ANSWERING REVIEWERS

April 7, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 8758-review.doc).

Title: MicroRNA-218 is upregulated in gastric cancer patients after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, and increases chemosensitivity to cisplatin.

Author: Xiang-liang zhang, hui-juan shi, ji-ping wang, hong-sheng tang, yin-bing wu, zhi-yuan fang, shu-zhong cui.

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO:8758

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 reviewers

Reviewer 1: This manuscript describes the identification of microRNA-218 (miR-218) as a tumor suppressor for gastric cancer. miRNA microarray and q-RT-PCR studies of 5 gastric cancer patients showed the upregulation of miR-218 after CRS+HIPEC. Overexpression of miR-218 in gastric cancer cell line SGC7901 suppressed its growth rate, increased the chemosensitivity to cisplatin, and impaired the tumor growth in a xenograft mice model. The results are convincing. The manuscript is worthy of consideration for publication. However, several issues need to be addressed before publication.

Major issues:

1. Fig.2: a correlation between miRNA microarray and q-RT-PCR data for the four micro-RNAs is needed to support the conclusion that the RT-PCR results were consistent with the microarray data, given that miR-96 data was not provided in Table 2.

Answer: Thank you for your careful review, its my carelessness to miss miR-96 data in Table 2. Actually, we have the relative data and we have put the data in table 2 already.

2. Fig.3: the authors need provide the data regarding to the expression levels of miR-218 in the parental SGC7901 and the SGC7901/miR218 stable cell line.

Answer: Thank you for your constructive suggestion, we have add the expression levels of miR-218 in the parental SGC7901 and the SGC7901/miR218 stable cell line in Fig 3c.

3. Fig.4: it seems that the authors use mTOR transition transfection to reveal the synergistic effect of mTOR and miR-218. mTOR is mammalian target of rapamycin. The description in results section is confusing and need to be rewritten for clarity.

Answer: Thank you for your careful review, we have rewritten the results section on page 9.

4. Page 11 Discussion: the authors discuss their bioinformatics results, but neither reference was cited nor data was shown.

Answer: Thank you for your good suggestion, we have add reference[35-36] to this part on page 11.

5. Table 2: is there any miRNAs down-regulated significantly?

Answer: Though there has some miRNAs were down-regulated by CRS+HIPEC significantly, this is not the emphasis in this manuscript, so, we has not mention it.

Minor issues:

1. Title: the title is confusing. please revise to “MicroRNA-218 is upregulated in gastric cancer patients after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, and increases chemosensitivity to cisplatin”

Answer: Thank you very much, according to your suggestion, we have revised the title into "MicroRNA-218 is upregulated in gastric cancer patients after cytoreductive surgery and hyperthermic intraperitoneal chemotherapy, and increases chemosensitivity to cisplatin"

2. Introduction: details about the HIPEC regimen should be provided.

Answer: The details about the HIPEC regimen has been provided on page 3 of the manuscript.

3. Accuracy is essential to the validity of all scientific papers. a. Introduction: the authors state that "…the finding that 50% of miRNA-encoding genes are located in cancer-associated genomic regions or fragile sites" (Page 5; also in Page 11). Is this applicable to all tumors? Please restrict the statement to the specific cancers reported in these references. b. Page 9: the authors state that "miR-218 is commonly downregulated in gastric cancers…". Please cite references for this.

Answer: Thank you very much, according to your suggestion, we restrict the statement to the specific cancers reported in these references on page 4. This sentence on page 11 was omitted according to the overall style of the discussion part. We have cited the reference 31 after the sentence" miR-218 is commonly downregulated in gastric cancers"

4. Replace all DDP with cisplatin. Too many abbreviations reduced the clarity of the manuscript.

Answer: We have replaced all DDP with cisplatin according to the opinion of reviewer.

5. Grammar errors: the authors need pay special attention to eliminate these errors and to improve clarity of the paper. e.g., Line 9 page

Answer: We have tried our best to correct the grammar errors and misspellings with the help of a native speaker. If there still have any errors, please do not hesitate to contact me via e-mail at zhxl7229@163.com.

6: "…research is needed to the contribution…" should be "…research is needed to examine the contribution…".

Answer: The sentence "…research is needed to the contribution…" has been changed into "…research is needed to examine the contribution…" on page 5 .

Reviewer 2: In this manuscript, Zhang and coauthors try to demonstrate that miRNA-218 regulation is involved in gastric cancer growth, by using clinical, in vivo and in vitro models. Three different approaches that would provide an interesting picture if they had developed to a sufficient depth.

Major concerns

1. At a conceptual level, many miRNAs are overexpressed (from 3 to 5 fold) in serum after surgery. Aside from the miRNA-218, the others disappear from any consideration. Moreover, How can we be sure that these miRNAs in serum are produced by tumor cells and not, for example, by the immune system? The number of patients is really low to draw conclusions;

Answer: Thank you for your constructive opinion, aside from the miRNA-218, there are other miRNAs were overexpressed indeedly such as miR-135a, miR-409, miR-96. Because miR-218 was upregulated 8-folder after CRS+HIPEC, it is more higher than other miRNAs, so, we decided to put emphasis on miR-218. If we achieved a positive results, we would continue to study the mechanisms of other miRNAs such as miR-135a, miR-409, miR-96. In the next work, we will expand the sample size as many as possible.

Besides, we have searched the previous studied covering immune system and miRNA, and found that the immune system had no expression of miRNA-218. However, there are other miRNA involved in the immune system, such as miR-155, miR-21, et al. [J Viral Hepat. 2014 Feb;21(2):99-110. doi: 10.1111/jvh.12126. Epub 2013 Sep 11.MicroRNA-155 controls Toll-like receptor 3- and hepatitis C virus-induced immune responses in the liver. Jiang M(1), Broering R, Trippler M, Wu J, Zhang E, Zhang X, Gerken G, Lu M, Schlaak JF.] [PLoS Pathog. 2013;9(4):e1003248. doi: 10.1371/journal.ppat.1003248. Epub 2013 Apr 25.HCV-induced miR-21 contributes to evasion of host immune system by targeting MyD88 and IRAK1. Chen Y(1), Chen J, Wang H, Shi J, Wu K, Liu S, Liu Y, Wu J.]

2. Methods are not detailed enough to understand how the experiment described were performed. For example, "specific primer" is not enough. Their sequence should be indicated, at least in supplementary materials. How many BALB/c nude mice were used?

Answer: Thank you for your good advice, we have described the meaning of specific forward and reverse primer on page 6 as follows: forward 5'-AAGACACCCTGGACGAAGCC-3', reverse 5'-ACAACCAGAGTCCACCGGCG-3'. 24 BALB/c nude mice were used in this study on page 10.

Minor points

1. "300 nm of each specific forward and reverse primers" nm is a length unit. Maybe nM? Specific primer is not enough. Their sequence should be indicated, at least in supplementary materials.

Answer: Thank you for your useful advice, we have added specific forward and reverse primer on page 6 as follows: forward 5'-AAGACACCCTGGACGAAGCC-3', reverse 5'-ACAACCAGAGTCCACCGGCG-3'. It was our negligence to misuse nm and nM, we have changed nm into nM on page 6.

2. Literature cited regarding gastric cancer and miRNA is not well updated. For example, miR-101 is completely missing. It should be added in the introduction section (Carvalho et al., Lack of microRNA-101 causes E-cadherin functional deregulation through EZH2 up-regulation in intestinal gastric cancer. J Pathol. 2012 Sep;228(1):31-44.; He et al., Downregulation of miR-101 in gastric cancer correlates with cyclooxygenase-2 overexpression and tumor growth.FEBS J. 2012 Nov;279(22):4201-12)

Answer: Thank you for your good advice, we have add the above two references in the introduction at 21,22 on page 4.

3.This is an interesting study with both in vivo and in vitro data. The discover of miR-218 was based on microarray results, hence the clinical significance is good. For a minor comments, we suggest that you should verify the microarray results (not only miR-218, but also including the first five or more up-regulated microRNAs) in more than 5 blood samples.

Answer: Thank you for your good advice, aside from the miRNA-218, there are other miRNAs were overexpressed indeedly such as miR-135a, miR-409, miR-96. Because miR-218 was upregulated 8-folder after CRS+HIPEC, it is more higher than other miRNAs, so, we decided to put emphasis on miR-218. If we achieved a positive results, we would continue to study the

mechanisms of other miRNAs such as miR-135a, miR-409, miR-96. In the next work, we will expand the sample size as many as possible.

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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