Reviewer's report

**Title:** High-saturate-fat diet delays development of diethylnitrosamine-induced hepatocellular carcinoma

**Version:** 3  
**Date:** 31 May 2014  
**Reviewer:** Steven Dooley

**Reviewer's report:**

**Major Compulsory Revisions**

The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

Authors showed that high fat diet can delay HCC development in a rat DEN model. The finding is very interesting and important for HCC research. The current finding is contrary to the finding from Hill-Baskin AE et al, Hum Mol Genet. 2009. Park EJ et al Cell. 2010, Blanche C. Ip et al. The Journal of Nutrition. 2014. However, different models were used. In this paper, authors used a rat DEN HCC model as contrasting with mice DEN HCC models in the other above mentioned papers. Further, a different genetic background might cause different responses to high fat diet. But, still several major concerns are relevant. These papers should be carefully discussed in the context of the authors results.

1. In the current paper, the authors showed that high fat diet can delay HCC. However, the low fat diet group should also be included, to see whether low fat diet could promote HCC. This will be a valuable information to understand how fat diet can affect HCC development in rats.

2. Authors showed reduced proliferation and increased apoptosis in high fat diet DEN mice at 10 and 12 weeks. Here, more molecular details should be addressed to find out the mechanism behind the observation. In figure 3, at week 14, apoptosis rates in high fat diet DEN mice is reduced instead of decreased. This should be clarified.

3. In figure 1, pictures of the whole liver should be provided to show the difference.

4. In figure 2a, data from all individual mice should be shown as immunoblot for PCNA.

5. In figure 2a and 3, immunohistochemistry staining of PCNA and Caspase 3 should be shown in order to find out the cell type and expression area in the animals. Proliferation markers such as ki67 or Tunel staining should also be provided.

6. In tables 2 and 3, representative photos of fibrosis and differentiation should be included. Furthermore, inflammation should also be analyzed. Park EJ et al Cell. 2010 showed that enhanced production of IL-6 and TNF is import for high
fat diet induced HCC in mice. Here, the authors should find out whether the situation is different in rats and include mRNA/ELISA data for IL6 and TNFa.

7. How many animals were used per time point in each of the analyses? This should always be stated in the figures.

Minor Essential Revisions

The author can be trusted to make these. For example, missing labels on figures, the wrong use of a term, spelling mistakes.

1. In figure 2a, molecular weight of PCNA and actin should be mentioned on the picture.
2. In figure 2b, 2c and 3, dot graph would be better than column to show individual animals.
3. Reference 19 and 33 is exactly the same.


Discretionary Revisions

These are recommendations for improvement which the author can choose to ignore. For example clarifications, data that would be useful but not essential.

Level of interest: An article of importance in its field

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

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

Declaration of competing interests:

I declare that I have no competing interests