Author's response to reviews

Title: miR-221 Facilitates the TGFbeta1-Induced Epithelial-Mesenchymal Transition in Human Bladder Cancer Cells by Targeting STMN1

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Author's response to reviews: see over
Dear reviewer:
I am very grateful to your comments for the manuscript entitled “MS: 1829324592150159-miR-221 Facilitates the TGFbeta1-Induced Epithelial-Mesenchymal Transition in Human Bladder Cancer Cells by Targeting STMN1”. According with your advice, we carefully revised our manuscript. Now I answer the questions one-by-one.

REVIEWER 1

Major Compulsory Revision:

1. Figure 4- results do not appear to be impressive with mir-221 inhibition. Is it possible to quantify the degree of invasion better because the pictures do not Look that different.

   **Answer:** Thank you for your suggestion, and it's a good advice that could be adopted. Since the result of fig.4 has demonstrated the role of miR-221 in bladder cancer cell lines induced by TGF-β1, and consideration about the grouping for figs. 3 to 7 was the same, so if I set a dose groups only for fig.3, it seems not to be standardized and unified. We will incorporate your kindly comments in our future studies, and we’re very appreciate for your suggestions.

2. Figure 7- please explain why your western blot shows mesenchymal markers In RT4 (an epithelial line) and epithelial markers in t24 (a mesenchymal line). Most papers show that these are not expressed at baseline in these cell lines. Were the films over exposed to get these results?

   **Answer:** Yes, and you’re very professional. There are two factors leading to this results: 1) we chose larger sample volumes (50ug) than usual (35ug) because of the low expression levels of these markers; 2) The films were indeed over exposed to get these results.

Minor Essential Revisions:
They were revised in highlight in the newly uploaded manuscript.

REVIEWER 2

Generality Major Compulsory Revisions

1. It is very difficult to understand why authors refer to breast (line 45) or ovarian (line 79, 194, 197, 212) cancer in the text while experiments have been performed on bladder cancer.

   **Answer:** I'm very sorry for our stupid mistakes. Since English is not our mother tongue, we are not very confident about our language editing. So we learned the language presentation from other English literatures which were similar to our methods, and contents in this study. But I’m very sorry that we forgot to correct some words leading to these unforgivable mistakes. We have modified the manuscript carefully and will pay much more attention to our language editing work. Thank you
for your reminding.
   Answer: Thank you for the suggestion. Yes, and we have already made the corrections in the manuscript.
3. The authors should justify why there is no in vivo studies to prove the role of miR-221 during metastasis.
   Answer: Yes, and thank you for your point advice, it is actually part of the plan in our future study.
Statistics Major Compulsory Revisions
4. Statistical analyses is not appropriate. Authors have used Student t test while One-way ANOVA should be performed.
   Answer: We'll adjust it in the article.
5. Figure 7 : lack of statistics .
   Answer: We have added statistics in figure 7.

Introduction
6. The problem being investigated and hypothesis is clearly stated.

Discretionary Revisions
7. At the end of the introduction, the authors should briefly describe the general experimental design and the main results obtained.

Minor Essential Revisions
8. As is the central subject of the article, epithelial to mesenchymal transition should be better explained and how the TGFb1 induces EMT also.
   Answer: we have made changes in the corresponding sections.
9. Normally, the introduction should summarize relevant research but here, there is no mention of this important article: Lu, Q., et al. MicroRNA-221 silencing predisposed human bladder cancer cells to undergo apoptosis induced by TRAIL. Urol Oncol. 2010 Nov-Dec;28(6):635-41.
   Answer: Thank you for the suggestion. I have added article in the introduction.

Method Minor Essential Revisions
10. Some experiments are not described in sufficient detail : Cells culture conditions should be mentioned (temperature, CO₂, medium composition). Quantitative RT-PCR conditions should be mentioned (temperature, cycle, time). Western blot antibody dilutions should be mentioned.
   Answer: Thank you for the suggestion. I have added the experimental details as your suggestion.

Results

Discretionary Revisions
11. Figure 2B : left graph y axe should be identified.
   Answer: Thank you for the suggestion. I have added y axe in Figure 2B : left graph.
12. Figure 5: The time point ‘0h’ in the measurement of cell migration by wound-healing assay is not required and could not appear in the graph.

Discussion/Conclusion

13. Claims in this section is well supported by the results.