Author's response to reviews

Title: MicroRNA-27a promotes proliferation and suppresses apoptosis by targeting PLK2 in laryngeal carcinoma

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Author's response to reviews: see over
Dear Editors,

Thanks for your kind consideration. I have revised my manuscript (m/s) word by word according to reviewers’ opinions. All the revised sections are marked as red words.

Reviewer 1
Question: The authors proposed in their cell experiments that miR-27a significantly decreased PLK2 expression at protein level (Figure 3D) but not at mRNA level (data not shown) in laryngeal cancer cells. PLK2 protein level in laryngeal cancer tissues, which can best describe the correlation between miR-27a and PLK2, is therefore needed to be detected.

Answer:
We have detected PLK2 protein level in all the cases with miR-27a up-regulation and analyzed the correlation between PLK2 and miR-27 expression levels. These results and related descriptions have been inserted in the corresponding parts of Abstract (line 4 to 6 of Results), Methods (first line of Western blot; line 4 to 5 of Statistical analysis), Results (line 13 to 18 of the third section) and Discussion (line 10 to 11 of the third paragraph).

Reviewer 2
[Major Compulsory Revisions]
Question: If the authors emphasize the significance of dysregulation of miR-27a and PLK2 in LSCC, they should analyze the down-regulation of PLK2 in clinical samples of LSCC (for example, IHC). If impossible, they should clearly state the limitation that they could not find the down-regulation of PLK2 expression in LSCC. They might mention about the indication of down-regulation of PLK2 in LSCC or HNSCC from any other articles in discussion.

Answer:
We have detected PLK2 protein level in all the cases with miR-27a up-regulation and analyzed the correlation between PLK2 and miR-27 expression levels. These results and related descriptions have been inserted in the corresponding parts of Abstract (line 4 to 6 of Results), Methods (first line of Western blot; line 4 to 5 of Statistical analysis), Results (line 13 to 18 of the third section) and Discussion (line 10 to 11 of the third paragraph).

[Minor Essential Revisions]
Question: (1) In discussion, the authors mistakenly used 'miR-24a' with 'miR-27a' in several
times. For example, 'miR-24a itself and silence of PLK2...'. etc. They need to correct them.
Answer: These mistakes have been corrected.

Question: (2) In Fig1D, in X-axis, what do 'T' and 'R' indicate?
Answer: 'T' and 'R' indicate tumor and the paired adjacent tissues, respectively.

Question: (3) In Fig2B, 'mir-27a mimics' should be 'miR-27a mimics'. (r->R)
Answer: 'mir-27a mimics' has been corrected to 'miR-27a mimics'

Question: (4) In Fig3A, 'Has-miR-27a' is mistake with 'Hsa-miR-27a'?, and its sequences are not shown.
Answer: 'Has-miR-27a' has been corrected to 'Hsa-miR-27a' and its sequence has been added.

Question: (5) In Fig4E and 4F, the result overlaped with Fig2D and 2F, respectively. The images and bars of 'miR-27a inhibitor' and 'miR-27a mimics' might be omitted.
Answer: Actually, the results come from different times of experiments. If it is required, we will omit the overlapped images and bars in the final revision.

[Discretionary Revisions]
Question: Fig1A, 1B, 3B might be supplemental figures.
Answer: It is a good suggestion and we have moved Fig1A, 1B, 3B to the supplemental section.

Reviewer 3
Specific points that should be answered:
Question: (1) Authors should describe somewhere the reason for choosing PLK2 out of 1211 predicted targets of miR-27a.
Answer: The reason for choosing PLK2 out of 1211 predicted targets of miR-27a includes three aspects which we have already explain them in corresponding parts of the manuscript. Firstly, we found miR-27a is up-regulated in laryngeal cancer and plays oncogenic role in laryngeal carcinogenesis (please see the first and second sections of Results), so the targets of miR-27a should be tumor suppressors. PLK2 is reported a presumably tumor suppressor gene even though it plays an oncogenic role in several tumors (please see the fourth paragraph of Discussion). Secondly, prediction results using three different programs indicates that as a candidate target, PLK2 sequence binging to miR-27a is highly-conserved (please see the third section of Results). In addition, the prediction scores are relatively quite high which we do not describe in
Question: (2) As miR-27a is part of a cluster miR-23a~miR 24a~ miR-27a, the expression of all these should also be checked individually in Hep2 cells at least to define the role of miR-27a clearly because this was used as basis for the study.
Answer:
We have mentioned in the second paragraph in Discussion "In our group, we also detected the expression of miR-23a and miR-24-2 in LSCC. The results showed that the two members are significantly up-regulated in general in LSCC (data not shown)."

Question: (3) Results section says “Significant down-regulation on luciferase activity was found in the presence of miR-27a in the HEK293 cells when transfected with pGL3-3’UTR of PLK2” whereas discussion says these results have been done in Hep2. Authors should describe where the luciferase experiment has been done HEK293 or Hep2 or both?
Answer: . It is a mistake. The luciferase experiment has been done in HEK293. We have corrected the mistake in Discussion (the third paragraph).

Question: (4) Higher resolution figures should be included for colony formation assays. Images at low resolution is not sufficient.
Answer:
Resolution of the images related to colony formation has been adjusted.

Question: (5) What is Box-and-whiskers plot. Fig. 1D explain.
Answer:
"Box-and-whiskers plot" has been changed by "Statistical analysis of miR-27a expression in LSCC (please see Figure 1B)".

Question: (6) The authors have described only few validated targets of miR-27a so they should do a proper literature search and include them in discussion.
Answer:
We described in discussion "At presence, ZBTB10/RINZF[25], FOXO1[26], and FBW7[33] are confirmed as the miR-27a target genes." In the sentence, we want to give several examples of miR-27 targets. Therefore, we have changed the sentence to "At presence, some genes such as ZBTB10/RINZF[25], FOXO1[26], and FBW7[33] have been confirmed as the miR-27a target genes."(Please see the third paragraph of Discussion).

Reviewer 4
Major points
Question: (1) As a normal cell line the authors used HCK293. The cell is inappropriate for representing larynx, therefore, cell lines originated from the tissue or
related tissues are recommended, such as bronchial epithelial cell lines for multiple cell lines.
Answer:
HEK293 is a commonly used as a tool cell line, which is accepted in different cancer investigation. The author comment is very suggestive. We will consider it in our further study.

Question: (2) It is well described that miR-27a is up-regulated in cancer. However, to complete the relation between miR-27a and PLK2, the expression of PLK2 in normal and cancer tissue should be given. Also, association study in statistics between the two genes’ expression should be given.
Answer:
We have detected PLK2 protein level in all the cases with miR-27a up-regulation and analyzed the correlation between PLK2 and miR-27 expression levels. These results and related descriptions have been inserted in the corresponding parts of Abstract (line 4 to 6 of Results), Methods (first line of Western blot; line 4 to 5 of Statistical analysis), Results (line 13 to 18 of the third section) and Discussion (line 10 to 11 of the third paragraph).

Question: (3) The authors described that PLK2 was downregulated by miR-27a in the protein level but not the RNA level. The way of regulation by miRNA is important concept and therefore the result for RNA should be given not in a form of “data not shown”. The result can be placed in the supplementary section.
Answer:
We have placed the result in the additional file 4.

Minor points
Question: (1) Fig.1A and B are not essential. So it is better to be moved to supplementary section.
Answer:
We have moved Fig. 1A and B to the additional file 1.
Question: (2) Fig. 1C; In addition to the bar graph, adding a box plot graph would be helpful to understand the result.
Answer:
We have added a box plot graph in Figure 1.

By the way, some words which were not clear or native in our original manuscript have been revised and marked as red color in the corresponding places of our revised manuscript.
In the revised manuscript, Figure 4 and additional files are added. Therefore, we moved Figure 1A and B in the original manuscript to the additional file 1, Figure 3 B in the original manuscript to the additional file 3 and Figure 4A in the original manuscript is still in Figure 4A. The left images in Figure 4A in the original manuscript are placed in Figure 5. Meanwhile, statistical analysis result is inserted in Figure 2D.

Finally, two references (reference 12 and 15) have been replaced by two new ones which have been published in 2014 on BMC cancer because the original manuscript was submitted on October of 2013.

Looking forward to your kind reply.

With my best regards

Sincerely yours

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