Reviewer’s report

Title: Gene expression, regulation of DEN and HBx induced HCC mice models and comparisons of tumor, para-tumor and normal tissues

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Reviewer: Vikash Verma

Reviewer's report:

The molecular mechanism of Hepatocellular carcinoma (HCC) induced by chemicals and viruses is not clearly understood. Two mouse models, DEN and HBx, have been generated to gain a better understanding of the HCC. Tanq Q et. al., performed and analyzed the RNA-seq data for DEN mouse model, while for the HBx mouse model microarray expression data was downloaded from the GEO database and analyzed. Authors conclude that DEN and HBx mice models have different gene expression profiles but they share the same pathways. The main problem with the manuscript is the comparison of gene expression data from two different platforms. I encourage authors to address the following points before it is suitable for publication.

Major Issues:

1. Comparison of microarray gene expression data with RNA-seq data is not ideal, it can often give rise to misleading information. Especially, when the aim is to precisely map the changes in gene expression between DEN and HBx mouse models. Several studies done on the comparison of gene expression profiles between two platforms (Microarray and RNA-seq) suggest that overall dynamic range is always higher in the case of RNA-Seq, and RNA-seq is much better at capturing any changes in the gene expression level, so I would recommend authors to use a similar platform either microarray or RNA-seq for their data analysis. How difficult it is to obtain the RNA-seq data for the HBx mouse model? Also, it is not clear from the text how many biological replicates were used to obtain the RNA-seq data.

2. With bioinformatics data in hand, I would recommend authors to do some qPCR and quantitative western blotting experiments to verify their claim.

3. While the data for the DEN mouse model was obtained in-house, it is not clear from the text whether the microarray data used for this analysis were obtained under the similar conditions. A lot of observed changes in different cancer samples can just result from different cell types and different age groups. A clarification on this part would certainly strengthen the claim authors are trying to make.
4. Authors conclude that "several miRNAs such as miR-381-3p, miR-142a-3p, miR-214-3p and TFs such as Egr1, Atf3 and Klf4 are the core regulators in HCC". Many of these regulators are already known in the field, authors should explain what is the novel part in their analysis in relation to the published literature, and how that can be exploited to develop new therapies.

5. Authors write "Chemical induced HCC is somewhat different: it leads to high levels of DNA damage and cause a failing to block the cell cycle before the damaged DNA repaired." However, it is surprising that authors didn't find any checkpoint / cell cycle regulatory proteins expressing at higher level in the DEN mouse model. However, these genes were highly expressed in the HBx mouse model which is exactly opposite of what I would expect. Am I missing something here?

6. Authors further quantified the microRNA expression level in DEN mouse model which is again surprising. Authors write- "Most of the miRNAs showed the highest expression level in para-tumor tissue and the lowest level in normal tissue;" I would have expected if not the highest then at least a similar expression of miRNAs in tumor cells. Authors should at least provide some justification for this observation.

Minor issues:

The whole manuscript needs considerable editing for grammatical errors, and the discussion section requires some meaningful explanation of the results presented in the manuscript.

Authors did a good job by mentioning the genetic background of the mice. However, it is not clear whether the RNA-seq data was just obtained from one experiment or authors acquired the data from at least three different experiments?

At what stage, the histopathological samples were collected? What kind of parameters were considered while doing the microscopic examination?

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

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

No
Are the conclusions drawn adequately supported by the data shown?
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No

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