Author's response to reviews

Title: MiR-181a contributes to bufalin-induced apoptosis in PC-3 prostate cancer cells

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

Thank you for your and reviewers' kindly suggestions. We are glad to submit this revision for your consideration. This revision has been edited by Edanz as recommended by BioMed Central. Point-to-point responses were listed below.

Reviewer 1

Major Compulsory Revisions

1. *Please use the help of the professional English speaking science writer. There are too many parts of the results section, that needed to be re-written in the clear manner indicating the experiment design and data obtained with sufficient details in the text and Legends.*
   
   A: We have made this manuscript edited by Edanz as recommended by BioMed Central to improve English writing.
   
2. *Please avoid the conversational style, such as "obvious" instead of pointing out the degree of the bufalin effect on the miR-181a expression. Again not "given" but "Since" or "Because". Also, not "as showed", but "as shown". p.9.*
   
   A: We have corrected these points as suggested.
   
3. *Please provide quantitative data about the effect (e.g. fold change, etc.).*
   
   A: We have analyzed some blots by ImageJ software to show the approximate fold change in this revision.
   
4. *Please describe in more details the obtained results, not just "confirmed by"*
   
   A: More detailed description on results has been included in this revision.
   
5. *Please described in more details the statistical data for Figure legends, such as "p" is such and such compare sample "A" to "B", how many samples (n=xx).*
   
   A: We have specified how many replications included in our statistics in this revision. Samples from which p value was got were indicated in figures with "bridge" lines under “p”.
   
6. *What is MiR-NC? Is it the scrambled one? Where is it obtained from?*
A: Sequence of miR-NC was from *C. elegans* and has no known similar sequence in human genome. The supplier didn’t give us any more information about the sequence of miR-NC. We have added this information in methods section.

7. **Please indicate in the immunoblot. Figure 3 and 4.**

A: We have indicated the molecular sizes of proteins in these figures.

8. **Please indicate the p values in Figure 4**

A: We have indicated p values in this figure as suggested.

9. **How much microRNA inhibitor was added? p. 5**

A: We have specified the miRNA inhibitor amount we used in section 1 of methods.

10. **Please state how many cells were used for transfection and other treatment. throughout the whole manuscript**

A: We didn’t count cells accurately in our study. However, we routinely followed the experiential starting cell density in this study and indicated this information in section 1 of methods in this revision.

Minor essential revisions:

1. **It seems that in conclusions’ section (Abstract, p. 2) line has a different font/size/ please correct.**

2. **Please separate the words after comma in the last sentence on p. 3.**

3. **Please avoid statements, like "so on" on p.4, end of the paragraph 2.**

4. **Please replace Here, we reported" with "Here, we report". p. 55.**Page 5.**Methods. Should state "Cells were treated with the indicated concentrations of bufalin for 24 hours"**

6. **When PBS designation used for the first time in the manuscript, please give a full name. p. 6**
8. Student is a name. p.14

A: These points have been corrected in this revision.

7. Please specify details about Flowjo software

A: Flowjo is a software that can processes Flow cytometry data and was developed by Tree Star, Inc., Ashland, OR, USA. This information has been included in methods section.

Reviewer 2

1. qPCR, used to screen miRNA, is low-efficient. Please take about application of miRNA microarray in cancer.

A: Thank you for forward-looking suggestion. It will be very helpful to us.

2. Mir-181a have multiple target genes apoptosis-related, such as RalA. It is needed for more target analysis.

A: In this revision, we validated that RalA was negatively regulated by miR-181a as a previous study. This result was used as positive control in our Figure 3.

3. Why didn’t author try to transfer mir-181a into PC-3?

A: When we tried to validate RalA and Bcl-2 as target genes of miR-181a, we transferred miR-181a into PC-3. This result was now shown in Figure 3.

Sincerely,

Xiaofeng Zhai