Effect of Upregulated DNA Replication and Sister Chromatid Cohesion 1 Expression on Proliferation and Prognosis in Hepatocellular Carcinoma

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Abstract

Background: DNA replication and sister chromatid cohesion 1 (*DSCC1*) (also called *DCC1*) is a component of an alternative replication factor C complex that loads proliferating cell nuclear antigen onto DNA during S phase of the cell cycle. It is located at 8q24 and frequently amplified in hepatocellular carcinoma (HCC). However, the role of DSCC1 in the carcinogenesis and progress of HCC has not been fully investigated. Here, we aimed to assert the importance of DSCC1 in the HCC.

Methods: In this study, copy number variation data and RNA sequencing data were used to calculate the DNA copy number and mRNA expression of *DSCC1* in HCC. Quantitative polymerase chain reaction, Western blotting, and immunohistochemistry analysis were used to determine the mRNA and protein level of *DSCC1* in HCC. The Kaplan–Meier analysis and univariate and multivariate Cox regression analysis were used to assess the association of DSCC1 with the overall survival (OS) of HCC patients. Moreover, lentiviral shRNA was used to knockdown *DSCC1*, and then, colony-forming assay, cell cycle assay, and cell proliferation assay were performed to evaluate the impact of *DSCC1* silencing on HCC cell lines.

Results: We found that *DSCC1* was amplified and highly expressed in HCC tumor tissues than in nontumor tissues. We then found that the overexpression of both mRNA and protein of *DSCC1* was linked to the bad prognosis of HCC patients. Astonishingly, the protein level of DSCC1 was an independent prognostic factor for OS (hazard ratio, 1.79; 95% confidence interval, 1.17–2.74; $P = 0.007$). Furthermore, the clonogenic capacity of *DSCC1*-amplified HCC cell lines (MHCC-97H, MHCC-97L, and Hep3B) was significantly inhibited by transduction of a lentiviral shRNA that targets *DSCC1*. We also showed that knockdown of *DSCC1* induced G0–G1 cell cycle arrest (increased from 60% to more than 80%) and greatly inhibited the proliferation of HCC cell lines.

Conclusion: These results suggest that *DSCC1* is a putative HCC driver gene that promotes proliferation and is associated with poor prognosis in HCC.

Key words: Cell Cycle; Cell Proliferation; DNA Replication and Sister Chromatid Cohesion 1; Hepatocellular Carcinoma

Introduction

Although considerable progress has been made in the diagnosis and treatment of hepatocellular carcinoma (HCC) over the last decade, advanced-stage HCC in most patients still progresses to metastasis and leads to a poor prognosis. Hence, it is of critical importance to explore the genomic events that occur in HCC, with the prime goals of understanding the genetic basis of the disease and identifying new therapeutic targets.

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The human chromosome 8q24 locus contains a famous oncogene, MYC, and is amplified in nearly half of HCC patients.[1-3] DNA replication and sister chromatid cohesion 1 (*DSCC1*, also known as *DCC1*), which locates in 8q24, is one of the components of an alternative clamp loader/unloader Ctf18-Dcc1-Ctf8-replication factor C (Ctf18-RFC) complex.[4-7] This protein complex plays important roles in sister chromatid cohesion, DNA replication, spindle checkpoints, DNA repair, and genome stability during the S phase of the cell cycle.[8-13] The most recent studies revealed that DSCC1 and Ctf18-RFC complex also play a key role in the cell cycle checkpoint control. The C-terminal of DSCC1 is required for full recruitment of the complex to chromatin and correct activation of the replication checkpoint.[14] The association of Ctf18-RFC with Pol ε at defective replication forks is a key step in activation of the S-phase checkpoint.[15]

As deregulation of cell cycle checkpoint control represents a common cause in cancers, we would like to know the role of *DSCC1* and Ctf18-RFC complex in cancer. Several publications have reported that RFC family members may play important roles in various malignant tumors.[16] Moreover, a recent study has identified that *DSCC1* has anti-apoptotic properties in colorectal cancer cells and promotes the survival of cancer cells in response to genotoxic stimuli.[17] However, the expression pattern and role of *DSCC1* in HCC have not yet been determined. In this study, we aimed to determine the expression pattern and the importance of DSCC1 in HCC.

Methods

Copy number profiling and RNA sequencing data

Copy number variation (CNV) data and RNA sequencing (RNA-seq) data of liver hepatocellular carcinoma (LIHC) in The Cancer Genome Atlas (TCGA) project (http://cancergenome.nih.gov) were obtained from the UCSC cancer browser (https://genome-cancer.ucsc.edu/). The CNV profiling was performed using an Affymetrix Genome-Wide Human SNP Array 6.0 platform, and the RNA-seq was performed using an Illumina HiSeq 2000 RNA-seq platform. The CNV data of HCC cell lines were obtained from the Gene Expression Omnibus database (GSE38207).[3] The copy number segmentation data of GSE38207 were kindly provided by the author and displayed using an Integrative Genomics Viewer (IGV2.3.52).[18]

Ethical approval

The study was conducted in accordance with the *Declaration of Helsinki* and was approved by the Ethics Committee of Guilin Medical University (No. 2011GMA031). Informed written consent was obtained from all patients before their enrollment in this study.

Tissue samples and cell lines

All HCC cancer tissues and adjacent noncancerous tissues were obtained from patients who underwent surgery at the affiliated hospital of Guilin Medical University. The human HCC cell lines, i.e., MHCC-97H, MHCC-97L, Hep3B, Huh7, HepG2, SK-HEP-1, and PLC/PRF/5 were purchased from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (Life Technologies, Carlsbad, CA, USA).

Quantitative real-time polymerase chain reaction

In order to validate the DSCC1 overexpression in HCC, quantitative real-time polymerase chain reaction (QRT-PCR) was used to measure the DSCC1 mRNA levels in an independent HCC cohort (Guilin cohort). Then, the expression level of DSCC1 mRNA was analyzed in seven HCC cell lines (MHCC-97H, MHCC-97L, Hep3B, Huh7, HepG2, PLC/PRF/5, and SK-HEP-1) using QRT-PCR analysis. Total RNA was extracted using TRIzol Reagent (Invitrogen, Carlsbad, USA), and cDNA synthesis was performed using High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, USA) according to the manufacturer's instructions. QRT-PCR amplification was performed on a LightCycler 480 instrument using LightCycler 480 SYBR Green I Master Mix (Roche Diagnostics Ltd., Mannheim, Germany). The thermal profile consisted of 1 cycle at 95°C for 10 min followed by 45 cycles of 15-s denaturation at 95°C, 15-s annealing at 55°C, and 15-s extension at 72°C. The sequences of the primers used were as follows: DSCC1, 5'-AAAGTTCCTTTGAACACATGCCT-3' and 5'-CGCATTCTGAAGTAGCATTCGT-3', and GAPDH, 5'-CCACATCGCTCAGACACCAT-3' and 5'-GGCAACAATATCCACTTTACCAGAGT-3'. Relative DSCC1 mRNA expression was calculated by normalization to the mRNA expression level of GAPDH.

Western blotting analysis

We then performed Western blotting analysis to further determine the protein level of DSCC1 in HCC and adjacent nontumor tissues. Fifteen pairs of tumor tissues and corresponding adjacent nontumor tissues of HCC patients in Guilin cohort were included in Western blotting analysis. Frozen tissue samples were homogenized and lysed in RIPA lysis buffer containing a 1× protease inhibitor cocktail (Thermo Scientific, USA). Protein concentrations were determined using a Pierce™ BCA Protein Assay Kit (Thermo Scientific, USA); 20-μg protein was denatured and separated in a NuPAGE Novex 10% Bis-Tris Gel (Invitrogen Life Technologies, Carlsbad, CA, USA) and then transferred to nitrocellulose membranes using iBlot Dry Blotting System (Invitrogen, Carlsbad, USA). After membrane was blocked in 5% milk for 1 h at room temperature, it was incubated with an anti-DSCC1 antibody (Abnova) at 4°C overnight. An horseradish peroxidase (HRP)-conjugated goat anti-mouse antibody (Santa Cruz Biotechnology, CA, USA) served as the secondary antibody, with which membranes were incubated for 1 h at room temperature. Immunostaining intensity was detected using a Western Lightning® Plus-ECL (PerkinElmer, Boston, MA, USA) and visualized on X-ray film.

Immunohistochemistry

To further determine the protein expression pattern and the location of DSCC1 in HCC, we performed immunohistochemistry (IHC) analysis of DSCC1 with HCC tissue microarray (TMA) containing tumor section and nontumor section of 144 patients from Guilin cohort. Paraffin-embedded tissue slides and TMA slides were deparaffinized with Histoclear and hydrated in graded ethanols and were heated in ethylenediaminetetraacetic acid buffer (pH 9.0) at 95°C for 25 min for antigen retrieval. Nonspecific signal was blocked by hydrogen peroxide block for 10 min at room temperature, followed by blocking with 5% bovine serum albumin for 30 min at room temperature. Mouse anti-human DSCC1 antibody (Abnova, Taiwan, China) at a working dilution of 1:50 was incubated at 4°C overnight, followed by HRP-conjugated secondary antibody incubation for 30 min at room temperature. Then, 3, 3'-diaminobenzidine reaction was used to reveal antibody binding with EnVision detection system (Dako, Denmark). Sections were then counterstained with hematoxylin, dehydrated in graded ethanols, and mounted. Negative controls were performed for each run of IHC. DSCC1 expression was scored by two pathologists independently according to the percentage of tumor cells with nuclear staining (0, <5% of tumor cells stained positive; 1, 5–30% of tumor cells stained positive; 2, 31–50% of tumor cells stained positive; 3, 51–80% of tumor cells stained positive; and 4, >80% of tumor cells stained positive).

Gene knockdown using shRNA

We then constructed DSCC1-specific lentiviral shRNAs to effectively silence the expression of DSCC1. Three *DSCC1*-specific shRNAs (DSK1: CCATGAAATTGGCTGAATAAT, DSK2: GAAGAAGATATTGCTCCATAT, and DSK3: GAAGACGTAGACCCAAGTTAA) and a nonsilencing control shRNA (NS: CTGAGGTGATAAACAGTTACA) were cloned into a lentiviral shRNA expression vector (HIV-H1; GeneCopoeia, USA) that contained a H1-driving shRNA expression cassette and a GFP reporter gene. Lentiviruses were produced by transient cotransfection of the shRNA plasmids and the packing helper plasmids, i.e., pLP1, pLP2, and pVSV-G into 293T cells. Viral supernatants were harvested after 48 or 72 h posttransfection and then filtered through a 0.45-μm filter. Viral supernatants were then concentrated by centrifugation in protein purification tubes (PALL, USA) at 4500 ×*g* for 60 min. The infectious titers of the purified viruses were determined using fluorescence-activated cell-sorting analysis of GFP-positive 293T cells. The viral titers were in the range of 10^9 transducing units/ml medium. The HCC cell lines were transduced with shRNA lentiviruses in an optimal multiplicity of infection in the presence of 4 μg/ml polybrene. Infected cells were quickly selected using puromycin for 3–5 days before use. The silencing efficiency of *DSCC1* was assessed using real-time PCR.

Colony-forming assay

Colony-forming assay was used to determine the impact of DSCC1 expression silencing on HCC clonogenic ability using lentiviral shRNA-transduced HCC cell lines. Cells were trypsinized, and the number of total and dead cells was counted manually in the presence of trypan blue. A total of 10^3 viable cells were placed in one well of a six-well plate and incubated for a minimum of 14 days. The DMEM growth medium was changed every 3rd day. The medium was removed and, following a rinse with phosphate-buffered saline (PBS), the cells were fixed to the plate using 1% paraformaldehyde in PBS for 15 min. The colonies were stained for 1 h with a solution of 1% crystal violet followed by several washes with ddH_2O. Stained colonies that contained more than 25 cells were counted.

Cell cycle analysis

Since DSCC1 plays important roles in insister chromatid cohesion, DNA replication, and cell cycle, we performed cell cycle analysis with 5-bromo-2'-deoxyuridine (BrdU) flow cytometry kit to determine the impact of DSCC1 silencing on cell cycle transition in HCC cell lines. For cell cycle analysis, the BrdU flow cytometry kit for cell cycle analysis (BD Biosciences, USA) was used according to manufacturer's instructions. BrdU incorporation (FITC-labeled anti-BrdU antibodies) was measured along with DNA content (using 7-AAD) in fixed and permeabilized cells. The analysis was gated on viable cells that were identified based on scatter morphology.

Cell proliferation assay

To determine the effect of DSCC1 knocking down on the proliferation of HCC cells, we performed MTS assay to measure the growth rate of MHCC-97H and Hep3B cells transduced with lentiviral DSCC1 shRNAs or control shRNA. Cell proliferation was evaluated by MTS assay using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay Kit (Promega, USA) according to the manufacturer's instructions. Briefly, 5000 cells/well were seeded in a 96-well plate after transduced with lentiviral shRNA for 12 wells for each shRNA-transduced group. At 1, 2, 4, and 7 days after seeding, 20 μl of CellTiter 96 Aqueous One Solution was added to each well, and the plate was incubated for 1–4 h. Plates were read at 490 nm and 650 nm (background) in a microplate reader (Molecular Devices, USA). After subtraction of background, the relative cell number was calculated.

Statistical analyses

The *t*-test was used to compare continuous variables between two groups. One-way analysis of variance was used to test the equality of means between three or more groups simultaneously. The correlation significance between *DSCC1* copy number and mRNA expression level was determined by Pearson correlation analysis. The Chi-square test was used to examine the association between *DSCC1* gene expression level and clinicopathological parameters. The Kaplan–Meier method followed by the log-rank test was used for survival analysis in both TCGA LIHC cohort and Guilin cohort. In order to determine the prognostic value of DSCC1 protein

expression in HCC, we performed univariate and multivariate Cox regression analysis of prognosis factors, including DSCC1 expression, among patients in the Guilin cohort. Univariate and multivariate Cox proportional-hazards regression model was performed to identify factors having a significant impact on overall survival (OS). Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Software, La Jolla, CA, USA) and R Statistical Software (Foundation for Statistical Computing, Vienna, Austria), a free software environment for statistical computing and graphics.[19] All data are representative of at least three independent experiments. A two-sided $P < 0.05$ was considered to indicate statistical significance for all analyses.

Results

DNA replication and sister chromatid cohesion 1 is frequently amplified and overexpressed in hepatocellular carcinoma tumors and cell lines

The 8q24 locus is frequently amplified in HCC patients, and it harbors a famous oncogene, MYC, which has been shown to be involved in both carcinogenesis and development of HCC. We noticed that the locus of another gene, *DSCC1*, has a minimum 1.5-fold increase in copy number in 32% (115/364) of HCC patients when compared to nontumors by analyzing the LIHC CNV data from the TCGA [Figure 1a].

While the normal liver and bile ducts expressed the lowest levels of *DSCC1* mRNA among all 14 normal tissues (liver, bile ducts, pancreas, colon, rectum, lung, breast, cervix, endometrium, bladder, kidney, prostate, thyroid, and brain), the mRNA level of *DSCC1* was dramatically highly expressed in HCC tumor tissues than in normal liver tissues according to the RNA-seq data in the TCGA project [Figure 1b]. We also found that the mRNA level of *DSCC1* was significantly associated with its DNA copy number ($r = 0.327$, $R^2 = 0.107$, $P < 0.001$), which suggested that the overexpression of *DSCC1* in HCC was partially attributed to copy number gain [Figure 1c].

Remarkably, we found that the mRNA expression level of *DSCC1* significantly correlated with the prognosis of HCC patients in TCGA LIHC dataset. Patients with higher *DSCC1* mRNA expression level tend to have a shorter OS time ($\chi^2 = 19.29$, $P < 0.001$) [Figure 1d]. QRT-PCR analysis of the *DSCC1* mRNA levels in an independent HCC cohort (Guilin cohort) showed that the relative expression level of *DSCC1* mRNA was significantly higher in tumor tissues than in nontumor adjacent tissues, which is consistent with the LIHC RNA-seq data in TCGA [Figure 2a].

Western blotting analysis of DSCC1 protein in HCC and adjacent nontumor tissues showed that DSCC1 protein was detected in nearly half of tumor tissues (7 of 15) but in none of these nontumor tissues [Figure 2b]. Both QRT-PCR and Western blotting results support that *DSCC1* had an undetectable level in normal liver tissue but was dramatically overexpressed in HCC tumors.

We also found that nuclear DSCC1 staining presented in 27% (39/144) of the analyzed HCC patients by IHC analysis of DSCC1 with HCC TMA containing tumor section and nontumor section of 144 patients from Guilin cohort, and its staining had no correlation with clinicopathological parameters of these patients [Figure 2c and Table 1]. But, a Kaplan-Meier analysis showed that positive staining of DSCC1 in HCC primary tissue was significantly linked to shorter OS time of HCC patient [Figure 2d].

Furthermore, a univariate analysis showed that DSCC1 staining, along with age, tumor size, tumor, node, and metastasis stage, multinodularity, venous infiltration, and metastasis, was associated with OS [Table 2]. However, none of these factors were associated with recurrent-free survival (data not shown). The limited number of the cases with available recurrent-free survival data may account for this negative result. In the following multivariate Cox regression analysis, the protein level of DSCC1 was an independent prognostic factor for OS (hazard ratio for death, 1.79; 95% confidence interval, 1.17–2.74; $P = 0.007$) [Table 3]. All these data presented above suggested that the overexpression of *DSCC1* might be involved in HCC development.

DNA replication and sister chromatid cohesion 1 oncogene dependency in human hepatocellular carcinoma cell lines

We have found that the overexpression of both *DSCC1* mRNA and protein was linked to the bad prognosis in HCC. We then sought to determine whether *DSCC1* is functionally involved in the development of HCC. We first found that all of the seven analyzed cell lines (MHCC-97H, MHCC-97L, Hep3B, Huh7, HepG2, PLC/PRF/5, and SK-HEP-1) had detectable *DSCC1* mRNA level. MHCC-97H had the highest *DSCC1* mRNA level and SK-HEP-1 had the lowest level [Figure 3a].

Among these HCC cell lines, seven out of eight cell lines have detectable *DSCC1* mRNA level. MHCC-97H has the highest *DSCC1* mRNA level and SK-HEP-1 has the lowest level [Figure 3a].

We then constructed *DSCC1*-specific lentiviral shRNAs and got two shRNAs, DSK1 and DSK2, which could effectively silence the expression of *DSCC1*. We found that endogenous *DSCC1* expression in HCC cell lines could be significantly knocked down after transduction of lentiviral DSK1 and DSK2 [Figure 3b]. We then found that MHCC-97H and MHCC-97L showed oncogene dependence on *DSCC1* expression by performing colony-forming assay to determine the HCC clonogenic ability after lentiviral shRNA transducing. The clone number of these two cells significantly dropped after *DSCC1* knocking down. However, the clone number of HepG2 and SK-HEP-1 largely remained unchanged after *DSCC1* knocking down [Figure 3c and 3d].

Interestingly, the oncogene dependency of HCC cell lines on *DSCC1* expression perfectly matched the copy

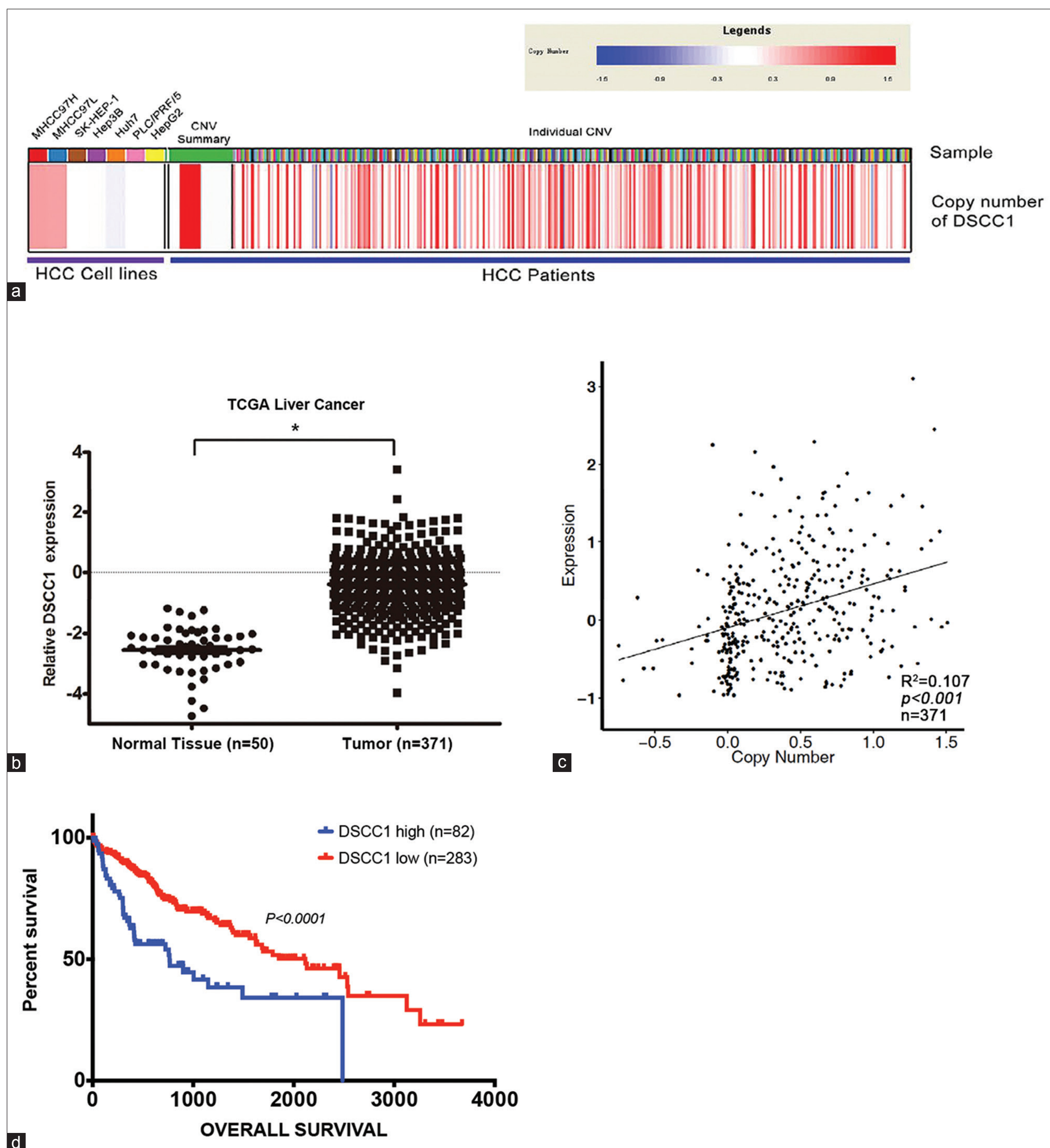

Figure 1: Amplification and overexpression of DSCC1 in HCC. (a) The copy number of DSCC1 DNA locus in HCC tissues and HCC cell lines that determined by SNP genotyping data from TCGA and GEO. (b) The mRNA expression data (log2 (x + 1) transform of TPM value) of DSCC1 in HCC tissues determined by analyzing RNA-seq data from TCGA LIHC dataset. (c) The correlation of DNA copy number and mRNA expression of DSCC1 in HCC patients from TCGA LIHC dataset. (d) The correlation between DSCC1 mRNA expression level and the OST of HCC patients in TCGA LIHC dataset (*$P < 0.001$). GEO: Gene Expression Omnibus; OST: Overall survival time; DSCC1: DNA replication and sister chromatid cohesion 1; HCC: Hepatocellular carcinoma; TCGA: The Cancer Genome Atlas; RNA-seq: RNA sequencing.

number status of these cell lines on the *DSCC1* locus. Cell lines with *DSCC1* locus amplification, MHCC-97H and MHCC-97L, showed oncogene dependency on *DSCC1* expression, while cell lines without *DSCC1* amplification, HepG2 and SK-HEP-1, can still maintain colony growth ability when the *DSCC1* expression was silenced [Figures 1a, 3c, and 3d].

DNA replication and sister chromatid cohesion 1 knocking down induced cell cycle arrest and inhibited proliferation

We found that, after knocking down of *DSCC1*, the percentage of cells in the G0/G1 phase increased significantly from nearly 60% to more than 80%, while the percentage of S-phase cells and G2/M-phase cells both decreased to half of the level in

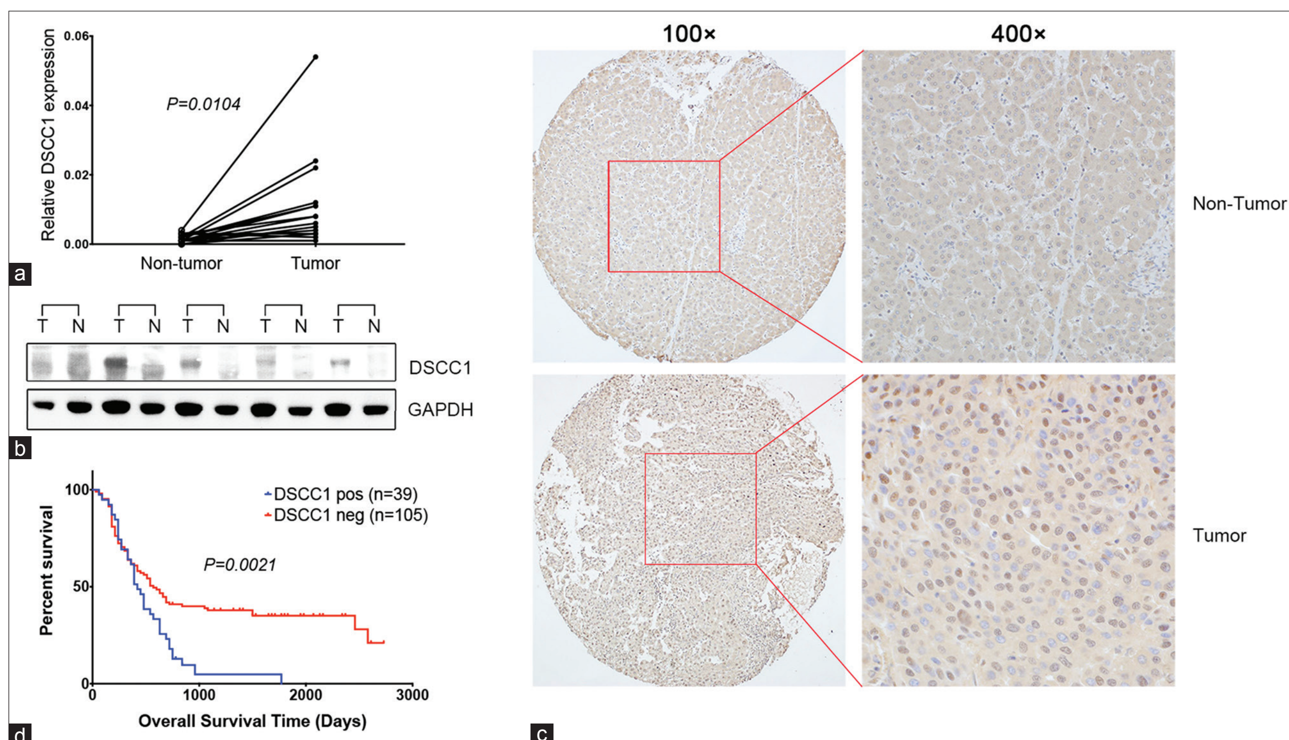

Figure 2: DSCC1 overexpressed in HCC and linked to prognosis. (a) The mRNA level of DSCC1 in HCC tissues and adjacent normal tissues determined by QRT-PCR analysis. (b) DSCC1 protein expression in HCC tissues determined by Western blotting analysis (T: Tumor tissue; N: Nontumor tissue). (c and d) The expression of DSCC1 protein determined by IHC analysis and its correlation with OST of HCC patients (IHC, ×100 [left] and ×400 [right]). IHC: Immunohistochemistry; OST: Overall survival time; DSCC1: DNA replication and sister chromatid cohesion 1; HCC: Hepatocellular carcinoma; QRT-PCR: Quantitative real-time polymerase chain reaction.

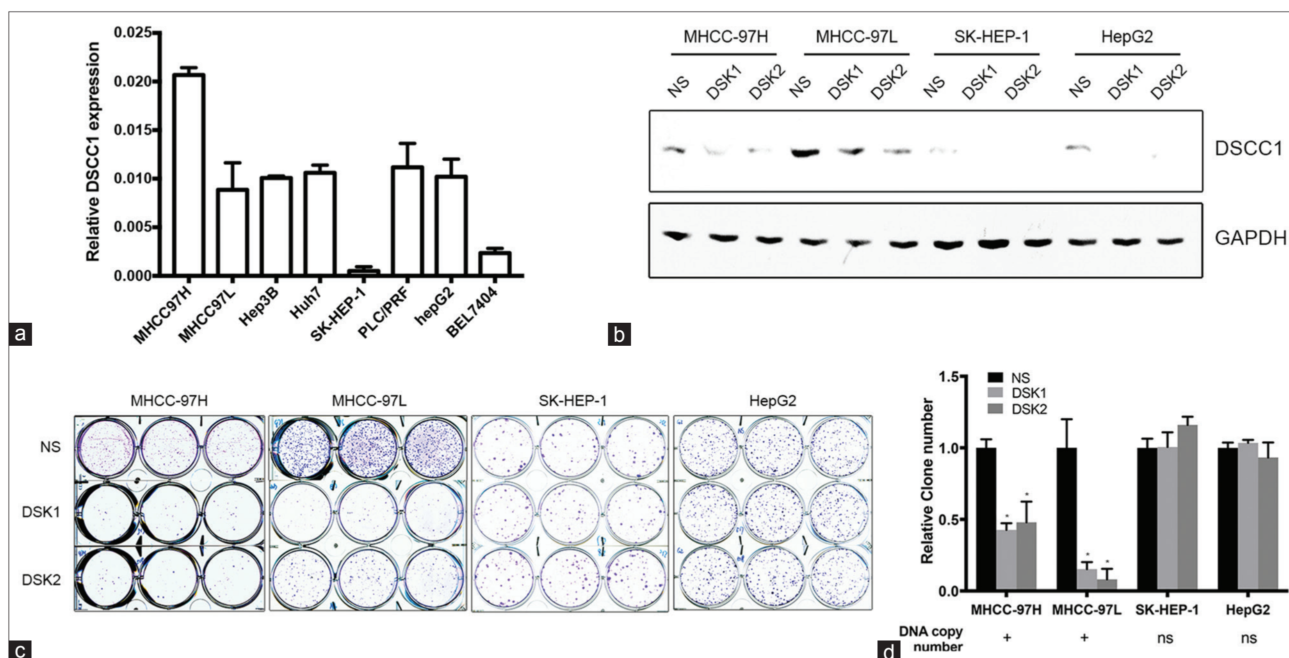

Figure 3: DSCC1 oncogene dependency in human HCC cell lines. (a) The DSCC1 mRNA level determined by real-time PCR in HCC cell lines. (b) Western blotting analysis of DSCC1 protein after it was knockdown by shRNA silencing. (c and d) The clone-forming assay of cell lines after DSCC1 silencing. Clonogenic assay of MHCC-97H, MHCC-97L, SK-HEP-1, and HepG2 infected with lentiviral shRNAs against nonsilencing control and DSCC1 (DSK1 and DSK2) (c) and the quantification of clonogenic assay of seven HCC cell lines from three independent experiments (d) (*$P < 0.001$). DSCC1: DNA replication and sister chromatid cohesion 1; HCC: Hepatocellular carcinoma; PCR: Polymerase chain reaction.

the control group [Figure 4a and 4b]. This finding indicates that cell cycle distribution was blocked significantly in the G0/G1 phase when knocking down of *DSCC1* in both MHCC-97H and Hep3B cells.

Table 1: Clinical characteristics of patients with HCC included in analyses of DSCC1 expression by IHC

Characteristics	Frequency, *n* (%)	DSCC1 IHC staining (*N* = 144)		
		Negative (*n* = 105)	Positive (*n* = 39)	*P*
Gender				
Male	127 (88.2)	92	35	0.952
Female	17 (11.8)	13	4	
Age				
<55 years	101 (70.1)	76	25	0.447
≥55 years	43 (29.9)	29	14	
Alcohol				
No	61 (42.4)	47	14	0.443
Yes	83 (57.6)	58	25	
Tumor size				
<6 cm	37 (25.7)	30	7	0.266
≥6 cm	106 (73.6)	74	32	
AFP				
<20.0 ng/ml	42 (29.2)	29	13	0.643
≥20.0 ng/ml	102 (70.8)	76	26	
HBsAg				
Negative	32 (22.2)	19	13	0.089
Positive	111 (77.1)	85	26	
Cirrhosis				
No	21 (14.6)	16	5	0.966
Yes	122 (84.7)	89	33	
TNM stage				
Early (I, II)	41 (28.5)	34	7	0.134
Late (III, IV)	103 (71.5)	71	32	
Multinodularity				
No	70 (48.6)	55	15	0.194
Yes	74 (51.4)	50	24	
Lympho-invasion				
No	131 (91)	97	34	0.522
Yes	11 (9)	8	5	
Venous infiltration				
No	108 (75)	79	29	1
Yes	36 (25)	26	10	
Metastasis and invasion				
No	100 (69.4)	73	27	1
Yes	44 (30.6)	32	12	

HCC: Hepatocellular carcinoma; DSCC1: DNA replication and sister chromatid cohesion 1; IHC: Immunohistochemistry; TNM: Tumor, node, and metastasis; AFP: Alpha-fetoprotein; HBsAg: Hepatitis B virus surface antigen.

MHCC-97H and Hep3B cells transduced with lentiviral *DSCC1* shRNAs or control shRNA were subjected to MTS assay. Compared with the control shRNA-transduced cells, the cell density of the groups treated with DSK1 and DSK2 increased significantly slower in both MHCC-97H and Hep3B cells, which indicates that *DSCC1* knocking down inhibits the growth of HCC cells [Figure 4c].

Discussion

In this study, we found that the *DSCC1* DNA locus is commonly amplified and the *DSCC1* mRNA and protein are overexpressed in HCC. However, the CNV status is only partially involved in overexpressing *DSCC1* mRNA in HCC. Only a weak connection between the mRNA level and the copy number of the *DSCC1* coding gene was found in HCC tissue. The CNV and mRNA expression pattern of *DSCC1* in HCC cell lines also supported this finding. Six of seven HCC cell lines contain overexpressed *DSCC1*, but only three of them harbor *DSCC1* amplifications.

We also found the overexpression of both *DSCC1* mRNA and protein is associated with the bad prognosis of HCC, which indicates that *DSCC1* overexpression may play a role in HCC development. We then proved that some HCC cell lines exhibit oncogene dependence on *DSCC1*. The clonogenic ability of these cells was dramatically inhibited when *DSCC1* was silenced using lentiviral shRNAs. Despite the limited contribution of CNV status to *DSCC1* overexpression in HCC, it appears that CNV status does have a vital impact on the oncogene dependence of HCC cells on *DSCC1*. Cell

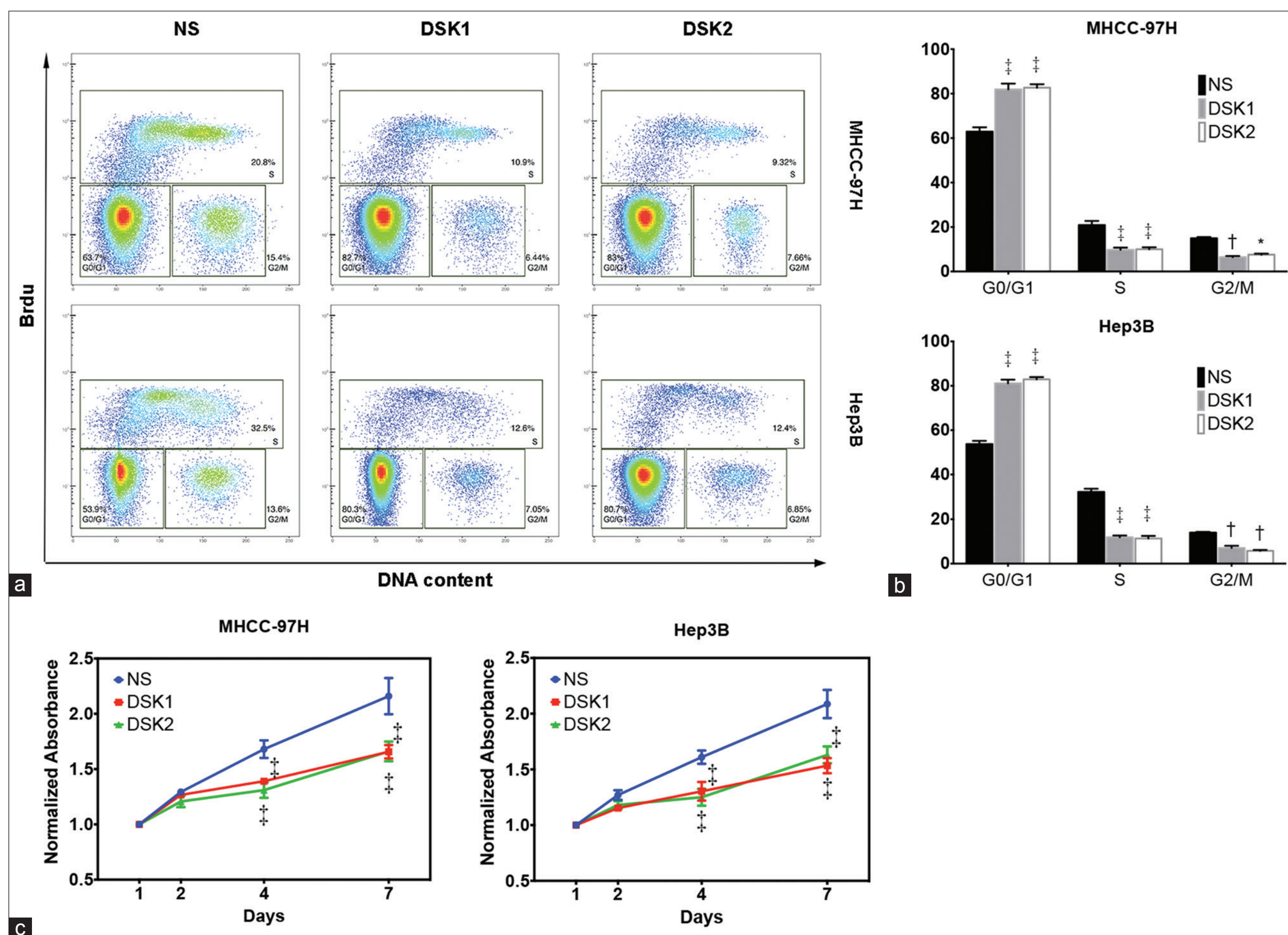

Figure 4: Effect of DSCC1 knockdown on cell cycle kinetics. (a and b) Cell cycle distribution was analyzed by flow cytometry with FITC-conjugated anti-BrdU and 7-AAD staining after lentiviral shRNA transduction (a) and the numerical analysis was performed for each experiment (b). Results are the mean ± SD in three independent experiments. (c) Equivalent number of NS, DSK1, and DSK2 lentiviral transduced cells was plated in 96-well plates, and cell proliferation assays were performed at the indicated time points with MTS assay (**P* < 0.01, †*P* < 0.001, ‡*P* < 0.0001). DSCC1: DNA replication and sister chromatid cohesion 1; SD: Standard deviation.

lines with amplified *DSCC1* locus are dramatically affected in clonogenic assay by *DSCC1* silencing, while cell lines without *DSCC1* amplification maintain normal clonogenic ability after *DSCC1* silencing. These data suggested that *DSCC1* is amplified and overexpressed in HCC and is required for clonogenicity of HCC cell lines.

Moreover, we showed that knockdown of *DSCC1* induced cell cycle arrest and inhibited proliferation in HCC cell lines. Cell cycle distribution in HCC cells was blocked significantly in the G0/G1 phase when knocking down of *DSCC1*. At the meantime, cell growth of HCC cell lines was also greatly inhibited after *DSCC1* silencing. We already know that DSCC1 is a component of the Ctf18-RFC complex and participates in DNA repair, mitosis, and cell cycle regulation.[1,2,4,5,9,10,13-15] We also know that *DSCC1* has anti-apoptotic properties and involved in the development of colorectal cancer.[17] Taken together previous knowledge and findings in this study, we could conclude that *DSCC1* overexpression probably promotes the cell cycle transition and cell division and consequently promotes cell growth in HCC.

With the development of high-throughput genomic technologies, the number of cancer genomic resources is growing at an unprecedented pace. For example, TCGA (http://cancergenome.nih.gov/) project generates comprehensive genome-wide datasets that include data for CNV, somatic mutations, DNA methylation status, gene and exon expression, protein expression, pathway inference, and phenotypes. Taking full advantage of these data, like what we do in this study, could help us to get better understanding of the molecular mechanism of HCC and identify possible treatment targets.

However, several limitations of this study should be noted. First, a limited number of cases were enrolled in this study, which diminished the statistical power of the results. Second, the exact mechanism of how *DSCC1* regulated cell cycle and proliferation was not explored. Moreover, if *DSCC1* could promote HCC development through some other pathways, such as apoptosis or autophagy, that should also be conducted in further studies.

In conclusion, this study successfully identified and validated *DSCC1* as a putative HCC driver gene, and extensive studies

Table 2: Univariate Cox regression analysis of prognostic factors for OS in HCC patients

Items	*HR*	95% *CI*	*P*
Clinicopathological parameters			
Gender (female vs. male)	1.4	0.73–2.50	0.340
Age (<55 vs. ≥55 years)	0.98	0.97–1.00	0.035
Alcohol (no vs. yes)	0.95	0.65–1.40	0.810
Tumor size (<6.2 vs. ≥6.2 cm)	1.1	1.00–1.10	<0.001
Serum AFP (<20.0 vs. ≥20.0 ng/dl)	0.88	0.58–1.30	0.560
HBsAg (negative vs. positive)	0.81	0.52–1.30	0.340
Cirrhosis (no vs. yes)	1.0	0.58–1.70	0.980
TNM (I and II vs. III and IV)	1.5	1.20–2.00	0.001
Multinodularity (no vs. yes)	1.7	1.10–2.50	0.008
Lympho-invasion (no vs. yes)	1.9	1.00–3.40	0.044
Venous infiltration (no vs. yes)	2.6	1.70–4.00	<0.001
Metastasis and invasion (no vs. yes)	2.2	1.50–3.30	<0.001
IHC staining			
DSCC1 (negative vs. positive)	1.9	1.20–2.80	0.003

HCC: Hepatocellular carcinoma; *HR*: Hazard ratio; *CI*: Confidence interval; AFP: Alpha-fetoprotein; TNM: Tumor, node, and metastasis; IHC: Immunohistochemistry; DSCC1: DNA replication and sister chromatid cohesion 1; HBsAg: Hepatitis B virus surface antigen; OS: Overall survival.

Table 3: Multivariate Cox regression analysis of prognostic factors for OS in HCC patients

Items	*HR*	95% *CI*	*P*
Clinicopathological parameters			
Lympho-invasion (no vs. yes)	1.54	0.74–3.20	0.243
Tumor size (<6.2 vs. ≥6.2 cm)	1.09	1.04–1.13	<0.001
TNM (I and II vs. III and IV)	0.91	0.63–1.30	0.592
Venous infiltration (no vs. yes)	2.10	0.84–5.24	0.113
Multinodularity (no vs. yes)	1.18	0.78–1.79	0.441
Metastasis (no vs. yes)	1.02	0.38–2.70	0.969
IHC staining			
DSCC1 (negative vs. positive)	1.79	1.17–2.74	0.007

HCC: Hepatocellular carcinoma; *HR*: Hazard ratio; *CI*: Confidence interval; IHC: Immunohistochemistry; TNM: Tumor, node, and metastasis; DSCC1: DNA replication and sister chromatid cohesion 1; OS: Overall survival.

should be conducted in the future to investigate the role of PPPDE1 in HCC.

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Conflicts of interest

There are no conflicts of interest.

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原发性肝细胞癌中过表达DSCC1促进增殖并与不良预后相关

摘要

背景：DSCC1（也称为DCC1）是在细胞周期S期负载增殖细胞核抗原（PCNA）到DNA的替代复制因子C（RFC）的组成部分。它所在的染色体8q24区段在原发性肝细胞癌(HCC)中存在高频率的扩增，但是DSCC1在HCC发生发展中的作用尚不清楚。

方法：利用HCC患者的DNA拷贝数变异数据(CNV)和转录组测序数据（RNA-Seq），分析DSCC1的拷贝数变异和mRNA转录水平；使用荧光定量PCR和Western Blot分析该基因mRNA和编码蛋白的表达水平。进一步使用慢病毒shRNA敲减DSCC1进行功能学验证，分析敲减DSCC1对HCC细胞系增殖和克隆形成能力的影响。

结果：我们发现HCC中DSCC1的拷贝数显著扩增、mRNA转录水平显著升高；并且其mRNA和蛋白的表达水平与患者的不良预后显著相关。更重要的是，我们发现使用shRNA敲减部分HCC细胞系（Hep3B，MHCC97H和MHCC97L）的DSCC1能显著抑制其克隆形成能力和增殖能力部分，且这些细胞系均存在DSCC1基因区段的扩增。最后，我们还发现敲减DSCC1能够使HCC细胞停滞于G0/G1期和并显著地抑制细胞增殖。

结论： DSCC1是一个潜在的原发性肝细胞癌驱动基因，其过表达能促进增殖并与HCC患者的不良预后相关。