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RAPID COMMUNICATION

The landscape of *UBE2S* in hepatocellular carcinoma: Prognostic significance, immuno-oncology feature and drug response

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide.[1] E2 ubiquitin conjugating enzymes (UBE2) are potential therapeutic targets in tumors arising from genomic instability and tumor microenvironment (TME).[2,3] UBE2S, an important UBE2, has demonstrated strong oncogenic activities in various malignant cancers, including HCC. However, a comprehensive study regarding its role in HCC is still absent, and its association with immunology and drug response of HCC is still unclear. In this study, we conducted a pan-cancer analysis of *UBE2S* expression and prognosis, carried out enrichment analysis of *UBE2S*-associated genes, and analyzed association between *UBE2S* expression and HCC microenvironment, stemness and drug response. Collectively, our results demonstrated that *UBE2S* expression was significantly increased in multiple types of cancer, including HCC, and harbors prognostic values for HCC. Potential function of *UBE2S* involves modulation of ubiquitin mediated proteolysis and cell cycle progression. Furthermore, in HCC, *UBE2S* expression was positively correlated with TME infiltration, microsatellite instability (MSI), DNA methylation, stemness and drug response. These findings highlighted the possible pivotal roles of *UBE2S* in HCC prognosis, precision immunotherapy and drug response.

To explore the clinical significance of *UBE2S* gene, a pan-cancer analysis of its expression and association with prognosis was conducted. As shown in Figure S1A, compared with normal samples, mRNA levels of *UBE2S* gene was significantly changed in 20 cancer types based on The Cancer Genome Atlas (TCGA) dataset; when additional normal tissues from Genotype-Tissue Expression (GTEx) dataset was included, the number of changed cancer types reached to 24 (Fig. 1A; Fig. S1B). Combined results revealed that *UBE2S* expression was statistically increased in 15 cancer types, including HCC; while it was not remarkably decreased in any cancer type. Survival analyses among these 15 cancer types demonstrated that only in HCC, higher *UBE2S* expression experienced both shorter overall survival (OS) and disease-free survival (DFS); while no statistical significance of *UBE2S* expression for predicting both survival rates of patients in other cancer types (Fig. 1B; Fig. S2). These data suggest that *UBE2S* expression harbors prognostic values for HCC.

To further understand its potential biological functions and associated signaling pathways in HCC, *UBE2S*-related genes, including 100 interacted and 100 co-expressed genes, were obtained by Search Tool for the Retrieval of Interacting Genes (STRING) and Gene Expression Profiling Interactive Analysis (GEPIA), respectively. Intersection analysis between the two sets generated four overlapping genes, including *CCNB1*, *CDN20*, *TPX2* and *UBE2C*, higher expression of which was observed in HCC tissues and correlated with poor prognosis of HCC (Fig. 1D; Fig. S3). Further Gene ontolog (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analyses of *UBE2S*-related genes indicated that *UBE2S* may be involved in ubiquitin mediated proteolysis and cell cycle progression (Fig. 1E, F).

To gain insights into its role in immune response within the TME of HCC, relationship of *UBE2S* expression with immune infiltration subtypes and TME scores was analyzed by R software. Among five immune subtypes of HCC, including C1 (wound healing), C2 (IFN-gamma dominant), C3 (inflammatory), C4 (lymphocyte depleted) and C6 (TGF-beta dominant), *UBE2S* expression varied, with C1, C2 and C4 comparatively high whereas C3 and C6 low (Fig. 1G). Furthermore, *UBE2S* expression was negatively correlated with stromal scores, while it was positively correlated with

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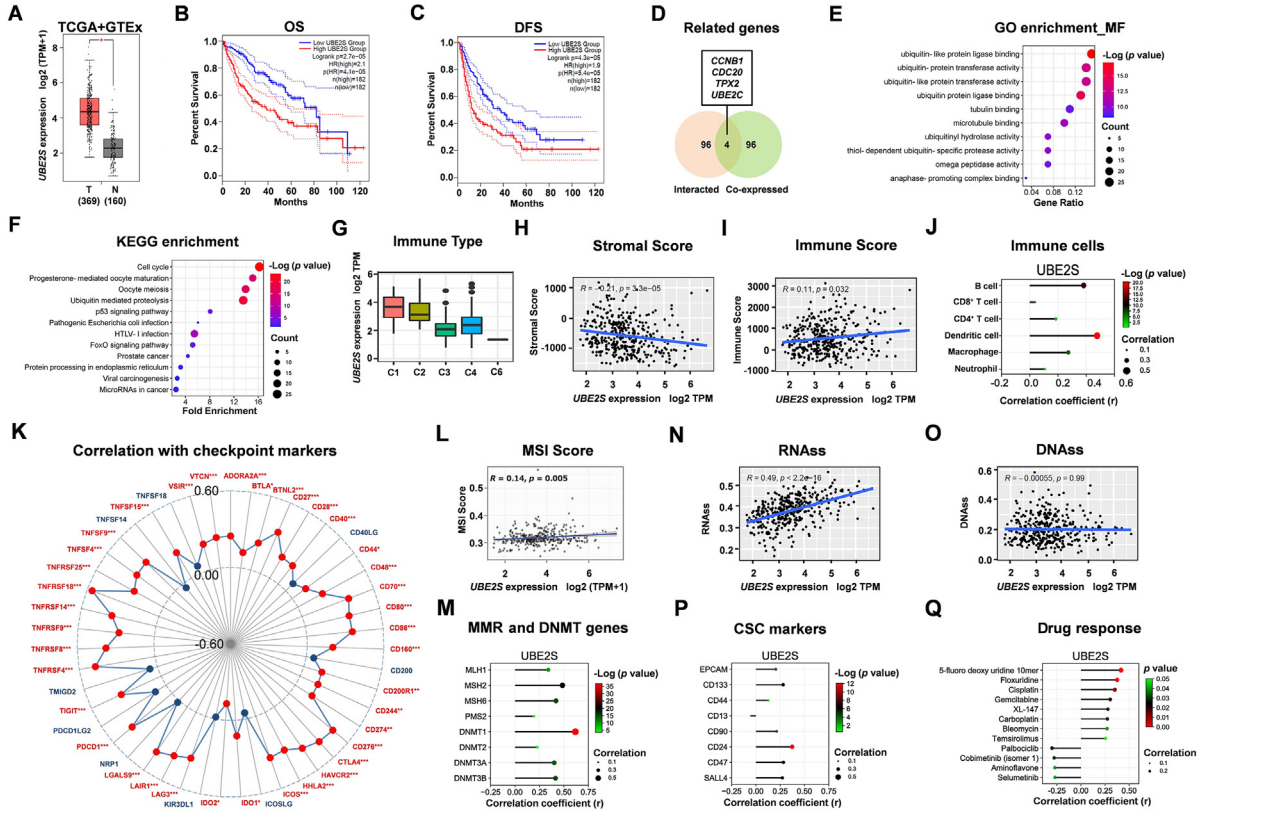

Figure 1 The prognostic significance, immuno-oncology feature and drug response of *UBE2S* expression in hepatocellular carcinoma (HCC). **(A)** mRNA levels of *UBE2S* gene in HCC and normal tissues from both The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) datasets by online tool GEPIA. T: tumor; N: normal. The corresponding number of cases of tumor or normal tissues was shown in brackets. $^{*}P < 0.05$. **(B, C)** Kaplan–Meier plots comparing overall survival (OS) and disease-free survival (DFS) of HCC patients with high and low expression of *UBE2S* by Gene Expression Profiling Interactive Analysis (GEPIA). **(D)** Top 100 proteins experimentally supported to interact with UBE2S, and top 100 genes clinically co-expressed with *UBE2S* in HCC were obtained by Search Tool for the Retrieval of Interacting Genes (STRING) and GEPIA, respectively. A Venn diagram represents the overlap of four genes interacted and co-expressed with *UBE2S*, including *CCNB1*, *CDN20*, *TPX2* and *UBE2C*. **(E)** Molecular function of Gene ontolog (GO) enrichment analysis result of *UBE2S*-related genes. Top ten enriched terms were shown. **(F)** Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis result of *UBE2S*-related genes. **(G)** Association between *UBE2S* expression and immune subtypes in HCC of TCGA database analyzed by R software. **(H, I)** Relationship of *UBE2S* expression with stromal scores and immune scores in HCC tissues of TCGA dataset analyzed by R software. **(J)** Association between *UBE2S* expression and infiltrations of immune cells in HCC of TCGA dataset analyzed by Tumor Immune Estimation Resource (TIMER). **(K)** Association between expression of *UBE2S* and 47 immune checkpoint genes in HCC of TCGA dataset analyzed by TIMER. Correlation values of $+0.60$ and -0.60 are marked. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. **(L)** Positive correlation between mRNA levels of *UBE2S* and microsatellite instability (MSI) scores in HCC of TCGA dataset obtained from Sangerbox. **(M)** Positive correlation between mRNA levels of *UBE2S* and four mismatch repair genes, including *MLH1*, *MSH2*, *MSH6* and *PMS2*, as well as four DNA methyltransferases (DNMTs), including *DNMT1*, *DNMT2*, *DNMT3A* and *DNMT3B* in HCC of TCGA dataset analyzed by TIMER. **(N, O)** Association between *UBE2S* expression and cancer stemness, including mRNA expression-based stemness index (RNAss) and DNA methylation-based stemness index (DNAss), in HCC of TCGA dataset analyzed by R software. **(P)** Association between expression of *UBE2S* and cancer stem cell (CSC) markers in HCC of TCGA dataset analyzed by TIMER. **(Q)** Significant positive or negative correlation between *UBE2S* expression and sensitivities of different drugs predicted by CellMiner database and R software.

immune scores (Fig. 1H, I). Based on positive correlation between *UBE2S* expression and immune scores, relationship of *UBE2S* expression with immune cell infiltration and expression of associated immune cell markers in HCC was further assessed by tumor immune estimation resource (TIMER). Results showed that *UBE2S* expression was significantly positively correlated with infiltration of B cells, $CD4^{+}$ T cells, dendritic cells, macrophages and neutrophils (Fig. 1J; Fig. S4). Although no significant correlation was found between *UBE2S* expression and $CD8^{+}$ T cell infiltration, *UBE2S* expression exhibited significantly positively correlation with multiple markers of the six types of immune cells, including $CD8^{+}$ T cells (Fig. S4 and Table S1). Since immune checkpoints, including PD-1 (PDCD1), PD-L1 (CD274) and CTLA-4, are responsible for tumor immune escape and are targets for the treatment of malignant tumors,[4] relationship of expression of *UBE2S* with 47 immune checkpoint genes in HCC of TCGA dataset was assessed by TIMER. Results showed that expression of 37 immune checkpoint genes was significantly positively correlated

with that of *UBE2S*, including *CTLA4* and *PD-1*; and expression of one gene, *IDO2*, was negatively correlated with *UBE2S* expression (Fig. 1K; Fig. S5). These data suggest that TME infiltration and tumor immune escape may be involved in *UBE2S*-mediated HCC progression.

Considering other tumor cell intrinsic factors, such as MSI and DNA methylation, could also impact on the effect of anti-cancer therapy, the relationship of *UBE2S* expression with MSI and associated genes as well as DNA methyltransferases (DNMTs) in HCC tissues of TCGA database was analyzed using Sangerbox and TIMER. Results revealed that *UBE2S* expression was positively correlated with MSI scores of HCC tissues and four mismatch repair genes, including *MLH1*, *MSH2*, *MSH6* and *PMS2*, as well as four DNMTs, including *DNMT1*, *DNMT2*, *DNMT3A* and *DNMT3B*, indicating that statuses of MSI and DNA methylation might partially responsible for the oncogenic function of *UBE2S* in HCC (Fig. 1L, M; Fig. S6).

Since cancer stem cells (CSCs) are a major source of drug resistance and are associated with poor therapeutic outcomes,[5] relationship of *UBE2S* expression with stemness of HCC cells and drug response was assessed using R software and TIMER. As shown in Figure 1N and O, in TCGA database, *UBE2S* expression was positively correlated with mRNA expression-based stemness index (RNAss), while it showed no significant correlation with DNA methylation-based stemness index (DNAss). Furthermore, expression of *UBE2S* exhibited significantly positively correlation with that of multiple HCC stem cell markers, including *CD24*, *CD47*, *CD133*, *SALL4*, *CD90*, *EPCAM* and *CD44* (Fig. 1P; Fig. S7). Drug response prediction by CellMiner database and R software suggested drug sensitivity of twelve drugs were positively or negatively correlated with *UBE2S* expression (Fig. 1Q; Fig. S8). These data support that *UBE2S* expression is linked to the phenotypes of cancer stem cells and drug resistance.

In the present study, we found that *UBE2S* was significantly increased in multiple types of cancer, including HCC, and it may serve as a prognosis biomarker for HCC patients. Furthermore, *UBE2S* may manipulate carcinogenesis and HCC progression by multiple strategies, including regulation of tumor microenvironment infiltration and tumor immune escape, induction of microsatellite instability and DNA methylation, as well as modulation of stemness properties and drug resistance of HCC cells. Thus, *UBE2S* may play pivotal roles in HCC prognosis, precision immunotherapy and drug response, and could be served as a prognostic biomarker and immune-related therapeutic target for HCC.

Author contributions

Conceptualization: LQ, ZT and HL; Data extraction, analysis and visualization, and writing: LQ, YW, ZL, ZT and HL. Funding acquisition: LQ, YW, ZT and HL.

Conflict of interests

The authors have declared no conflict of interests.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.04.010.

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Lipeng Qiu [a,***], Yue Wang [a], Zhihuan Li [a], Zhigang Tu [a,**], Hanqing Liu [b,*]

[a] *School of Life Sciences, Jiangsu University, Zhenjiang, Jiangsu 212013, China*
[b] *School of Pharmacy, Jiangsu University, Zhenjiang, Jiangsu 212013, China*

*Corresponding author.

**Corresponding author.

***Corresponding author.
E-mail addresses: tulip_lipeng@163.com (L. Qiu), zhigangtu@ujs.edu.cn (Z. Tu), hanqing@ujs.edu.cn (H. Liu)

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