

Dear Editors,

Thank you very much for the review of our manuscript entitled: "miR-34a demethylation up-regulates MPP2 expression and promotes liver cancer cell apoptosis". We sincerely appreciate all valuable comments and suggestions, which helped us to improve the quality of the article. Our responses to the Reviewers' comment are described below in a point-to-point manner.

We hope that our manuscript will be acceptable for publication in **World Journal of Gastroenterology**.

Yours sincerely,

Ting-Yong Fan

Reviewer # 1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing) Conclusion: Minor revision

Specific Comments to Authors: In the experimental study methylation of miR-34a is shown as molecular mechanism in regulation of MPP2 in liver cell carcinogenesis. The authors demonstrate that miR-34a demethylation is associated with an increase in MPP2, which belongs to the membrane palmitoylated proteins. These multidomain proteins are involved in several important cellular activities. The manuscript is well written and in the scope of the Journal. The experimental setting is well and provides essential data.

Comments

1. HCCs are investigated. The classification and entity should be given.

Reply: Thank you for your opinion. The expression of miR-34a or MPP2 in stage I/II tumor, stage III/IV tumor, primary liver cancer tumor and metastatic tumor was provided again. The details were listed in Figure 1.

2. Page 5: MPP is for membrane palmitoylated proteins and not palmitoyl membrane protein.

Reply: Thank you for your opinion. Palmitoyl membrane protein has been corrected to membrane palmitoylated. The details were shown in line 130 of this manuscript.

3. MPPs are important in lateral membrane organization and cell adhesion. In the study apoptosis and diminished proliferation, invasion and migration of liver cells were found. It should be of interest to investigate cellular adhesions after after miR-34a demethylation and the si-MPP2 rescue.

Reply: Thank you for your opinion. In this study, the levels of intercellular adhesion molecules E-cadherin and N-cadherin were detected, which can be used to evaluate cell adhesion.

Cancer cells are often accompanied by down-regulation of E-cadherin and up-regulation of N-cadherin. In this paper, the demethylation of miR-34a or the up-regulation of MPP2 leads to the up-regulation of E-cadherin and down-regulation of N-cadherin. However, when miR-34a is demethylated and MPP2 is down-regulated, E-cadherin is down-regulated and N-cadherin is up-regulated. The above results indicated that demethylated miR-34a may promote cell adhesion through MPP2, and finally inhibit cell migration and invasion. A discussion on cell adhesion has been added to the manuscript discussion section (line 446-454).

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing) Conclusion: Minor revision

Specific Comments to Authors: The study "miR-34a demethylation up-regulates MMP2 expression and thus promotes liver cancer cell apoptosis" has been submitted to the World Journal of Gastroenterology by Li et al. The study explores the role of miR-34a and MMP2 in liver cancer and adjacent normal tissues. They subsequently find miR-34a methylation to govern the expression of MMP2, and thus propose miR methylation as a potential method for liver cancer treatment. The study is interesting but the authors should address the following concerns.

1. Apart from MMP2, please discuss other biological factors that promote the metastatic nature of liver cancer. Any redundancy between proteins of the MMP family should also be taken into account.

Reply: Thank you for your opinion. Cell adhesion plays an important role in cell migration and invasion, that is, cell adhesion inhibits epithelial-mesenchymal transition, which is the basis of cancer cell migration and invasion. In this study, the levels of intercellular adhesion molecules E-cadherin and N-cadherin were detected, which can be used to evaluate cell adhesion.

Cancer cells are often accompanied by down-regulation of E-cadherin and up-regulation of N-cadherin. In this paper, the demethylation of miR-34a or the up-regulation of MMP2 leads to the up-regulation of E-cadherin and down-regulation of N-cadherin. However, when miR-34a is demethylated and MMP2 is down-regulated, E-cadherin is down-regulated and N-cadherin is up-regulated. The above results indicated that demethylated miR-34a may promote cell

adhesion through MMP2, and finally inhibit cell migration and invasion. A discussion on cell adhesion has been added to the manuscript discussion section (line 446-454).

2. Apart from MMP2, were any other downstream targets of miR-34a found to be altered?

Please elucidate

Reply: Thank you for your opinion. miRDB and TargetScan databases predicted that MMP2 might be the downstream target protein of miR-34a, and the double luciferase reporter gene verified that there was a targeting relationship between them. However, other downstream target genes of miR-34a were not mentioned in this study. This is also the limitation of this paper. Other downstream targets of miR-34a will be discussed in future studies. This deficiency has been stated in the manuscript (line 459-462).

3. Mention the distance of resection margins from the site of tumors? Did the expression of miR-34a, MMP, caspase follow any specific trend with increasing or decreasing distance from tumor site should be discussed thoroughly.

Reply: Thank you for your opinion. According to Figure 1, miR-34a and MMP2 are statistically different between normal adjacent tissues and tumor tissues, indicating that miR-34a and MMP2 expressions seem to be related to the distance between them and tumor. The lack of exploration on this point is the deficiency of this paper. Therefore, this "expression-distance" relationship will be discussed in depth in future research. This limitation has been stated in the manuscript (line 462-464).

Reviewer #3:

Scientific Quality: Grade D (Fair)

Language Quality: Grade B (Minor language polishing) Conclusion: Major revision

Specific Comments to Authors: The title of this paper reflects the main content of the manuscript: miR-34a can up-regulate the expression of MPP2 and promote the apoptosis of liver cancer cells through demethylation, and inhibit proliferation, invasion and metastasis of cells. The idea of this paper is relatively complete, focusing on the effect of miR-34a demethylation on the biological function of liver cancer cells.

However, I think the key word "caspase3" is not accurate enough, and the methods in the manuscript is not clear enough. For example, when cell proliferation is measured by MTT assay, how is cell viability calculated? what is the dilution ratio of the antibody in western blot analysis? What's the concentration of 5-za?

Reply: Thank you for your comments. The keyword "Caspase 3" (line 90) has been deleted.

The concentration of 5-Za is 10 mol/L (line 181). The dilution and concentration of protein antibody have been supplemented in Western blot (line 238-241). The activity of cells was detected using MTT assay kit (Abacam). After the cells were dyed with water-soluble MTT, the absorbance was detected at the peak of 570 nm. Since the number of living cells is proportional to the absorbance, the absorbance value is applied as the cell activity (line 263-264).

In addition, in the Transwell experiment, NC demeth and NC pre groups images of HepG2 in figure S1 were the same, miR-34a demeth and miR-34a demeth + siMPP2 groups images of Hep3B in figure S4 were the same, please give a reasonable explanation for the above phenomena.

Reply: Thank you for your opinion. The article pictures have been thoroughly checked, and Figure S1 and S4 have been uploaded again.

And in the results of western blotting assay, there were only statistical graphs and no stripes, which I thought it was not rigorous enough. Finally, if experimental conditions and time permit, it is suggested to increase animal experiments, so as to better illustrate the research topic of this paper.

Reply: Thank you for your opinion. Protein blots have been provided in the result picture (Figure S5). In addition, due to time and cost reasons, this paper failed to launch a suitable in vivo tumor experiment to further study the regulation of demethylated miR-34a/MPP2 axis in solid hepatocellular carcinoma. The above deficiencies have been stated in the discussion section (line 457-459).

In the experimental study methylation of miR-34a is shown as molecular mechanism in regulation of MPP2 in liver cell carcinogenesis. The authors demonstrate in the further revised version that miR-34a demethylation is associated with an increase in MPP2, which belongs to the membrane palmitoylated proteins. These multidomain proteins are involved in several

important cellular activities. Comments 1. HCCs are investigated. The classification and entities according to WHO are not addressed in detail.

Reply:

Dear editor, Please find the attached revised manuscript for your kindly reference.