Identification and Verification of Ubiquitin D as a Gene Associated with Hepatitis C Virus-Induced Hepatocellular Carcinoma

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\textbf{Keywords}\nUbiquitin D · Hepatitis C virus · NS3 · Liver hepatocellular carcinoma · p53

\textbf{Abstract}
Introduction: Accumulated studies have suggested that hepatitis C virus (HCV) infection is one of the leading causes for hepatocellular carcinoma (HCC). However, the mechanisms underlying the effect of HCV on the occurrence of HCC are still poorly understood. \textbf{Methods:} HCV infection datasets (GSE82177 and GSE17856) and HCC datasets (The Cancer Genome Atlas Liver Hepatocellular Carcinoma and GSE89377) were downloaded from Gene Expression Omnibus or TCGA for analysis. The common differentially expressed genes in the above four datasets were identified by R software. The expression of ubiquitin D (UBD) in HCV-infected HepG2 cells was detected by RT-qPCR and Western blot, respectively. The interaction between NS3 and p53 was detected by co-immunoprecipitation. The influence of UBD on the proliferation and migration ability of HepG2 cells was evaluated by CCK-8 and wound healing assay, respectively. \textbf{Results:} UBD was upregulated in both HCV-infected samples and HCC samples. HCV NS3 interacted with p53 and inhibited its expression. HCV NS3-induced UBD promoted the proliferation and migration of HepG2 cells. \textbf{Conclusion:} Our results suggest that HCV NS3-induced UBD is positively correlated with the development of HCV-related HCC during HCV infection. Targeting UBD could be a potential strategy for preventing and treating HCV-induced HCC.

\textbf{Introduction}

Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer in adults as well as a leading cause of cancer-related death worldwide [1], with approximately 700,000 deaths each year. As the fastest rising cause of cancer-related death, the average 5-year survival rate of HCC <15%, there remains great difficult to manage HCC [2]. The majority cases of HCC (approximately 80%) are associated with chronic hepatitis B virus or hepatitis C virus (HCV) infection [3]. Underlying HCV infection is the major risk factor for chronic liver disease and HCC in most Western countries [1], which caused almost 20–70% of HCC cases in European countries and the USA [4].

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HCV is a hepatotropic positive-sense, single-stranded RNA (+) virus of the Flaviviridae family. The 9.6-kb genome of HCV encodes a large polyprotein consisting of 3,010 amino acids. This polyprotein is cleaved by cellular and viral proteases into ten different proteins including the structural proteins (core, E1, and E2), the hydrophobic peptide p7, and the nonstructural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) [5, 6]. Various HCV proteins, including the core [7–10], envelope [11], and nonstructural proteins [12, 13], have been reported to play an essential role in HCC development. Core was reported to exacerbate disease progression by inducing cell apoptosis and causing liver damage [14]. Similarly, NS5A can lead to liver damage through inhibiting the activated protein kinase R or interfere with antiviral treatment through encompassing the interferon sensitivity-determining region [15].

In contrast to hepatitis B virus, HCV cannot integrate into the human genome because of lacking reverse transcriptase activity. As a completely cytoplasmic-replicating virus, the main hypothesis for HCV to induce HCC is that it can cause persistent chronic inflammation and hepatocellular injury [16]. The implementation of direct-acting antiviral treatments allows a high rate of virus eradication and decreases the HCC rate in HCV patients. Even though the combination antiviral therapy with peginterferon and ribavirin can reduce HCV RNA levels, some patients still fail to achieve complete early virologic response and sustained virologic response, thus leading to a chronic HCV infection [17]. The exact molecular mechanism through which persistent HCV infection induced HCC is still unclear. Therefore, to further explore the potential carcinogenic mechanism of HCV is beneficial for the research of antiviral therapy and vaccine development, which will lower the mortality rate of HCC.

Ubiquitin D (UBD), also known as HLA-F adjacent transcript 10, is a ubiquitin-like modifier which directly targets proteins for proteasomal degradation. As an effector of the immune system, UBD is strongly induced by pro-inflammatory cytokines [18, 19] or upregulated during the maturation of antigen-presenting cells [20–22]. Moreover, several recent reports have shown that UBD is highly upregulated in many different types of cancers including HCC, gastrointestinal and gynecological cancers, and glioma [22–26]. In addition, UBD regulates cancer development-related signaling pathways such as NF-κB, Akt, or Wnt signaling [19, 27, 28]. Meanwhile, UBD also directly interacts with and affects downstream targets such as p53 [29–31], β-catenin [28], SMAD2 [32], and MAD2 [33–35], leading to enhanced survival, proliferation, invasion, and metastasis of cancer cells as well as nonmalignant cells. Therefore, aberrant expression of UBD might cause the abnormal activation of signal pathways which contributes to HCC progression. However, the regulation of UBD expression and distinct functions of UBD in regulating HCC development remain largely unknown.

In this study, we found HCV NS3-induced UBD promoted the proliferation and migration of HCC cells. Our findings unveil a novel HCV-induced effector protein which accelerates the development of HCC and provides theoretical basis for UBD-based HCC targeting therapy.

**Materials and Methods**

RNA-Seq and Microarray Data Collection and Data Preprocessing

The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov/), an important cancer database, mainly contains clinical data of various human cancers (including subtypes). Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/geo/) is a database repository of high-throughput gene expression data and hybridization arrays, chips, and microarrays. In the present study, Liver Hepatocellular Carcinoma (LIHC) dataset is RNA-seq gene expression data download from TCGA, while GSE82177 [36], GSE17856 [37], and GSE89377 from GEO.

TCGA-LIHC comprised 374 HCC samples and 50 normal samples; GSE82177 comprised 9 HCV-infected samples and 10 normal samples; GSE17856 comprised 44 HCV-infected samples and 8 normal samples; GSE89377 comprised 35 HCC samples and 13 normal samples. Probe IDs in the download file were converted to gene symbol names with R software. All gene expression data were standardized and transformed with log2.

Identification of Common Differentially Expressed Genes in HCV Infection and HCC

Analysis of differentially expressed genes (DEGs) was performed with the DESeq2 and Limma package of R software [38]. We set adjusted p value (adj.p.val) < 0.05 and log2 fold change (log2.fc.) >1 as the cutoff criteria. The common DEGs were identified by integrating each DEG in the four datasets with the Robust Rank Aggreg package of R software.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes Pathway Enrichment Analyses of Common DEGs

Cluster Profiler offers genetic classification and enrichment analysis, namely, group gene ontology (GO), enrich GO, and enrich Kyoto Encyclopedia of Genes and Genomes (KEGG) for genetic classification and enrichment analysis [39]. Cluster Profiler is easy to use, and it provides a visual output of the analysis results. To explore the biological functions of common DEGs, GO and KEGG pathway enrichment analyses were performed on the common DEGs with the Cluster Profiler package. Adjusted p value <0.05 was considered as statistically significant.
Cell Culture, Viral Infection, and Reagent

HepG2 cells were purchased from the China Center for Type Culture Collection (CCTCC, Chinese Academy of Sciences, Shanghai, China) and cultured in DMEM medium (Gibco, Waltham, MA, USA) with 10% FBS (HyClone Laboratories, Logan, UT, USA) at 37°C with 5% CO₂. HepG2 cells in 24-wells plate were infected with 1×10⁸ copies HCV cc (Japanese fulminant hepatitis 1, genotype 2a) at 37°C for 4 h. The supernatants were discarded, and the infected cells were washed twice with phosphate-buffered saline and incubated in DMEM containing 10% FBS for each experiment.

Plasmids and Transfection

Myct/His-tagged expression plasmids for HCV core, p7, E1, E2, NS2, NS3, NS4A, NS4B, NS5A, NS5B, p53 proteins were constructed by cloning into pcDNA3.1 expression vector using standard cloning techniques. Restriction enzymes and primers for cloning are listed in online supplementary Table S1a (for all online suppl. material, see www.karger.com/doi/10.1159/000525543). Cells were grown to 50% confluence in 6-wells plate, and transfections were conducted with Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer’s instructions. In all co-transfection experiments, corresponding vectors were used as negative controls (NCs) to ensure similar DNA concentrations. Cells were detected either at 24 h post-transfection for migration assay or at 36 h for Western blot.

RNA Interference Analysis

Short interfering RNA (siRNA) against UBD siRNA (si-UBD) or p53 (si-p53) and control siRNA (si-NC) with nonspecific targets were synthesized by Gene Pharma Co. (Shanghai, China). The sequences were as follows: si-UBD-1 (5′-GCAUC-AGAAAGGGCAACUAC-3′); si-UBD-2 (5′-GCUGGGCCUCC-AAGAAUCUA-3′); p53 siRNA-1 (5′-GAAAUGUUCUUGCAG-UUAAGG-3′); p53 siRNA-2 (5′-GCAGUUAAGGGUUAUGGU-A-3′); NC siRNA (5′-UUUCUCCGAACGUUCACGU-3′). HepG2 cells were grown to 40% confluence in 6-well plates and transiently transfected with 100 nM siRNAs using Lipofectamine 2000. The cells were analyzed either at 24 h post-transfection for in vitro migration assay or at 36 h post-transfection for Western blot.

RNA Extraction and Quantitative Reverse-Transcription PCR

Total RNA was extracted with TRIzol (Invitrogen). Reverse transcription was performed with 500 ng of the purified RNA template. The obtained cDNA samples were subjected to PCR with a PCR Kit (Bio-Rad, Hercules, CA, USA). Quantitative reverse-transcription PCR (qRT-PCR) was carried out on a Step One Plus Real-Time PCR System. Specific primers for detection of target genes are listed in online supplementary Table S1b. The relative mRNA expression levels of each gene were normalized based on GAPDH. Relative fold differences were determined using the method of ΔΔCT. All experiments were performed at least three separate times.

Immunoblotting and Immunoprecipitation

For immunoblotting (IB), collected cells were lysed in RIPA buffer supplemented with protease inhibitor cocktail for 30 min on ice. Cell lysates were separated by SDS-PAGE and transferred to a polyvinylidene fluoride membrane by electroblootating. After blocking with 5% BSA in TBST, the membrane was incubated with target protein-specific primary antibody and then with HRP-conjugated secondary antibody. GAPDH was used as a control. All bands were displayed with an ECL Western blotting kit and normalized to GAPDH. Three independent experiments were performed for each analysis. For immunoprecipitation, cells were lysed in RIPA buffer supplemented with protease inhibitor on ice for 30 min. Primary antibody was incubated with protein agarose A/G beads for 4 h at 4°C, followed by incubation with cell lysates for 5 h with rotation at 4°C. The beads were washed four times with lysis buffer and analyzed by IB. Antibodies against UBD (A5491; Abclonal, China), NS3 (ab13830; Abcam, Boston, MA, USA), p53 (60283-2-1g; Proteintech, China), Myc-tag (60003-2-1g; Proteintech, China), and GAPDH (60004-1-1g; Proteintech, China) were used in this study.

Cell Proliferation Assay

For proliferation assay, after being transfected with pcDNA3.1-NS3 or si-UBD, HepG2 cells were collected and modulated cell density to 1×10⁴/mL, and 100 μl suspended cells were seeded into 96-well plate. Cells were incubated for 24 h in an incubator at 37°C with 5% CO₂. Next, each well was added with 10 μl CCK-8 solution and cultivated at room temperature for 4 h. Absorbance was measured at 450 nm. The experiment was repeated three times successive 6 days.

Migration Assay

To examine the migratory ability, after being transfected with pcDNA3.1-NS3 or si-UBD, HepG2 cells were incubated in plates. Three straight scratches across each well were created in the cell layer by a pipette tip; then, cells were exposed to the medium containing 2% FBS after being washed by phosphate-buffered saline. Cell migration was observed under a microscope after 48 h of incubation. The migratory activity was assessed by measuring the area of migration into the scratches in each well by Image-Pro Plus, and the migration rate was calculated as the ratio of the relative migration area in the experimental group to the relative migration area in the control group.

Statistical Analysis

All data were presented as mean ± SEM of at least three independent experiments. Unpaired t test or one-way ANOVA followed by Newman-Keuls post hoc test was performed for data analysis. Two-sided p values under 0.05 were considered as statistically significant (*p < 0.05; **p < 0.01; ***p < 0.001). ns represented no significance. All statistical analyses were performed with GraphPad Prism V.8.0 (GraphPad Software, San Diego, CA, USA).

Results

Identification of Common DEGs in HCV-Infected Patients and HCC Patients

To get the DEGs in HCV-infected samples and HCC samples, two RNA-seq datasets (GSE82177 and TCGA-LIHC) and two mRNA microarray datasets (GSE17856 and GSE89377) were processed and normalized, and...
Table 1. Common DEGs in HCV-infected samples and HCC samples

| Common DEGs | Gene symbol |
|-------------|-------------|
| **Upregulated** | |
| UBD | AKR1B10 |
| CXCL10 | TMEM45B |
| KRT23 | SCGN |
| SPINK1 | GPC3 |
| MMP9 | ISG15 |
| SPP1 | MUC13 |
| TOP2A | LAMP3 |
| STAT1 | DTL |
| HKDC1 | CDC20 |
| IFI27 | CXCL11 |
| PTTG1 | CDCA5 |
| CDKN3 | ASPM |
| PRC1 | CCNB2 |
| BAIAP2L2 | MDK |
| AURKA | THY1 |
| BAIAP2L2 | CDKN3 |
| MCM2 | TRIM31 |
| ZIC2 | NCAPG |
| NCAPG | MELK |
| MELK | KIF20A |
| KIF20A | HMMR |
| PBK | ANLN |
| ANLN | MND1 |
| LRRC1 | MMP11 |
| MMP11 | CCNA2 |
| MMP11 | IGF2BP3 |
| IGF2BP3 | COL15A1 |
| COL15A1 | UBE2T |
| UBE2T | PLVAP |
| PLVAP | TTK |
| TTK | CENPM |
| CENPM | RECQL4 |
| RECQL4 | HHJURP |
| HHJURP | BLM |
| BLM | KIF4A |
| KIF4A | CDT1 |

| **Downregulated** | |
| CNDP1 | SRD5A2 |
| KCNN2 | SRD5A2 |
| ANXA10 | SRD5A2 |
| CYP39A1 | BCHE |
| BBOX1 | EXPH5 |
| AOX1 | MT1F |
| MT1M | CYP2C19 |
| SLC3A1 | NEU4 |
| NAT2 | MBL |
| F9 | HP |
| PZP | IGFALS |
| DHODH | FCN3 |
| FCN3 | MT1H |
| HAMP | HSD17B13 |
| HSD17B13 | SLC38A2 |
| PIK3C2G | CFHR3 |
| CFHR3 | TRPM8 |
| TRPM8 | TMEM45A |
| TMEM45A | RCL1 |
| RCL1 | HKD178 |
| HKD178 | 24768 |
| HKD178 | 1301 |
| 1301 | 150 |
| 150 | 8713 |
| 8713 | 74 |
| 74 | 198 |

**Fig. 1. a–d** Volcano plots of the four datasets. Red represents upregulated genes, and green represents downregulated genes (adj.p.val < 0.05, |log2.fc.| > 1).
DEGs among each dataset were identified with the threshold adj.p.val < 0.05 and |log2.fc| > 1. 156 DEGs were screened from GSE82177, including 82 upregulated genes and 74 downregulated genes (shown in Fig. 1a). 200 DEGs were screened from GSE17856, including 150 upregulated genes and 50 downregulated genes (shown in Fig. 1b). 2,901 DEGs were screened from TCGA-LIHC, including 1,301 upregulated genes and 1,600 downregulated genes (shown in Fig. 1c). 391 DEGs were screened from GSE89377, including 91 upregulated genes and 300 downregulated genes (shown in Fig. 1d). The common DEGs among the four datasets were identified with the Robust Rank Aggreg package of R software. All the upregulated and downregulated common DEGs mentioned above are listed in Table 1.

Fig. 2. GO and KEGG analysis of the common DEGs among the four datasets. a–c Enriched pathways in molecular function (a), cellular components (b), and biological process (c) by GO analysis were shown. d Enriched pathways of common DEGs by KEGG analysis.
**GO and KEGG Pathway Enrichment Analyses of Common DEGs**

GO analysis was performed on the common DEGs with the Cluster Profiler package of R software. The screening criterion for GO analysis was \( \text{adj.} p \text{ val} < 0.05 \). As shown in Figure 2a–c, the common DEGs were involved in three aspects, including molecular functions, cellular components, and biological process. In molecular functions, the common DEGs were enriched in oxidoreductase activity, mainly acting on paired donors with incorporation or reduction of molecular oxygen, monoxygenase activity, and heme binding (shown in Fig. 2a). In cellular components, the common DEGs were enriched in collagen-containing extracellular matrix, blood micr-
roparticle, and plasma lipoprotein particle (shown in Fig. 2b). In biological process, the common DEGs were enriched in steroid metabolic process, response to xenobiotic stimulus and hormone metabolic process (shown in Fig. 2c). KEGG pathway enrichment analysis on the common DEGs was performed with the Cluster Profiler package of R software. The screening criterion for KEGG pathway enrichment analysis was adj.p.val <0.05. The KEGG pathway enrichment analysis results for common DEGs are shown in Figure 2d. The signaling pathways of common DEGs were mainly enriched in the chemical carcinogenesis, retinol metabolism, and drug metabolism – cytochrome P450 (CYP). These results suggested that HCV infection promoted the oxygen utilization and metabolism of host cells which contributes to the development and chemotherapeutic drug resistance of HCC.

**HCV NS3 Protein Promoted the Expression of UBD**

To find out the critical genes which connect HCV infection and HCC, we verified the upregulated genes

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**Fig. 4.** NS3 upregulated UBD by interacting with p53. a–c After HepG2 cells were transiently transfected with pcDNA3.1-NS3 or vector. co-IP was performed to identify the interaction between NS3 and UBD or p53. d After HepG2 cells were transfected with p53 or co-transfected p53 and NS3 expressing plasmids for 36 h, the expression of UBD, p53, and NS3 was analyzed by Western blot. e After HepG2 cells were transfected with 100 nM si-UBD (si-p53) or si-NC for 36 h, the expression of p53 was detected by Western blot. f After HepG2 cells were transfected with si-p53-2 or pcDNA3.1-NS3 as indicated, the expression of UBD, p53, and NS3 was analyzed by Western blot.
shown in Figure 3a by RT-qPCR. As shown in Figure 3b, UBD and AKR1B10 were significantly upregulated by HCV infection. Owing to overexpressed AKR1B10 in chronic hepatitis C patients which has been reported to promote the development of HCC [40], we selected UBD for the follow-up research. Consequently, HCV infection promoted the mRNA and protein expression of UBD in a time-gradient-dependent manner in HepG2 cell line model (shown in Fig. 3c, d). Then, HepG2 cells were transfected with overexpressing plasmids which encode myc/his tag-labeled HCV proteins to identify the candidate HCV proteins for upregulating UBD. As shown in Figure 3e, NS3 but not higher level expressed NS2 upregulated UBD expression. Notably, NS3 but not p7 increased UBD expression in a dose-dependent manner in HepG2 cells (shown in Fig. 3f, g). Thus, these results indicated that NS3 mediated HCV-induced UBD expression.

**NS3 Upregulated UBD by Interacting with p53 and Inhibiting the Expression of p53**

To explore whether there is a direct interaction between NS3 and UBD, we performed a co-IP assay. As shown in Figure 4a, UBD did not combine with overexpressed NS3, suggesting that NS3 regulates the expression of UBD through an indirect pathway. Consistent with the previous research that NS3 played an important role in the hepatocarcinogenesis of HCV by interacting with p53 [41], we find NS3 inhibited the expression of p53 by upregulating UBD (shown in Fig. 3f). Therefore, we wonder whether NS3 will interact with p53 to affect the expression of p53, ultimately upregulating UBD expression. In order to prove this, we tested the interaction between NS3 and p53 in HepG2 cells. co-IP results showed that overexpressed NS3 formed a complex with endogenic p53 (shown in Fig. 4b, c). Moreover, overexpressed NS3 reversed the suppression of overexpressed p53 on UBD (shown in Fig. 4d). However, the promotion of disrupting

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**Fig. 5.** Downregulation of UBD reversed NS3-mediated promotion of HCC proliferation and migration. a After HepG2 cells were transfected with 100 nM si-UBD or si-NC for 36 h, the expression of UBD was detected by Western blot. b Proliferation of HepG2 cells transfected with indicated plasmids was assayed by CCK-8 (***p < 0.001; n = 3). c, d Migration ability of indicated HepG2 cells was analyzed by wound healing assay, and relative migration rates were shown. Data were presented as mean ± SEM in b, d, n = 3; ***p < 0.001.
p53 on UBD was not affected by NS3 (shown in Fig. 4e, f). These results suggested that the interaction between NS3 and p53 upregulated the expression of UBD in HepG2 cells, which is dependent on the inhibition of p53 expression.

**Downregulation of UBD Reversed NS3-Mediated Promotion of HCC Proliferation and Migration**

Considering the combination between NS3 and p53 upregulates UBD expression and UBD is involved with tumorigenesis and malignancy [25, 26, 31, 42], we wonder whether interfering UBD with RNAi can reverse NS3-mediated development of HCC. To confirm this, we disrupted the expression of UBD with UBD-specific siRNAs in HepG2 cells (shown in Fig. 5a). Then, the proliferation and migration capacities were determined by CCK8 and wound healing assay, respectively. As shown in Figure 5b, siRNA-induced depression of UBD-blocked NS3 promoted proliferation of HepG2 cells. Moreover, wound healing assay showed that although overexpressed NS3 enhanced cell migration, co-transfection of pcNDA3.1-NS3 and si-UBD reversed this promotional effect (shown in Fig. 5c, d). Overall, these results demonstrate that HCV NS3-induced UBD promotes the proliferation and migration of HCC cells and supports inhibiting UBD as a potential therapeutic strategy against HCV-related HCC.

**Discussion**

Based on techniques such as high-throughput gene screening, microarray, and RNA-seq, aberrantly significant genes and pathways have been found in HCC. However, there is no study about common DEGs among HCV infection and HCC. These DEGs are critical for the development of HCV-related HCC and meaningful for further understanding the pathogenesis of HCV-related HCC.

In this study, we identified common DEGs which associated with HCV infection with HCC using bioinformatics methods. 324 common DEGs among one TCGA dataset and three GEO datasets were screened out. It is consistent with the established concept that metabolic abnormalities are significantly associated with HCV-related HCC [43]. GO analysis of the common DEGs showed that the common DEGs were mainly involved in steroid metabolic process, response to xenobiotic stimulus, and hormone metabolic process. Similarly, KEGG pathway enrichment analysis showed that the signaling pathways of the common DEGs were mainly enriched in the chemical carcinogenesis, retinol metabolism, and drug metabolism – CYP. Retinol is an important nutrient for humans and must be provided by the diet. It regulates specific nuclear receptors (retinoic acid receptors and retinoid X receptors) which in turn affect the expression of genes that are associated with cell growth, differentiation, development, and homeostasis [44, 45]. Altered retinol metabolism has been identified as one of the different pathways involved in the process of hepatic fibrosis; meanwhile, retinol metabolism-related enzymes have been reported to associate with HCC [46]. CYP enzymes are essential for the production of cholesterol, steroid, prostacyclin, and thromboxane A2, the detoxification of foreign chemicals, and the metabolism of drugs. CYP enzymes are key enzymes associated with cancer development [47]. Significant change of CYPs activities has been found in HCC patients [48]. Consequently, the common DEGs identified in this study were shown to be significantly involved in the disruption of metabolism which contributes to the progress and resistant of HCC.

Except for disrupting CD4 (+) T cells-mediated antiviral immune response, HCV could also alter host regulatory pathways involved in energy metabolism, angiogenesis, epithelial-mesenchymal transition, DNA repair, apoptosis, and proliferation [49] through direct viral factors such as NS3 to induce HCC [50]. Among the common DEGs, ubiquitin-like modifier UBD has been reported to directly involve in cell cycle/apoptosis regulation and esophageal cancer development [51]. In addition, overexpressed UBD facilitated HCC proliferation through degrading Wnt-induced secreted protein-1 [52]. At the same way, NS3, a serine protease, which is responsible for the cleavage of at least four sites (NS3/4A, NS4A/4B, NS4B/5A, and NS5A/5B junctions), has been reported to contribute the development of HCC through inhibiting p53-mediated apoptosis [42]. In this study, UBD was significantly upregulated by NS3 in HepG2 cells. Moreover, we observed that NS3 upregulated UBD by inhibiting the expression of p53, which negatively regulates the expression of UBD mRNA through binding to an inhibitory site within the UBD promoter [30]. We found that HCV NS3-induced UBD promoted the proliferation and migration of liver cancer cells by inhibiting p53 for the first time. However, how UBD promotes the proliferation of HCC cells is still unclear. We hypothesize that UBD might accelerate the proliferation of HCC cells by upregulating glycosylation-related kinase such as HK2 [53]. Owing to the multifunction in regulating tumor biological process, UBD is more competitive as a candidate gene associated with HCV-induced HCC.

Among the HCV-encoded proteins, core, E2, NS3, and NS5A can activate cellular proliferative RAF/MAPK/
ERK kinase pathways and E2F1 pathway, which are associated with a more aggressive HCC phenotype [54–57]. Meanwhile, NF-kB was reported to mediate the promotion of UBD on the proliferation of HCC [58]. Therefore, it is of great significance to further explore the signaling pathways which regulate HCV NS3-induced UBD in HCC cells. In summary, we clarified a new potential strategy adopted by HCV to induce the development of HCC and targeting NS3-upregulated UBD might be a feasible method to treat HCV-related HCC.

Statement of Ethics

As no clinical samples were used in this research, this paper is exempt from Ethical Committee approval according to the decision of Ethics Committee of First Affiliated Hospital of Kunming Medical University.

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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