Author’s response to reviews

Title: MicroRNA-200a confers chemoresistance by antagonizing TP53INP1 and YAP1 in human breast cancer

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Dear Editor:

We are most grateful for your decision letter and for the reviewers’ valuable comments concerning our manuscript entitled “MicroRNA-200a confers chemoresistance by antagonizing TP53INP1 and YAP1 in human breast cancer” (Manuscript No: BCAN-D-17-00643). The comments were all very helpful and provided great guidance in revising and improving our paper. We studied the comments carefully and improved our manuscript according to reviewers’ kind suggestions. We hope that our revised manuscript will be clearer than the original version. Please find our point-by-point responses as follows:
Reviewer #1 (Reviewer Comments to the Author):

In this manuscript, Yu et al investigated the potential oncogenic impact of miR-200a in breast cancer. Overall, this is a well-designed and performed study. The authors have demonstrated that miRNA-200a may induce chemoresistance by inhibiting TP53INP1, YAP1 and consequently p53 family protein mediated apoptosis. The authors further demonstrated the overexpression of miR-200a in chemoresistant breast cancers to suggest the potential clinical relevance. However, this manuscript still contains some gaps in the mechanism studies which need to be addressed by supplying additional experimental data before the consideration of acceptance.

Specific comments:

1. The authors need to declare the matured miRNA sequence - e.g. miR-200a-5p or miR-200a-3p.

   Response: We appreciate the reviewer’s kind recommendations. We apologize for our insufficient descriptions regarding this point in our previous manuscript. MicroRNA-200a in the current study represents miR-200a-5p and the sequence was illustrated in Fig. 3B. We have added the details in the materials and methods section of the revised manuscript (Page 4, line 9-10).

2. It is highly recommended the author recapitulate their discovery in Fig. 4D using anatago-miR-200a in the gemcitabine resistant MDA-MB-231 cells.

   Response: Thanks a lot for the reviewer’s suggestion. As instructions, we test expression of TP53INP1 in MDA-MB-231 GR cell line and MDA-MB-231 cell line. In MDA-MB-231 GR cell line, expression of miR-200a was upregulated and correspondingly, expression of TP53INP1 decreased compared with MDA-MB-231 cell line. We further rearranged Fig. 3 and Fig. 4 to make it more reasonable. In MDA-MB-231 GR cell line, anatago-miR-200a promoted expression of TP53INP1 and enhanced the sensitivity of gemcitabine-resistant cells to gemcitabine (Fig. 4C). Detailed information was illustrated on page 8-9.

3. The downregulation of p73 in Figure 4D is not well discussed - please clarify the potential mechanism. It is highly recommended the authors delineate whether the downregulation of p73 is due to the direct targeting effect of miR-200a at RNA level or the destabilization due to the loss of YAP1 at post-translational level. A good starting point is to check p73 mRNA level by qRT-PCR upon miR-200a overexpression.

   Response: We appreciate the reviewer’s kind suggestion. We have checked p73 mRNA level by qRT-PCR. As indicated in the manuscript, miR-200a expression was much higher in MDA-MB-231 GR cell line than that in MDA-MB-231 cell line. However, further experiment indicates no significant difference of p73 mRNA expression between MDA-MB-231 GR and MDA-MB-231 cell lines (Fig. S1), which means downregulation of p73 is not due to the direct targeting
effect of miR-200a at RNA level, and might due to the loss of YAP1 at posttranslational level. The detailed information was shown in line 2-3, page 12 in the revised version.

4. Similarly, the authors need to demonstrate p73 is associated with the alteration of Bim, Bax and PUMA upon miR-200a overexpression.

Response: We thanks for the reviewer’s reminder. We have indicated in Fig. 3, overexpression of miR-200a resulted in downregulation of p73, as well as Bim, Bax and PUMA expression.

5. Figure 5A: Since there is no CR patients in this cohort, please remove it from the legend. Please suggest the statistical method for p-value calculation as well.

Response: Thanks a lot for the reviewer’s kind reminder. We have remove “CR: complete response” from legend of Fig.5 in line 22, page 17.

The statistical method used in Fig. 5A was t-test. We have added the statistical method in legend of Fig. 5 in line 19, page 17.

6. Figure 5B: Please additionally test the prognostic impact miR-200a by Cox regression model. This avoid the subjective cut-off of a continuous variable (miR-200a expression level) used in Kaplan-Meier model, unless the authors can suggest the biological inference of such cut-off.

Response: We appreciated the reviewer’s kind recommendations. We agreed that the subjective cut-off of a continuous variable was not appropriate. In the revised manuscript, we choose X-tile program to determine the optimal cut-off value for miR-200a expression. The new survival curve was added in Fig. 5B.

7. Please discuss whether p53 participated in miR-200a mediated chemoresistance (ZR-75-30 harbors wt TP53).

Response: We appreciated the reviewer’s insightful recommendations. We used two breast cancer cell lines MDA-MB-231, which harboring p53 mutation and ZR-75-30 harboring wt TP53. MicroRNA-200a plays a role in mediating chemoresistance in both cell lines, regardless of status of TP53. Therefore, we believe p53 may not participate in miR-200a mediated chemoresistance. We have added this in discussion part in the revised version (page 12, line 7-9).

Reviewer #2 (Reviewer Comments to the Author):

Overall, this manuscript demonstrates the relationship between miR-200a expression and chemoresistance. The data are solid and convincing. The clinical correlation data in Table 1 implies that the miR-200a specifically correlates to chemoresistance and it is universal in the
population studied regardless of other clinical traits. This finding is helpful for more accurate prognosis and effective preoperative chemotherapy strategy.

In the manuscript, the author concluded that miR-200a was overexpressed in gemcitabine resistant breast cancer cells, and inhibition of miR-200a can restore sensitivity to these cells. However, the expression level of miR-200a and the pro-apoptosis marker in the cohort remain unknown (no data shown). It would be useful to at least choose 3-5 patient samples to examine those markers that were examined in the cell lines used in this manuscript. Overall, this finding is still very informative for a more suitable clinical application for those who are less responsive to preoperative chemotherapy.

Response: We appreciate the reviewer’s comments and suggestions. We have made some amendments in the revised manuscript and we hope that our revised version would be more convincing and reasonable.

Other minor issues:

1. Figure 1A error: Platinum should be Platin?

Response: Thanks a lot for the reviewer’s reminder and we apologize for such a mistake. We have replaced “Platinum” with “Platin” in Fig. 1A in the revised manuscript.

2. Figure 1B-1E legend does not match the figures. It is confused especially for Figure 1B because the cell line in the figure is MDA-MB-231 but in the legend it is ZR-75-30.

Response: We appreciated the reviewer’s reminder. We have changed legend of Fig. 1 in line 5-10, page 16.

3. Page 12, line 25: "directly bind" should be "directly binding".

Response: We appreciated the reviewer’s reminder. We have replaced "directly bind" with "directly binding" in page 11, line 11 in the revised version.

4. Page 12, line 45, "can clearly" should be "clearly".

Response: We appreciated the reviewer’s patience and we are sorry for the mistake. We have replaced "can clearly" with "clearly" in page 11, line 18 in the revised version.

5. Page 13, line 3 "bind" should be "binding".
Response: Thanks a lot for the reviewer’s reminder. We have changed “bind” to “binding” in page 12, line 5 in the revised version.

We sincerely hope that the revised manuscript will meet all criteria. It would be a great honor for our team if our manuscript can be accepted for publication in BMC cancer. Thank you once again for all your help and kind consideration.

Sincerely,

Zhi-Ming Shao