Author’s response to reviews

Title: MiR-199a-3p affects the multi-chemoresistance of osteosarcoma through targeting AK4

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Comments:
The manuscript is well designed study and the authors were able to show AK4 is the target gene, elucidated the possible mechanism and signaling pathway mediated by miR-199a-3p on multiple chemoresistance by testing on three OS cell lines. The manuscript can be published in its present form and I think it would improve the knowledge in better understanding of OS drug resistance and involvement of MicroRNAs.

1. I would like the authors to show how many animals were used in vivo study (n=?) and how many spots developed tumor or reduced tumor size?
A: 6 mice were used for each cell line in our initial experiment. 3 mice were injected with G-292 treated with Dox at 2.5 mg/kg once in 3 days, the remaining 3 mice were injected with G-292 treated with phosphate-buffered saline (PBS).

Vijay Pandey (Reviewer 2): Background information is superficial.
Authors should represent more information regarding miRNA, AK4 gene, and OS in separate paragraphs. The complete name for AK4 abbreviation is missing in the manuscript.
A: Thank you for your advice, we have added more information regarding miRNA, AK4, and OS in the introduction. In addition, we have added the full name of AK4 abbreviation in the manuscript.

Material and methods:
Authors should use proper nomenclature for the gene (mRNA and protein) as suggested by HUGO Gene Nomenclature Committee, or equivalent resources to ensure standardized nomenclature is used for species-specific gene and protein names.
A: Corrected.

The author should provide certificate number or details from IACUC committee approval of Anhui Medical University.
A: We have added the certificate number: LLSC20170464 in the manuscript.

Interpretation of results is not conclusive. As most of the conclusion addressed by authors are MIGHT BE….
A: We have carefully revised the manuscript to make clear interpretation of the results.

The discussion is superficial and not adequately written. Mostly repetition of results and introduction...
A: According to your suggestions, we have carefully revised the manuscript to avoid duplication.

Overall, the current version of the manuscript needs scientific proofread.

Results:
Authors should provide predictive analysis of the targets of miR-199a-3p using the following websites: Targetscan, miRDB, and microRNA.org. Moreover, rational for AK4 selection for further analysis in this study.
A: Added.

Figure 2E, authors should provide new WB for MNNG/HOS (NC and 3PA), as control blot (GAPDH) for not look substantial.
A: According to your suggestion, we have repeated all the WB experiments, and updated the Figures, the full-length gels of the western analyses used in the manuscript was also uploaded in the supplementary materials.

One of the primary concern in this study is in vivo analysis, the tumour volume. Authors should provide details regarding protocol in the supplementary information.

A: We have added the detailed information regarding the in vivo experiments in the supplementary.