Reviewer’s report

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

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Reviewer: Durga Attili

Reviewer's report:

The present manuscript by Lei et al., show that MiR-199a-3p by targeting AK4 is involved in the chemoresistance of Osteosarcoma. The authors established the IC50 values by testing cisplatin (CDDP), carboplatin (Carb), and doxorubicin (Dox) on OS cell lines (G-292, U2OS, and MNNG/HOS) supplemental fig1. In fig1, the authors show the relative expression ratio of miR-199a-3p in 3 cell lines using qPCR, similarly they show the AK4 is the target gene of MiR-199a-3p using qPCR and western. In fig 2 they showed the change in AK4 expression levels in miR-199a-3p mimic-transfected G-292 and U2OS cells and antagomiR-transfected MNNG/HOS cells. In Fig 3, the authors using luciferase assay established that AK4 is the target of miR-199a-3p (using reporter vector and luciferase gene). In fig 4, the authors using western and qPCR methods establish that the AK4 could be positively corelated with multi OS drug resistance using miR-199a-3p mimic or si-AK4 into G-292 or U2OS cells and miR-199a-3p antagomiR into MNNG/HOS. In fig 5, to understand the mechanism of OS drug resistance mediated by miR-199a-3p the authors measured the activities of 10 cancer related signaling pathways and found that NF-κB pathway might be involved in the OS drug resistance. In fig 6, using in vivo experiments the authors showed that miR-199a-3p inhibits both the growth and CDDP drug resistance of G-292-derived tumor xenografts in nude mice (measured tumor size, tumor proliferation by IHC analysis of AK4 and Ki67).

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?
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

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