Reviewer's report

Title: Cyproheptadine, an Antihistaminic Drug, Inhibits Proliferation of Hepatocellular Carcinoma Cells by Blocking Cell Cycle Progression through the Activation of P38 MAP Kinase

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Reviewer: Xinliang Mao

Reviewer's report:

Feng et al. tried to demonstrate that cyproheptadine inhibits proliferation of hepatocellular carcinoma cells by blocking cell cycle progression through the activation of P38 MAP kinase. It found that cyproheptadine arrested Huh-7 at the G1 phase while arrested another cell line in the S phase, which indicated that cyproheptadine induces hepatocellular cell apoptosis might be in a different manner depending on cell types. Also, in the results section, many results were overstated (see below for detail).

1. Figure 2. Cyproheptadine decreases the proliferation of both Huh-7 and HepG2 cell lines, however, it decreased the cleaved PARP at high concentrations which is different from the general understanding on cell apoptosis, why? The authors claimed that cyproheptadine induces hepatocarcinoma cell apoptosis, did the author found it induced the annexin-staining cells? How about its effects on caspase-3?

2. The time-course dependence was not impressive in almost all the examined signals, including PARP, p21, p27, p16, p-pRb, cyclin D1. Previous investigations suggested that cyproheptadine might act effectively within 48-72 hrs, the molecular action of cyproheptadine will be better depicted in a concentration manner at the time point such as 36, 48 or 72 hrs (Blood, 2008; Eur J Hematol, 2009; 2013).

3. In many cases, the signals were induced after cyproheptadine treatment for 18 hrs but decreased in the extended treatment, such as p16, pRB, cyclin D1 in HuH-7. How were these samples treated? Because the expression pattern of cell signals such as cell cycle-associated proteins might change according to the incubation time. It will be better to collect cells for each time point (including no treatment and treatment).

4. The authors claimed they previously observed that the combined treatment of thalidomide and cyproheptadine achieved complete remission, the reviewer personally suggested the authors should focus on the mechanisms behind this combined treatment. Did cyproheptadine enhance the cytotoxicity of thalidomide or vice verse, how about their action on the P38 signaling pathway and downstream cell cycle regulation?

Minor issues:
1. The results in The Background section should be shortened.
2. uM should be microM in both main text and in the figures, should be corrected.
3. Line 153, “control untreated cells”, control should be deleted.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I have No competing interests.