Response to Reviewers

Reviewer 00070422

Classification: good, Language evaluation: minor polishing, Conclusion: major revision

The authors have polished their language and made the required revisions in their manuscript.

HSPG was isolated from human liver. Preliminary results showed that it was detected by rabbit anti-glypican. The 1E4-1D9 was raised against human liver HSPG and specific antigen was characterized. Amino acid sequence analysis revealed that the antigen recognized by mAb 1E4-1D9 specific molecule contained no transmembrane region. It has 15 cysteines and 11 putative- and 6 predicted N-glycosylation sites. The sequence matched to all PDZ domain protein with 85.6% match to glypican-3. The studies of co-expression and coprecipitation demonstrated that mAb 1E4-1D9 could compete with antiglypican-3. The findings suggest that the antigen recognized by 1E4-1D9 is glypican-3. Moreover, findings revealed that FYCO1 co-precipitated with glypican-3 using mAb 1E4-1D9, suggesting that FYCO1 is a partner molecule of glypican-3.

The authors thank the reviewer for critically reading and evaluating our manuscript.

Although Author's preliminary results of mAb 1E4-1D9 showed that it could react with human HepG2 cells. 1. The question is why mAb 1E4-1D9 only react with glypican-3 in HepG2 cells or all HCC cells?

We concluded that 1E4-1D9 is specific to GPC-3 expressed in cell lines. We now make this point clearer in our revised manuscript.

2. How about other HCC cells that could produce GPC-3?

We have no other HCC cell lines in our lab except for Huh7 described in the text so that we are unable to broaden this conclusion.

Reviewer 02992983

Classification: excellent, Language evaluation: priority publishing, Conclusion: accept

The other reviewers recommended that we polish our language and we have done so in our revised manuscript.

The mAb will be a good tool to study the function of Glypican-3, I hope it works in the future study.

The authors thank the reviewer for critically reading and evaluating our manuscript.

Reviewer 03074879

Classification: very good, Language evaluation: minor polishing, Conclusion: accept

The authors have polished their language and made the required revisions in their manuscript.

Early detection of HCC is very important to study, the Glypican-3 is a good point to research, so topic of paper is novel and design of experiment is precise You can can express the research method and train of thought in content at length, but it is a little tedious.

The text has been improved to make it less tedious to the reader.

I think you can make a diagram to describe the methods, which can include most steps of experiment. So the reader can easy to understand your content with a diagram.

The methods described in these experiments vary slightly from one to another and are not amenable

to a single figure or diagram. We have carefully revised and rewritten the experimental section to ensure that these are understandable and easily repeatable.

In discussion, you just say the advantage of the Glypican-3 compared with the family of HSPG.

The major advantage to targeting glypican-3 is its presence at elevated levels in HCC. This is stated in our revised manuscript.

You should tell the method of diagnosis of the HCC except the HSPG's family, then compare with the Glypican-3, which can better highlight the advantage of the Glypican-3.

The development of a diagnostic approach using mAb 1E4-1D9 will be straightforward after these findings have been validated. We express this in our revised manuscript.