Reviewer's report

Title: FOXO/TXNIP pathway is involved in the suppression of hepatocellular carcinoma growth by glutamate antagonist MK-801

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Reviewer: Yasuo Takano

Reviewer's report:

In this paper, the authors demonstrated that stimulation of NMDA receptor (NMDAR) signaling augments hepatocellular carcinoma (HCC) cell proliferation. This conclusion is based on in vitro analysis of cell proliferation showing that NMDAR antagonist MK-801 by inducing cell cycle arrest. The study was further extended to mechanistic analysis of how MK-801 confers this growth inhibiting effect. They showed that known FOXO-TXNIP pathway is involved in the growth suppression mechanism. Although straightforward, it seems that the story is relatively small advance compared to already published works. Further, I have several concerns about contents of this manuscript.

Major comments

1) First of all, it is not clear why the authors focused on HCC this time. Can they generalize this mechanism for other NR expressing cancer cells? Otherwise, are there any evidences showing that expression level of NRs in HCC is higher than that of other cancer cells?

2) Authors performed experiments using only two HCC cell lines. If the authors want to show NMDR signaling is generally involved in HCC proliferation, more works using additional HCC cells are needed (at least, cell viability and cell cycle analysis).

Specific comments

1) Figure 1A; Expression levels of NRs is not clear. Again, are there any evidences showing that NRs are highly produced in HCC cells compared to other cancer cells?

2) Figure 4A; Are there evidences or reports showing that FOXO can bind to nucleotide sequence shown here. If so, it should be cited in the text.

3) Regarding comment#2, it is valuable to perform chromatin immunoprecipitation analysis of FOXO binding to TXNIP promoter region and show binding of FOXO is increased in response to MK801 stimuli. It strengthens the author’s conclusion.

4) Figure 4C; Western blotting data should be given.

5) Figure 4C&D; The authors performed experiments with a single TXNIP siRNA. At least, two different siRNAs should be tested to exclude the possibility that the results are due to off-target effect of used siRNA.
6) Does TXNIP-FOXO axis contribute to cell cycle arrest? Otherwise does it inhibit cell growth via independent pathway?

7) Description of figure legends is too busy. Majority of it should be moved to Methods section. Otherwise removed if redundant.

Overall, it was felt that the HCC story provided here is incomplete and additional works are needed to give solid conclusion to this manuscript. Plus, I recommend that they write the manuscript in ways that aim of the study is clear to the readers, such as in Background section summarizing why the authors are encouraged to start experiments with HCC cells. It is also valuable that they make it obvious what is new in this work and how it differs from and add existing works.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests.