Dear editors and reviewers,

Thank you for giving us a chance to revise our manuscript. We really appreciate the reviewers for their comments and advices, which is useful to improve the quality of our manuscript.

We have made a great revision for the manuscript. First of all, we added some contents about immunohistochemical analysis of GLUT1 for the HCC tissue samples and in vitro cellular assay of glucose metabolism in GPC3-expressing HepG2 and non–GPC3-expressing RH7777 cells. Secondly, we have made a great revision in the Discussion section to emphasize our findings. All the revisions were highlighted with red text in the revised version.

We will answer the comments of reviewers one by one as following:

For reviewer #1 (reviewer's code:01557574)

This article title with 'RETROSPECTIVE STUDY. PET/CT Finding: Low Glucose Metabolism in the Hepatocellular Carcinoma with GPC3 Expression'' it should be published at WJG. It has new informations and it makes a new contribution for understanding of hepatocellular carcinoma

Answer: Thank you very much for your appreciation. We are greatly encouraged by your affirmation for our manuscript.

For reviewer #2 (reviewer's code: 03538415)

What is not clear here is that the authors use correlative analyses to demonstrate that low glucose metabolism is associated with GPC3 expression. But the conclusions they made do not make sense to me. Cancers including HCC have high consumption of glucose. and therefore this should be positively correlated with GPC3 expression. In the text, the authors concluded and I report here: "Our research work confirmed that low glucose metabolism occurred in HCC tumors with GPC3 positivity on the patient study, suggesting that GPC3 may play a role in regulating the glucose metabolism in

HCC ".Is it a positive correlation or a negative one with glucose metabolism. The authors should pick a side, as this is not evident reading the entire manuscript.

Answer: Thank you very much for pointing out the shortcomings in our manuscript and gave us helpful instructions for the revision. In the revised manuscript, we added some contents to clarify our views, such as "There were an inverse relationship between GPC3 expression and SUV_{max} (Spearman correlation coefficient=-0.281; *P*=0.038)" in the Results section and "Present study demonstrated that GPC3 expression is inversely associated with glucose metabolism" in the Conclusions section.

In the previous study, we found ^{11}C-choline, as a probe of lipid metabolism, could be highly taken up by well and moderately differentiated HCC. So, we deduce that GPC3 may have a potential to promote the lipid metabolism in HCC, which may conversely reduce the glucose metabolism. In the future, we want to do more further research to confirm this hypothesis.

For reviewer #3 (reviewer's code: 03538415)

The manuscript is well written and figures are well prepared. Despite the retrospective nature, methodology of image interpretation and statistical analyses are solid. The paper fits well into the pages of World J Gastroenterology, and can be published following minor revision according to the below comments: M&M, Image Interpretation Please move the following sentence to discussion section: "T/NT ratio was reported to be more accurate to define 18F-FDG uptake in HCC because it was not influenced by serum glucose level, the uptake period and measurement variation, which often make the measurement of SUVmax inaccurate [25]." M&M, Statistical analysis: please correct "t testimg" and state type of t-test (paired/unpaired) Discussion: Please compare SUVmax and T/NT ratios of primary tumors with the literature. This may help to document that you have limited selection bias in your retrospective cohort

Answer: Thank you very much for your appreciation. In the revised manuscript, we have make some revisions according to the instruction of the reviewer. In the M&M, Statistical analysis section, we corrected "t testimg" to be “unpaired t-test”. We also move the following sentence in M&M, Image Interpretation section “"T/NT ratio was reported to be more accurate to define 18F-FDG uptake in HCC because it was not influenced by serum glucose level, the uptake period and measurement variation, which often make the measurement of SUVmax inaccurate [25]" into Discussion section. In addition, in the Discussion section, we added some contents to compare our findings with the literatures, but did not list the detailed values of SUVmax and T/NT ratios of primary tumors, as following: “In the present study, we found that SUVmax was actually lower in well- or moderately differentiated HCC than in poorly differentiated HCC, which consolidated the above views” and “Our study confirmed the above findings that low GLUT1 expressing tumors actually had a significantly low ^{18}F-FDG uptake than that of high GLUT1 expressing tumors (P<0.001) ”.

For reviewer #3 (reviewer’s code: 00070577)

Li et al reported that HCC with GPC3 expression may play a role in regulating the glucose metabolism in HCC. Its concept is very intriguing, however I felt that the information is insufficient to believe the conclusions.1) The mechanism between GPC and glucose metabolism is unclear. To show this in vitro analysis using GPC+ and – cell lines may be necessary2) The authors should show the results of the immunostaining of GLUT 1 and 2 etc.3) The showed the positivity of GPC3 as scores from 0-9. The authors do not analyze depending on the score.

Answer: Thank you very much for your comment and advice. According to your instruction, we have added some new experiment findings in the revised manuscript. Firstly, we had finished some cellular assays to evaluate the effect of GPC3 expression on the glucose metabolism. GPC3-expressing

HepG2 cells and non–GPC3-expressing RH7777 cells were incubated with ^{18}F-FDG for 60 min and the cellular uptake was measured. We found that HepG2 cells really had a significantly lower ^{18}F-FDG uptake than that of RH7777 cells (0.37±0.05 % vs. 1.03±0.04 % of inputted radioactivity, t=-20.352, P<0.001), which was consistent with the findings of the patients study. Secondly, we measured the immunostaining of GLUT 1 for HCC tumors and found that ^{18}F-FDG uptake in the high GLUT1 expressing tumors was signifcantly higher than that in the low GLUT1 expressing tumors (SUVmax : 13.58±3.44 vs. 5.57±3.49, t=6.898, P=0.000; T/NT ratio: 6.38±1.91 vs. 2.46±1.55, t=6.307, P<0.001), which was consistent with the findings of the published literatures. However, when we investigated the relationship between GPC3 and GLUT1 expression, although an inverse trend of relationship was observed between GPC3 and GLUT1 expression, their association did not reach a statistical significance (Spearman correlation coeffcient =-0.232, P= 0.088). Therefore, we think we have no enough evidence to say that GPC 3 inversely regulates the glucose via GLUT1. In the previous study, we found ^{11}C-choline, as a probe of lipid metabolism, could be highly taken up by well and moderately differentiated HCC. So, we deduce that GPC3 may have a potential to promote the lipid metabolism in HCC, which may conversely reduce the glucose metabolism. Further basic researches are warranted to uncover the mechanism. We did no perform the immunostaining for GLUT2 due to short of the anti- GLUT2 antibody. Thirdly, we did not do the analysis depending on the score of GPC3. The positivity of GPC3 as scores ranged from 0-9, if we do the analysis depending on the score, we needed a much larger of patient sample to ensure enough more cases in each group to meet requirement of statistical analysis.

Thank you for your useful comments and kind advice again.

Sincerely yours,

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