Correspondence on Editorial regarding “UBE2S promotes glycolysis in hepatocellular carcinoma by enhancing E3 enzyme-independent polyubiquitination of VHL”

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Abbreviations:
HCC, hepatocellular carcinoma; HIF-1α, hypoxia inducible factor-1α; PDX, patient-derived xenograft; UBE2S, ubiquitin conjugating enzyme E2 S; VHL, von Hippel-Lindau tumor suppressor
Dear Editor,

We would like to thank Martina Mang Leng LEI and Terence Kin Wah LEE\(^1\) for their interest in our previous study.\(^2\) The editorial provides a refined summary of our work in the construction of a ubiquitin-related gene risk prediction model for hepatocellular carcinoma (HCC) and elucidation of the mechanisms by which ubiquitin conjugating enzyme E2 S (UBE2S) promotes hypoxia inducible factor-1α (HIF-1α)-dependent glycolysis in HCC by enhancing E3 enzyme-independent polyubiquitination of von Hippel-Lindau tumor suppressor (VHL). In addition, the editorial also discusses several limitations in our study. We would like to focus our response on these items raised by the authors.

In our previous study,\(^2\) we found that UBE2S catalyzes K11-linkage ubiquitination of VHL and promotes the degradation of VHL by the proteasome, thereby indirectly stabilizing HIF-1α protein levels. Accumulated HIF-1α positively regulates glycolysis of HCC cells by up-regulating the expression of downstream glycolytic-related genes such as glucose transporter type 1 (GLUT1) and hexokinase 2 (HK2). Considering that UBE2S also positively regulates the HIF-1α signaling via stabilizing β-catenin,\(^3\) it is highly likely that β-catenin is involved in the mechanism of UBE2S-mediated glycolysis in HCC. In order to verify the above hypothesis, we explored the effect of β-catenin knockdown on the expression of glycolytic enzymes mediated by UBE2S. Our results demonstrated that the levels of GLUT1 and HK2 were significantly increased when UBE2S was up-regulated in HCCLM3 and MHCC-97H cells, which was decreased with β-catenin knockdown (Fig. 1A-B). In particular, the down-regulation of these glycolytic enzymes mediated by β-catenin knockdown was abolished after HIF-1α overexpression (Fig. 1A-B). Taken together, our results suggest that UBE2S promotes HIF-1α pathway dependent glycolysis in HCC cells by both disrupting VHL protein stability and enhancing β-catenin protein stability.

As an important anti-cancer drug target, HIF-1α has been reported to be closely related to tumor metastasis, cell survival, angiogenesis, poor patient prognosis, as well as tumor
resistance therapy. Previous studies found that UBE2S promotes epithelial-mesenchymal transition (EMT) of pancreatic cancer cells and enhances their tumorigenicity via the VHL-HIF-1α-STAT3 pathway. These results not only reinforce our data in the important role of UBE2S-mediated activation of HIF-1α signaling pathway in tumor progression, but also suggest that in addition to promoting glycolysis, UBE2S also regulates several other malignant biological behaviors in HCC cells through this pathway, such as EMT. Since HIF-1α activates the transcription of genes that are involved in crucial aspects of cancer biology, whether UBE2S can be regulated by HIF-1α, thus forming a positive feedback loop between UBE2S and HIF-1α, deserves further investigation.

Despite significant advances in systemic treatment options including tyrosine kinase inhibitors and immune checkpoint inhibitors over the past decade, liver cancer remains the third leading cause of cancer-related death globally. HCC is the most common type of primary liver cancer, accounting for 75-85% of total cases. In-depth exploration of the molecular regulatory network in HCC progression is of great significance for the development of novel strategies to address this global health challenge. Since our previous studies elucidated the mechanism by which UBE2S promotes HCC progression by positive regulation of HIF-1α-dependent glycolysis, we evaluated the therapeutic potential of combination strategies targeting UBE2S and HIF-1α in a xenograft models derived from human HCC cell lines. Considering the high tumor heterogeneity of HCC in pathogenesis, morphology, microenvironment, and molecular and signaling network, other mouse models that can stably maintain the characteristics of tumor cells in vivo are also worthy of being applied to evaluate the anti-HCC efficacy of the above combined strategies. Patient-derived xenograft (PDX) models are established by transplanting fresh tumor tissue from clinical patients into immunosuppressed mice. Compared with cell line-derived xenograft models, PDX models effectively maintain the molecular diversity, histologic characteristics and intratumor heterogeneity of primary tumors. These characteristics make PDX models more advantageous in cancer treatment researches, including preclinical trials of novel
drugs, validation of drug combinations, and exploration of drug resistance mechanisms. Therefore, further exploration of the therapeutic potential of combination strategies targeting UBE2S and HIF-1α using PDX models will be more helpful to evaluate the anti-tumor efficacy and screen drug-sensitive patients, thereby improving the clinical benefits of HCC patients.

**Authors’ contribution**

Manuscript drafting: Renyu Zhang, Ding Wei. Revision and supervision: Huijie Bian, Zhinan Chen. Final approval: All authors.

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**Conflicts of Interest**

The authors have no conflicts to disclose.
REFERENCES


Figure legends

Figure 1. The regulatory effects of β-catenin on UBE2S-mediated glycolytic metabolic enzymes in HCC. A-B. The protein expression of key metabolic enzymes was assessed by western blot assays in HCCLM3 cells (A) or MHCC-97H cells (B) with overexpression or knockdown of UBE2S, HIF-1α, and β-catenin. HCCLM3-Lv-Ctrl and HCCLM3-Lv-UBE2S indicate that HCCLM3 cells were stably infected with control lentiviruses or UBE2S overexpression lentiviruses, respectively. MHCC97H-CV702-Ctrl and MHCC97H-CV702-UBE2S indicate that MHCC-97H cells were transfected with control plasmid or CV702-UBE2S plasmid, respectively. β-catenin knockdown or HIF-1α overexpression was achieved by transfection of sh-β-catenin plasmid or CV702-HIF-1α plasmid in HCC cells. Antibodies against Flag-Tag (8146), HIF-1α (36169), and HK2 (2867) were purchased from Cell Signaling Technology; β-catenin (51067-2-AP), GLUT1 (66290-1-Ig), and α-tubulin (666031-1-Ig) were purchased from Proteintech.