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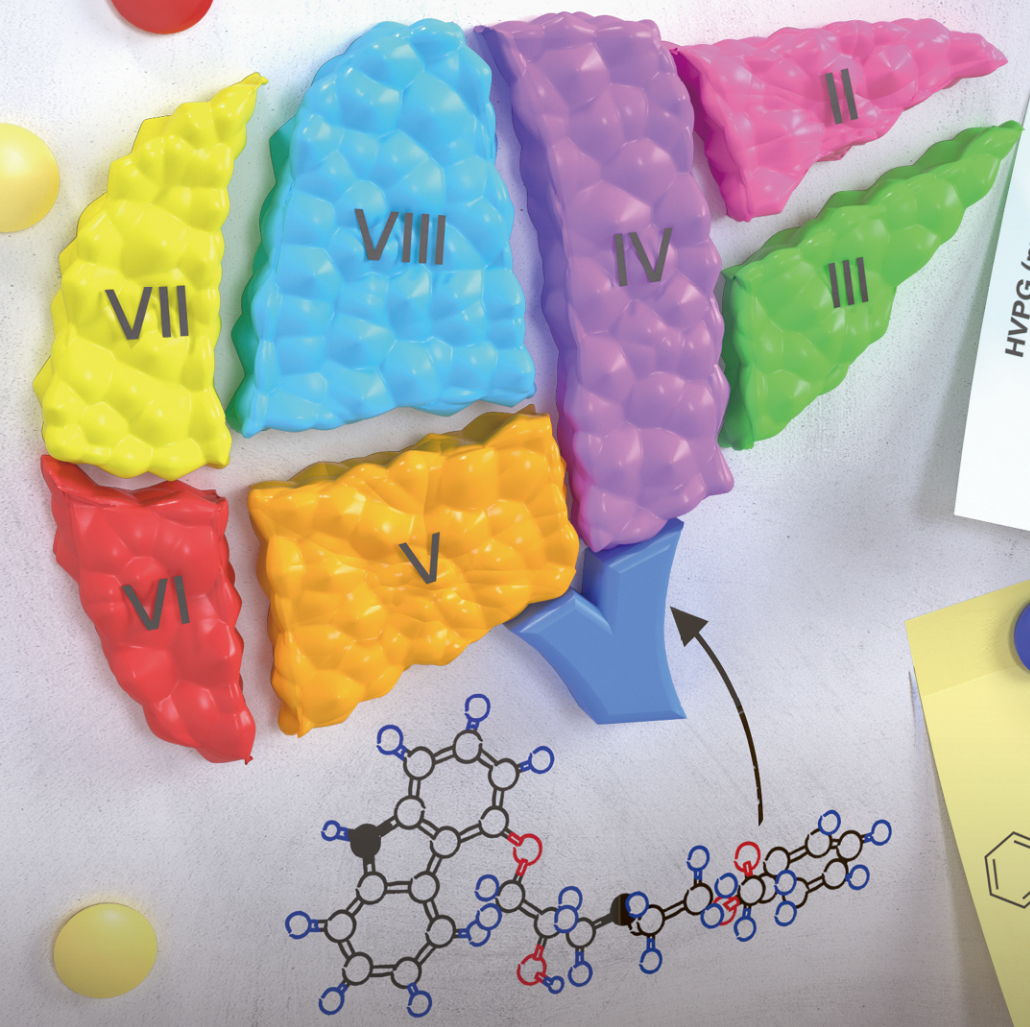

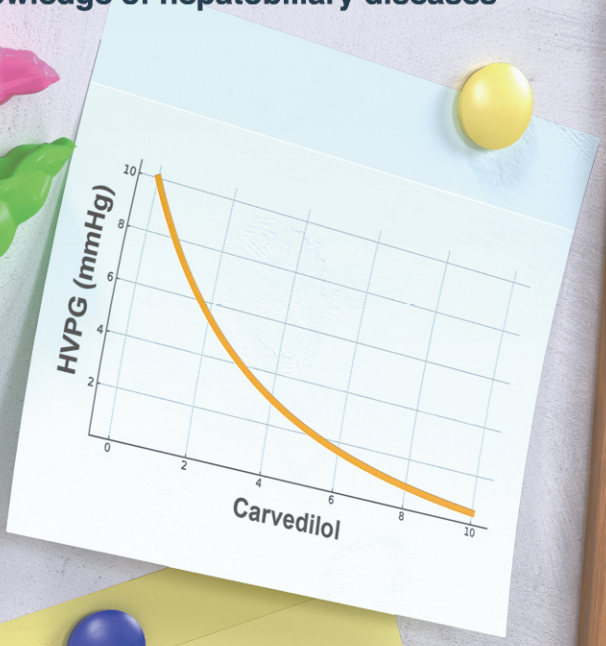

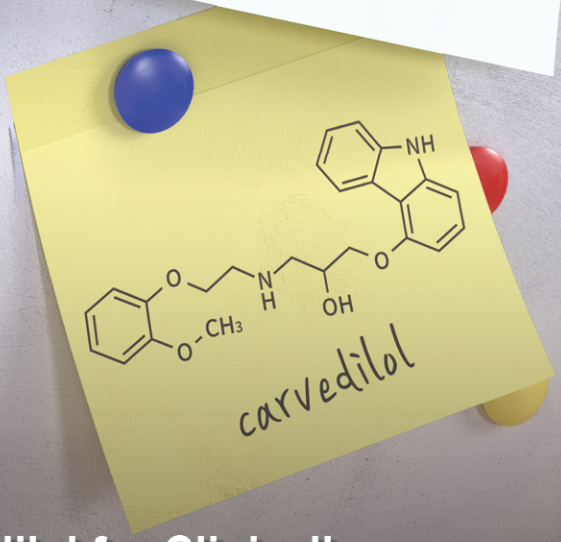

Non-invasive Model guiding Carvedilol for Clinically Significant Portal HTN

Intrapatient variability of tacrolimus on CKD in LT
HCV self-testing and disease burden reduction
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Editorial

UBE2S: A novel driver of HIF-1alpha-induced metabolic reprogramming in hepatocellular carcinoma: Editorial on "UBE2S promotes glycolysis in hepatocellular carcinoma by enhancing E3 enzyme-independent polyubiquitination of VHL"

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Ubiquitination is a series of enzymatic reactions in which ubiquitin molecules attach to substrate proteins via ubiquitin-activating enzymes (E1s), ubiquitin-conjugating enzymes (E2s), and ubiquitin ligases (E3s). This process is reversible and can be undone by deubiquitinating enzymes (DUBs). Deregulation of these enzymes leads to the post-translational modification of proteins that are vital for controlling various cellular processes, such as endoplasmic reticulum homeostasis, genomic integrity, epigenetic regulation, cell growth, cell death, autophagy, and metabolic reprogramming.[1-5] These modifications have a significant impact on the development and progression of several types of cancers, including hepatocellular carcinoma (HCC).[3-5] To date, two E1s, at least 40 E2s, over 800 E3s, and more than 100 DUBs have been identified in the human genome.[6-8] In recent years, small molecule inhibitors targeting these enzymes have been developed, and their antitumor effects have been proven in various clinical trials.[9] Notably, several of these inhibitors, such as bendamustine, thalidomide and mitoxantrone, have been Food and Drug Administration-approved for the treatment of cancers.[10] Nonetheless, the majority of current research on ubiquitination has focused on the investigation of certain enzymes, and a systematic approach for screening key enzymes that have clinical relevance is currently limited. Therefore, there is an urgent need to systemically identify key ubiquitin-related enzymes with clinical value.

HCC is one of the deadliest diseases and is the 4th leading cause of cancer mortality worldwide.[11] To prolong the survival of HCC patients, immense efforts have been made to identify molecules for diagnosis, prognosis and therapeutic intervention, with the ultimate goal of improving patient survival. In an issue of *Clinical and Molecular Hepa-*

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tology, Zhang et al.[12] reported the use of a ubiquitin-related gene risk prediction model to evaluate the prognosis of HCC patients by analyzing 1,423 ubiquitin-related genes via LASSO and multivariate Cox regression analyses. Among these genes, a four-gene signature comprising *SSR3*, *CYHR1*, *UBE2S*, and *SKP2* was established and serves as an independent prognostic factor for the prediction of overall survival in HCC patients. Further clinical analysis revealed that only CYHR1 and UBE2S are significantly overexpressed in HCC at both the mRNA and protein levels compared with adjacent normal tissues. These findings, along with the significant correlation between the expression level of *UBE2S* and the overall and disease-free survival of HCC patients, prompted a focus on UBE2S for functional characterization and pathway elucidation.

To gain insight into the molecular mechanism underlying UBE2S overexpression, Zhang et al. employed JASPAR database analysis and identified E2F2, a cell-cycle regulatory protein that is overexpressed in HCC, as the transcription factor that binds to the promoter region of UBE2S. This analysis was further supported by a luciferase promoter assay showing increased UBE2S promoter activity upon E2F2 overexpression. Further truncation analysis revealed three binding sites in the UBE2S promoter region from –500 to –250 bp, where E2F2 binds, as confirmed by chromatin immunoprecipitation and qPCR analysis. Clinically, E2F2 and UBE2S were positively correlated in HCC samples at both the mRNA and protein levels. In addition to E2F2, FOXM1, another transcription factor that regulates cell cycle progression, was previously reported to increase UBE2S expression via promoter activation in HCC.[13] Collectively, these findings highlight that E2F2 directly binds to the UBE2S promoter region and thereby stimulates the transcriptional upregulation of UBE2S expression.

Through the use of bulk RNA sequencing to characterize the genetic profiles of UBE2S knockout and control cells, several metabolic pathways, including glycerolipid metabolism, pentose and glucuronate interconversion, glycolysis/gluconeogenesis, pentose phosphate pathway, and phenylalanine, tyrosine and tryptophan biosynthesis, were found to be significantly reduced in UBE2S-knockout cells via Kyoto Encyclopedia of Genes and Genomes pathway analysis. Specifically, the levels of glucose-6-phosphate, fructose-1,6-diphosphate, dihydroxyacetone phosphate, 3-phosphoglycerate, 2-phosphoglycerate, and phosphoenolpyruvate in the glycolysis pathway were also significantly suppressed. Similarly, several parameters of glycolysis, including glucose uptake, lactate production, and the extracellular acidification rate, were suppressed upon the repression of UBE2S. Conversely, the overexpression of UBE2S resulted in increased proliferation of HCC cells, which aligns with previous studies that reported similar findings.[14,15] Interestingly, this enhanced proliferative effect was offset by treating UBE2S-overexpressing cells with 2-DG, a known glycolysis inhibitor, further confirming the novel role of UBE2S in the regulation of glycolysis in HCC. Gene set enrichment analysis of the changes in glycolytic enzymes upon UBE2S suppression revealed that these genes may be regulated by the HIF-1 signaling pathway. Furthermore, alterations in the expression of UBE2S affect the protein level of HIF-1α. The functional role of UBE2S in the regulation of HIF-1α-mediated glycolysis in HCC cells was further confirmed in UBE2S-overexpressing HCC cells via the suppression of HIF-1α via genetic and pharmacological approaches.

On the basis of the observation that there was no change in the HIF-1α mRNA level upon UBE2S alteration, the authors postulated that HIF-1α is regulated by UBE2S via posttranslational modification. Interestingly, Zhang et al. reported that UBE2S overexpression reduced the expression and protein stability of von Hippel-Lindau tumor suppressor (VHL), a classical ubiquitin ligase that mediates the ubiquitination of HIF-1α.[16] Further mutation and protein stability experiments further supported the regulatory role of UBE2S in VHL in a ubiquitin-proteasome-dependent manner. Importantly, surface plasmon resonance demonstrated that UBE2S directly binds to VHL rather than through another molecule. Screening of mutated ubiquitin forms for lysine ubiquitination revealed that only K11O (Ub with an intact Lys11 residue) could be linked to VHL by UBE2S, whereas its mutation form was not linked to VHL in HCC cells. An immunoprecipitation assay further revealed that

Abbreviations:

DUBs, deubiquitinating enzymes; DHAP, dihydroxyacetone phosphate; E1s, ubiquitin-activating enzymes; E2s, ubiquitin-conjugating enzymes; E3s, ubiquitin ligases; F-1,6-BP, fructose-1,6-diphosphate; HCC, hepatocellular carcinoma; VHL, von Hippel–Lindau tumor suppressor

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the K171R and K196R mutations abolished UBE2S-mediated ubiquitination, indicating that K171 and K196 are specifically responsible for the UBE2S-catalyzed ubiquitination of VHL. An *in vitro* ubiquitination assay demonstrated that UBE2S increases the ubiquitination of VHL and promotes its degradation without requiring an E3 enzyme, thereby increasing the protein stability of HIF-1α (Fig. 1). This finding provides a deeper understanding of the previously unanswered question of how UBE2S regulates sorafenib resistance by regulating the stability of VHL in HCC.[15]

To investigate the impact of UBE2S on the development of HCC *in vivo*, a hepatocyte-specific UBE2S knockout mouse model was created by breeding UBE2S$^{loxp/1oxp}$ mice with Alb-Cre mice. Zhang et al. reported that upon treatment with diethylnitrosamine/CCl4, there was a significant delay in the onset of HCC development in *UBE2S*$^{-/-}$ mice compared with that in their wild-type counterparts, and this delay was accompanied by a decrease in the secretion of several key enzymes involved in liver damage. Most importantly, HIF-1α and a number of glycolytic enzymes were significantly downregulated in *UBE2S*$^{-/-}$ mice. Based on these *in vivo* observations, UBE2S may act as an oncogene that drives HCC development via the regulation of HIF-1α-mediated glycolysis. With the previous successful experience in the use of cephalomannine for the inhibition of UBE2S, Zhang et al. consistently reported that cephalomannine inhibited the proliferation of HCC cells *in vitro* by effectively suppressing UBE2S. Given that HIF-1α is a downstream effector of UBE2S, the combined effect of cephalomannine and PX-478 was evaluated. They found that this combination exerts a maximal tumor suppressive effect in an HCC xenograft model, thus providing a potential novel therapeutic strategy for HCC patients.

Overall, this study provides new mechanistic insight with clinical implications. First, this study develops a risk prediction model and identifies UBE2S as a critical ubiquitination

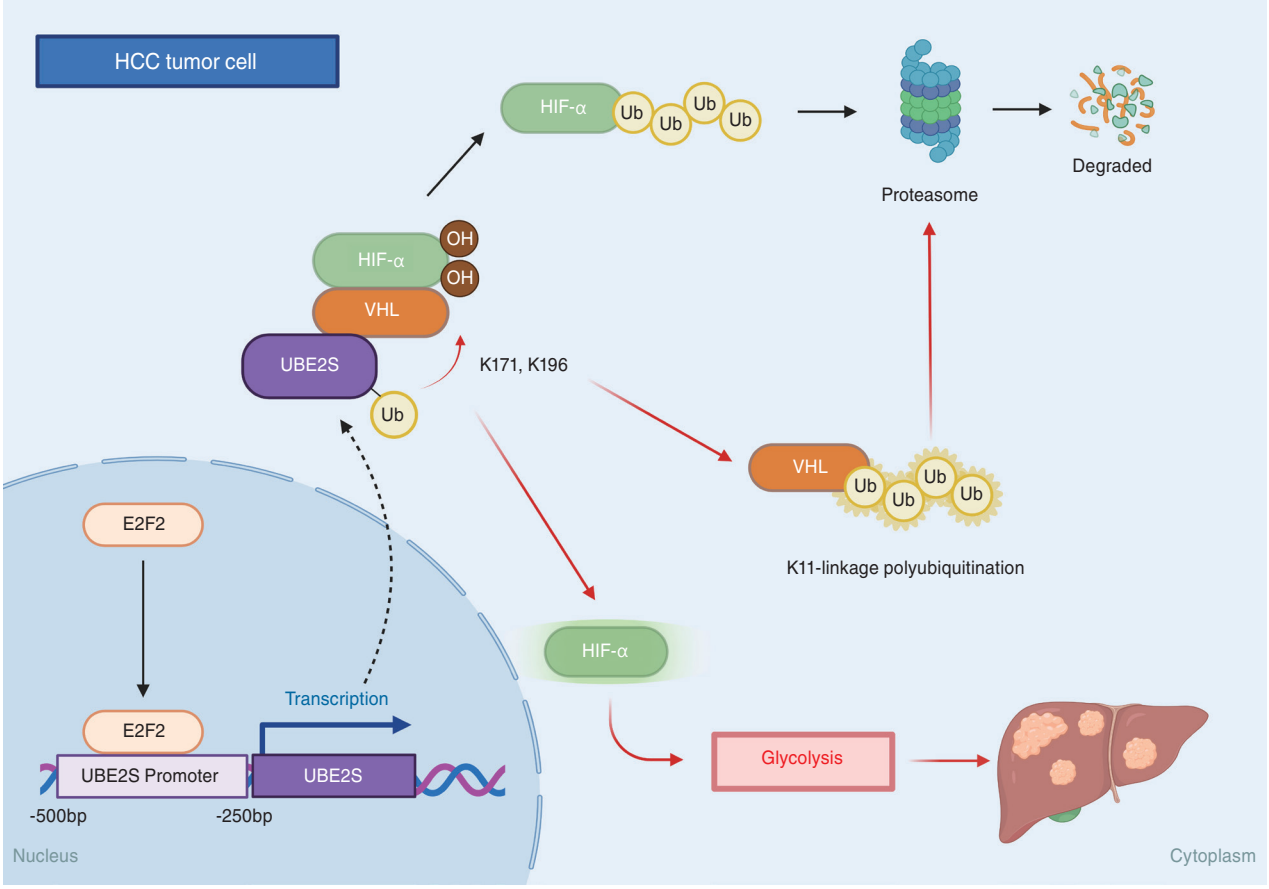

Figure 1. Schematic representation of the role of E2F2/UBE2S/VHL in regulating HIF-1α-driven glycolysis in HCC. VHL, von Hippel–Lindau tumor suppressor; HCC, hepatocellular carcinoma.

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enzyme for the prediction of the overall survival of HCC patients. Second, E2F2 was identified as a novel transcription factor upstream of UBE2S. Third, like other E2 enzymes, including UBE2T[17] and UBE2E[18] reported previously, UBE2S facilitates ubiquitin-mediated degradation in an E3 enzyme-independent manner. Finally, this study provides a novel strategy for the treatment of HCC patients by targeting UBE2S and HIF-1α. Although the current *in vitro* and *in vivo* data obtained by Zhang and colleagues are encouraging, this study has several limitations. A previous report showed that UBE2S stabilized β-catenin against myocardial ischemia/reperfusion injury by activating HIF-1alpha signaling.[19] Given the significant role of β-catenin in HCC pathogenesis and HIF-1α modulation, whether beta-catenin is independently involved in the mechanism of UBE2S-mediated promotion of glycolysis in HCC remains unclear. In addition, previous reports have suggested the presence of a positive feedback loop between UBE2S and HIF-1α, wherein the activity of UBE2S was found to be regulated by VHL/HIF-1α.[20] Finally, although it has been suggested that targeting UBE2S and HIF-1α has therapeutic potential, further investigations using a variety of mouse HCC models are warranted.

Authors' contribution

M.M.L drafted the manuscript. T.K.L reviewed and finalized the manuscript.

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Conflicts of Interest

The authors have no conflicts to disclose.

REFERENCES

1. Senft D, Qi J, Ronai ZA. Ubiquitin ligases in oncogenic transformation and cancer therapy. Nat Rev Cancer 2018;18:69-88.
2. Chen Q, Zheng W, Guan J, Liu H, Dan Y, Zhu L, et al. SOCS2-enhanced ubiquitination of SLC7A11 promotes ferroptosis and radiosensitization in hepatocellular carcinoma. Cell Death Differ 2023;30:137-151.
3. Wu H, Yang TY, Li Y, Ye WL, Liu F, He XS, et al. Tumor necrosis factor receptor-associated factor 6 promotes hepatocarcinogenesis by interacting with histone deacetylase 3 to enhance c-Myc gene expression and protein stability. Hepatology 2020;71:148-163.
4. Liu Y, Tao S, Liao L, Li Y, Li H, Li Z, et al. TRIM25 promotes the cell survival and growth of hepatocellular carcinoma through targeting Keap1-Nrf2 pathway. Nat Commun 2020;11:348.
5. Ge C, Vilfranc CL, Che L, Pandita RK, Hambarde S, Andreassen PR, et al. The BRUCE-ATR signaling axis is required for accurate DNA replication and suppression of liver cancer development. Hepatology 2019;69:2608-2622.
6. Vogel A, Meyer T, Sapisochin G, Salem R, Saborowski A. Hepatocellular carcinoma. Lancet 2022;400:1345-1362.
7. Piñero F, Dirchwolf M, Pessôa MG. Biomarkers in hepatocellular carcinoma: diagnosis, prognosis and treatment response assessment. Cells 2020;9:1370.
8. Komander D, Rape M. The ubiquitin code. Annu Rev Biochem 2012;81:203-229.
9. Dikic I, Schulman BA. An expanded lexicon for the ubiquitin code. Nat Rev Mol Cell Biol 2023;24:273-287.
10. Spano D, Catara G. targeting the ubiquitin-proteasome system and recent advances in cancer therapy. Cells 2023;13:29.
11. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2021;71:209-249.
12. Zhang R, Li C, Zhang S, Kong L, Liu Z, Guo Y, et al. UBE2S promotes glycolysis in hepatocellular carcinoma by enhancing E3 enzyme-independent polyubiquitination of VHL. Clin Mol Hepatol 2024;30:771-792.
13. Gui L, Zhang S, Xu Y, Zhang H, Zhu Y, Kong L. UBE2S promotes cell chemoresistance through PTEN-AKT signaling in hepatocellular carcinoma. Cell Death Discov 2021;7:357.
14. Zhang RY, Liu ZK, Wei D, Yong YL, Lin P, Li H, et al. UBE2S interacting with TRIM28 in the nucleus accelerates cell cycle by ubiquitination of p27 to promote hepatocellular carcinoma development. Signal Transduct Target Ther 2021;6:64.
15. Wu J, Xu X, Wu S, Shi W, Zhang G, Cao Y, et al. UBE2S promotes malignant properties via VHL/HIF-1α and VHL/JAK2/STAT3 signaling pathways and decreases sensitivity to sorafenib in hepatocellular carcinoma. Cancer Med 2023;12:18078-18097.
16. Maxwell PH, Wiesener MS, Chang GW, Clifford SC, Vaux

EC, Cockman ME, et al. The tumour suppressor protein VHL targets hypoxia-inducible factors for oxygen-dependent proteolysis. Nature 1999;399:271-275.
17. Ho NPY, Leung CON, Wong TL, Lau EYT, Lei MML, Mok EHK, et al. The interplay of UBE2T and Mule in regulating Wnt/β-catenin activation to promote hepatocellular carcinoma progression. Cell Death Dis 2021;12:148.
18. Sun L, Fang J. E3-independent constitutive monoubiquitination complements histone methyltransferase activity of SETDB1. Mol Cell 2016;62:958-966.
19. Chen X, Wang C, Yang P, Shi L, Wang H. Ube2s-stabilized β-catenin protects against myocardial ischemia/reperfusion injury by activating HIF-1α signaling. Aging (Albany NY) 2020;12:5716-5732.
20. Wang L, Liang Y, Li P, Liang Q, Sun H, Xu D, Hu W. Oncogenic activities of UBE2S mediated by VHL/HIF-1α/STAT3 signal via the ubiquitin-proteasome system in PDAC. Onco Targets Ther 2019;12:9767-9781.