RESPONSE LETTER

Dear Editors and Reviewers:

On behalf of my co-authors, we thank you very much for your positive and constructive suggestions on our manuscript entitled “STAT3 promotes the Warburg effect might by inducing pyruvate kinase M2 phosphorylation in the malignant transformation of hepatic progenitor cells”. (Manuscript NO: 45933).

We have studied reviewer’s comments carefully and have made correction which we hope meet with approval. A copy of the revised manuscript with the revisions highlighted in red text has been uploaded to the submission system. Also, please find our replies below to the comments made by the editors and reviewers.

Responds to the reviewer’s comments:

[The comments of Reviewer #02861035]

Comment 1

Although it is unclear how appropriate this model in modelling human

cancer formation and hepatic lesion, but the authors showed hepatocyte GST-π expression in some hepatocytes in their model.

Response:

Thank you for your helpful comments.

Primary liver cancer comprises hepatocellular carcinoma (HCC), intrahepatic cholangiocarcinoma (iCCA), and other rare tumors. A better understanding of the cell types originating liver cancer can aid in exploring molecular mechanisms of carcinogenesis and therapeutic options. The carcinoma might originate from mature liver cells or hepatic progenitor cells (HPC). Molecular studies have identified adult hepatocytes as the cell of origin. These cells have been proposed to transform directly into HCC cells. Alternatively, progenitor cells also give rise to HCCs and iCCAs with markers of progenitor cells[1]. Hepatic progenitor cells might generate primary liver tumors because, during liver development, hepatocytes and cholangiocytes each arise from a common progenitor (hepatoblasts). Studies have noted a progenitor cell phenotype in many HCCs[2]. Results from the detection of surface markers demonstrated that the newly developed tumors were derived from differentiated HPCs, suggesting that HPC may be involved in the occurrence of HCC[3]. Immunophenotyping analysis of liver tissue in patients with HCC has further indicated that 28-50% of HCC cells

express HPC surface markers, such as CK7 and CK19[4]. And liver cancers with stem cell features have more aggressive clinical behavior and a worse prognosis than those without stem cell features. Therefore, it is of great significance to investigate the role and mechanism of HPC in the formation of hepatocellular carcinoma.

The Solt-Farber model is an established hepatic precancerous animal model. Chemical carcinogen diethylnitrosamine (DEN) is the initiator of carcinogenesis; The presence of 2-acetylaminofluorene (2-AAF) after 70% partial hepatectomy (PH) renders mature hepatocytes unable to proliferate, resulting in oval cells playing an important role in liver regeneration. Rat hepatic oval cells, as the representative of hepatic stem cells, have been widely used in the study of the liver stem cell differentiation “blocked maturation” theory mechanism of hepatocellular carcinoma[5-7].

GST-π, an embryonic enzyme of the liver, is a glutathione S-transferase (GST) that detoxifies electrophiles through conjugation to thiol-reduced glutathione (GSH) and is overexpressed in the early stages of carcinogenesis, including hepatic precancerous lesions. It is a neoplastic marker in the early stages of carcinogenesis in HCC[8]. Our results showed that GST-π clearly labeled the heterocellular clusters in the model group. This indicates that the model of liver precancerous lesions has been successfully replicated.

Comment 2

The authors also showed p-STAT3 are expressed in the liver but the staining is unclear about the identity of cells that express p-STAT3, as it seems that OV-6 cells do not express p-STAT3 in their staining, but instead the cells adjacent to it. Double immunofluorescent staining should be used to confirm whether these cells are hepatocytes or other cells types.

Response:

Thank you so much for your comments.

OV-6 stains the cytoskeleton of bile duct cells, oval cells, and HCC but not that of hepatocytes[9] (green fluorescence). The stronger green fluorescence in Figure 1D may be labeled as bile duct cells. p-STAT3 is widely expressed in liver tissue (red fluorescence). Notably, OV6 and p-STAT3 were co-expressed in the same heterocellular clusters (yellow-green fluorescence). These results suggest that the activation of STAT3 is closely related to the activation of hepatic progenitor cells in the progression of liver precancerous lesions in rats.

Comment 3

Furthermore, level of STAT3 and p-STAT3 by Western blot should be performed to investigate the level of STAT3/p-STAT3 in their model.

Response: Thank you for the helpful comments. We have supplemented this result in Figure 1E.

Comment 4

The authors also showed the expression of PKM2 by hepatic progenitor cells in the model, but this is unclear whether this only specific to this model or to all models with hepatic progenitor cell activation.

Response: We appreciate your comments. The pyruvate kinase M2 (PKM2) isoform, which is commonly upregulated in many human cancers, has been recently shown to play a crucial role in metabolism reprogramming, gene transcription and cell cycle progression. Our results indicate that PKM2 is expressed in activated hepatic progenitor cells in Solt-Farber rat model of liver precancerous lesions. And a previous study observed remarkable higher expression levels of PKM2 in cirrhotic and HCC livers compared to normal livers[10]. Liver cirrhosis and non-alcoholic fatty liver disease (NAFLD) also accompanied with hepatic progenitor cell activation. However, it is not clear that whether PKM2 is expressed in all models with hepatic progenitor cell activation.

Comment 5

PKM expression and its level should be investigated also in model such as AAF-PH, like shown in Fig 1.

Response: Thank you for the helpful comments. We have provided the expression level of PKM2 in AAF+PH group in Figure 2C-E.

Special thanks to you for your good comments.

[The comments of Reviewer #02936520]

Comment 1

The manuscript needs minor copy editing.

Response: Thank you so much for your positive comments. We have corrected manuscript by native speakers of English.

Comment 2

The title needs to be modified to demonstrate the contents.

Response: Thank you so much for your comments. We have changed the title to: STAT3 promotes the Warburg effect might by inducing pyruvate kinase M2 phosphorylation in liver precancerous lesions.

Comment 3

The results are presented adequately. Although the histology photos need to be larger.

Response: We appreciate your suggestions and comments. We have revised the histological picture in Figure 1A, 1B and Figure 2C in the newly submitted manuscript.

Comment 4

The discussion needs to address the extrapolation of these results to human cases where stem cell proliferation is not a prominent feature. In those cases where hepatocarcinogenesis is the result of dedifferentiation of heptocytes.

Response:

Thank you for your helpful comments.

Primary liver cancer comprises hepatocellular carcinoma (HCC),

intrahepatic cholangiocarcinoma (iCCA), and other rare tumors. A better understanding of the cell types originating liver cancer can aid in exploring molecular mechanisms of carcinogenesis and therapeutic options. The carcinoma might originate from mature liver cells or hepatic progenitor cells (HPC). Molecular studies have identified adult hepatocytes as the cell of origin. These cells have been proposed to transform directly into HCC cells. Alternatively, progenitor cells also give rise to HCCs and iCCAs with markers of progenitor cells[1]. Hepatic progenitor cells might generate primary liver tumors because, during liver development, hepatocytes and cholangiocytes each arise from a common progenitor (hepatoblasts). Studies have noted a progenitor cell phenotype in many HCCs[2]. Results from the detection of surface markers demonstrated that the newly developed tumors were derived from differentiated HPCs, suggesting that HPC may be involved in the occurrence of HCC[3]. Immunophenotyping analysis of liver tissue in patients with HCC has further indicated that 28-50% of HCC cells express HPC surface markers, such as CK7 and CK19[4]. And liver cancers with stem cell features have more aggressive clinical behavior and a worse prognosis than those without stem cell features. Therefore, it is of great significance to investigate the role and mechanism of HPC in the formation of hepatocellular carcinoma.

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model, which has been widely used in the study of the "blocked maturation" theory mechanism of hepatocellular carcinoma.

We have added some explanations in the discussion section (at the beginning of the second paragraph of the discussion section).

Comment 5

The idea has been addressed previously.

The methodology is appropriate.

The use of an inhibitor of STAT3 and measuring its consequences is strong supportive evidence for the positive role of STAT3 in the pathogenesis of the Warburg effect in experimental HCCs.

Response:

Thank you for your helpful and positive comments. We have studied your comments carefully and have made correction which we hope meet with approval.

Once again, thank you very much for your comments and suggestions.

Responds to the editors' comments:

Response:

We read the editors' comments and suggestions carefully and completely. Corresponding modifications were made according to the required. We have supplemented the manuscript, responded to the comments of peer reviewers, produced audio core tips and uploaded documents according to the requirements of the journal. We have checked that there are no duplicate references. Thank you very much for your comments and suggestions.

References:

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We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you. Please feel free to contact us if you need any additional information.

Thank you and best regards.

Yours sincerely,

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