. In Figure 1, the case number should be included for each comparison.

Reply: We have revised our manuscript according to the comments of reviewer.(legend for the Figure1)

2. The standard for the low, middle, and high expression of miR-128 should be described

in the Methods.

Reply: We have revised our manuscript according to the comments of reviewer.(the last three lines in the section of RNA isolation and qRT-PCR).

3. Due to the limited case number, the conclusion of this study should be validated by further investigation. Especially, in Table 2, only small case number was compared to draw the conclusion.

Reply: As reviewer said, the conclusion of this study are based on a limited number of cases and clinicopathologic parameters and require confirmation from the analysis of larger cohorts and further investigation in the future.

4. The English should be edited by a native speaker to correct numerous spelling and grammar errors.

Reply: The manuscript have been edited by a native speaker according to the comments of reviewer.

Reviewer4:

This is a carefully done study and the findings are of considerable interest. This is the first report about the expression of miR-218 in serum of GC patients, which may make sense for the diagnosis, treatment and prognosis of gastric cancer. For better understanding of the readers, a few minor revisions listed below may be useful to your study.

1. Fig.1 It would be better if the gap between columns be narrowed, and the title of Y axis should be changed to "miR-218 relative expression".

Reply: We have revised the figure according to the comments of reviewer.

2. Table1 "low miR-218 expression($T/N < 1.2$); middle expression($T/N = 1.2-10$) ; highexpression($T/N > 10$)". Could you please give us a definition of "T/N"? Also the significance of P value should be elaborated.

Reply: We have revised our manuscript according to the comments of reviewer.(the last

three lines in the section of RNA isolation and qRT-PCR), and the significance of P value have been elaborated.

3. Para.1, page 6: "The expression level of miR-218 correlated with 3 year survival and stage of the patients." A survival curve of the patients in your studies is needed here to support your point of view.

Reply: We have added the Kaplan-Meier graph as Figure2.

4. The sentences is hard to understanding: "it miR-218 and miR-34a were considered to function as tumor suppressor miRs" (Line 14-15, page 7)

Reply: We are so sorry to make a clerical error in the sentence that reviewer mentioned and we have revised the sentences: " the miR-218 and miR-34a were considered to function as carcinogenic miRs."

5. Para. 3, page 8: "From the viewpoint of clinic, low expression of miR-218 might be considered as a risk factor of tumor progression, and thereby a strict systemic therapeutic strategy after operation such as immunotherapy, angiogenesis inhibitor drugs, chemotherapy, and radiotherapy with a regular investigation might improve prognosis." There is a good paper entitled "Self-assembled supramolecular nano vesicles for safe and highly efficient gene delivery to solid tumors" published in "International journal of nanomedicine", in which a simply tailored non-cytotoxic nano vesicle was fabricated and significant potential for use in practical gene therapy was showed. Please cite this paper and evaluate the prospect of gene-therapy using the similar nano-vicles and the microRNAs including miR-218.

Reply: We have cited the paper entitled "Self-assembled supramolecular nano vesicles for safe and highly efficient gene delivery to solid tumors" in our manuscript and evaluated the prospect of gene-therapy using the similar nano-vicles and the microRNAs including miR-218 in discussion (line 9-13 pagraph 3 page 10) .

6. It is strongly recommended that a professional reviewer major in Statistics should be

invited to review this study.

Reply: We have invited one professional reviewer major in Statistics to review the study according to the comments of reviewer.

Reviewer5:

Xin et al. evaluated the miR-218 in serum of gastric cancer patients and its correlation with tumor invasion and prognosis. Reduced expression of miR-218 correlated to cancer, histology, metastasis, stage, and prognosis. The results are positive, however, the manuscript was not check-up for mistakes.

1, I cannot find the Kaplan-Meier graph.

Reply: We have added the Kaplan-Meier graph as Figure2.

2, Results section Line 3: Table 1 is results of miR-218 expression, not “demographic and clinicopathologic variable for the cohort”.

Reply: We are so sorry for our clerical error. Results section Line 3: Table 1 would be Table 2.

3,miR-218 expression and GC features section, Line 2: Table 1 would be Table 2.

Reply: We have revised the manuscript according to the comments of reviewer.

4,miR-218 expression and GC features section, Line 7: This way of writing usually means positive relation of expression and tumorigenesis and tumor progression. Write clarifying the negative correlation.

Reply: We have revised the manuscript according to the comments of reviewer.

5, Discussion section, Line 7: Again, miR-218 associated with anti-carcinogenesis and cancer anti-development. In total, write the manuscript more carefully, and choose the words to avoid confusing. Carcinogenesis and cancer progression also be thought separately.

Reply: We have revised the manuscript according to the comments of reviewer.

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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