Author's response to reviews

Title: miR-141 and miR-200b is closely related to invasive ability and considered as decision-making biomarkers for extent of PLND during Cystectomy

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Author's response to reviews: see over
Dear editor Olino,

Thank you very much for your email with regard to our manuscript entitled “miR-141 and miR-200b is closely related to invasive ability and considered as decision-making biomarkers for extent of PLND during Cystectomy (MS: 6702757091446335)” together with the comments on 09-Dec-2014. Those comments are valuable and very helpful for revising and improving our manuscript. We have studied comments carefully and made a revision accordingly. We have tried our best to respond to the comments, point by point. Major revisions or additions are highlighted in red in the revised manuscript.

We would like to resubmit the revised manuscript to BMC Cancer and appreciate your reconsideration.

If there are any problems with our manuscript, please don’t hesitate to contact us.

With best wishes,

Yours sincerely,

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Point by point responses to reviewers:

Reviewer 1:

Reviewer: PRAVEEN JAISWAL

Reviewer's report:

MiRNAs is involved in various biological processes, including cell cycle control, apoptosis, cell proliferation and invasion. Authors investigate the role of miR-141 and miR-200b in context with decision-making biomarkers for PLND during Cystectomy. These two miRNA was further investigated in vitro to confirm their impact on invasive ability of bladder malignant cell and to explore whether they are involved in the regulation of EMT. The observations from present study are interesting. However, there are points remain to consider before publishing the manuscript:

1. Is there any specific reason to select only male patients for present study?

Thanks for your careful review. We are very sorry to have made you misunderstand. As shown in Table 1, 64 male and 14 female were included in our study.

2. Use the same notion for gene name throughout the manuscript.

Thanks for your careful review. Did you mean the different usage of "miR" and "miRNA". Accordingly, “miR” have been revised as "miRNA " in our revised manuscript.

3. What were the reasons to choose only these specific miRNAs viz. miR-141 and miR-200b among miR-200 family.

Thanks for the question. As present studies reported, all five members of miR-200 family were deregulated in bladder cancer. In our paper, we wanted to select two miRNA which are most relevant to invasive ability and metastasis of bladder cancer cells. Thus, we used qTR-PCR to measure the expression of five miRNA-200 family members in muscle-invasive BC specimens and corresponding
adjacent tissues. The most significantly regulated miRNAs in our series were miRNA-141 (3.25 fold) and miRNA-200b (4.28 fold). Therefore, we selected these two miRNA for further investigation (result section, paragraph 1).

4. What was time to process the fresh tissue from tumor site? And the tissue size for RNA extraction. Please add it.

Response: Fresh tissues were obtained when the bladder had been removed during the operation and stored in liquid nitrogen before use. For RNA extraction, bladder tissues with a diameter of about 3–4 mm were used for RNA extraction (added in page 4, line 17-18). Then, for each bladder specimen, the cDNA was synthesized from 2 µg total RNA (page 6, line 13-14).

5. Authors should add a statement from their ethics committee or institutional review board indicating the approval of the present project.

Thanks for the suggestion. In the present manuscript, we have mentioned "This study was approved by the Ethics Committee of Central South University" in the method section (page 4, line 8).

6. Authors should have mentioned a statement about the impact of confounding factors such as Smoking on PLND.

Thanks for the suggestion. We agreed with you that some potential confounding factors might have some impact on the results of AUC curves. However, it is hard to precisely evaluate the impact. Thus, we mentioned it in the limitation part (page 16, line 6-7).

7. Add the term year with Age ±SD throughout the manuscript including tables.

Response: According to your advice, Age ±SD have been added in the Table 1 and methods section(page 8, line 11).
8. Study limitation part should be elaborated as sample size is not good enough to withdraw a conclusion for decision making biomarker for PLND specially in case of subgroup analysis.

Thanks for the suggestion. We have added this into the limitations (page 16, line 7-9).

Reviewer 2:

Reviewer: Shikha Dubey

Reviewer's report: Accepted

Response: Thanks for your careful review and positive comments.

Reviewer 3:

Reviewer: Dhruva Kumar Mishra

Reviewer's report:

Article entitled “miR-141 and miR200b is closely related to invasive ability and considered as decision-making biomarkers for extent of PLND during cystectomy” reported the downregulation of miR-200 family miRNAs in bladder cancer tissues vs adjacent tissues and furthermore their (miR-141, miR200b) roles in invasive ability and EMT phenotype of bladder cancer. Additionally, authors found a relationship between urinary miR-200 expression with lymph node metastasis, which can predict the same. I have following major comments:

1. As authors have created the high expressing CRL1749 and low expressing HTB9 stable cancer cell lines by transfecting miR 200 and respective sponges, it would interesting to know their metastatic ability in nu/nu mice. It will further clarify its role in invasion and metastasis.
We would like to express our sincere thanks for your constructive comments that pointed us a good direction. The additional experiments you suggested were of great value because it will further clarify the role of miRNA-200 in invasion and metastasis in vivo. In the present study, we mainly focused on impact of the urinary miRNA-200b and miRNA-141 level on PLND. Thus, the animal-based experiment was not included in the present study. And there is another reason. The funding of animal experiments was quite difficult to be approved in our university. It is estimated that we have to wait for another 1.5 years to get the animal experiments started. We are hoping that we could accomplish the animal-based experiment in following experiments.

2. Though authors have shown in Fig 1B, the transduction efficiency of the miRs, I would suggest a figure showing the relative expression of miRs in these stable cell lines after transduction.

Thanks for the suggestion. We have added two new figures which indicated the relative expression of miR-200b and miR-144 in the manuscript as shown in Fig. 1C and 1D.

3. Authors should provide brief details about miRNA sponge used for this study to inhibit the miRNA function.

Thanks for the suggestion. The miRNA sponge was designed and constructed by a biotech company (Yinrunbio, Changsha, China). We have consulted them if there was any possibility of providing us the detailed information of the sponges such as the sequences. But they declined with a reason of “confidentiality”. Therefore, we only added the company’s name into the manuscript and attained the approval of the company (page 5, line 14).

Minor comments:

1. Authors should use the Bladder cancer instead of BC in Abstract -Line 5

According to your advice, BC has been revised to Bladder cancer in abstract -Line 5.
2. In Methods section (page 4, Line 7), authors should provide City & country name along with University.

Response: According to your advice, City & country name along with University have been added in the Methods section (page 4, Line 8-9). 

3. In discussion (Page 14, line 2), Is it HTB9 or it should be CRL1749?

Response: HTB9 has been corrected as CRL1749 in page 14, line 14.