Reviewer’s report

Title: Elevated levels of circulating ITIH4 are associated with hepatocellular carcinoma with nonalcoholic fatty liver disease: From pig model to human study

Version: 0 Date: 20 Apr 2019

Reviewer: Chuanrui Xu

Reviewer’s report:

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NAFLD is a widely spread disease in western countries and is rapidly growing in developing countries. Since NAFLD can evolve to HCC and there is no any evident symptoms at its early stage, effective diagnosis will be the key for early therapeutic intervention. In this study, Nakamura et al established a novel pig model to mimic human HCC with NAFLD background. Then they examined the serum marker and found that ITIH4, an protein secreted by hepatocytes, was elevated significantly in the swine serum. In concert, ITIH4 was also increased in HCC patients with NAFLD but not in the patients with hepatosteatosis, NASH or hepatic virus infections. These results indicate that ITIH4 holds the potential to be used as a early diagnosis biomarker for those population with NAFLD. This paper provides a new biomarker for diagnosis of HCC based on the data from a pig HCC model, which is convenient for multiple collections of serum. Both the finding and animal model will be helpful for the researchers or clinicians in this field. However, some concerns about the data or writing needs to be clarified before it is accepted for publication.

Major concerns:

1. The authors used many times in the figure legends and results section, but they failed to define what the control group refers to. Does that refer to the pigs injected with DEN but not fed with HFD? The authors should clarify it.

2. For the serum collection and MS-SPEC analysis, how much volume of blood was collected and how much serum/protein was used for HPLC-MS analysis? The details for this experiment will be critical for other group to repeat or to use it as reference.

3. The data in Figure 3 showed that ITIH4 increased rapidly from 36-60 weeks and the HCCs were formed in all the pigs they used at 60 weeks. This reviewer is wondering when actually those HCC started to originate during 36-60 weeks assuming ITIH4 is
reliable marker for HCC formation? If ITIH3 starts to rise around the HCC formation, then it will be an excellent marker for early diagnosis of HCC.

Minor questions

Figure 1. The authors may use multiple columns instead of single tandem column to quantify the pathological scores, since the former will easier for readers to interpret and compare.

Figure 3C. The replicate numbers and error bars should be shown or indicated in the graph or figure legend..

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.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

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