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Original Article

Upregulated expression of hematopoietic cell kinase inhibits tumor proliferation in hepatocellular carcinoma

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Abstract: This research focused on the role of HCK in hepatocellular carcinoma (HCC), which is one of the diseases with a high incidence and mortality. Hematopoietic cell kinase (HCK), as a Src family member of tyrosine kinases, has been reported in many tumors, such as human gastric cancer, breast cancer, colorectal cancer and non-small cell lung cancer. In our present study, we found elevated HCK in HCC specimens by western blot and immunohistochemistrical analyses. However, its role in HCC remains unclear. Furthermore, we obesrved that the expression level of HCK was positively correlated with the tumor grade, tumor size, number of tumor nodes, vein invasion and Ki-67 expression. Kaplan-Meier curve indicated that the high expression of HCK was significantly correlated with poor prognosis. In vitro, the depletion of HCK in HCC cell lines resulted in cells cycle arrest at G1/S phase. Meanwhile, the interference of HCK led to the reduction of proliferation using CCK-8 and plate colony assays. In addition, the reduced expression of HCK attenuated resistance to oxaliplatin in the HCC cells. All these findings prompted that HCK could exhibit an important role in HCC proliferation. In summary, HCK may serve as a novel prognostic marker and be a potential therapeutic target in HCC.

Keywords: HCK, hepatocellular carcinoma, prognosis, cell proliferation

Introduction

Hepatocellular carcinoma (HCC), the most major malignancy worldwide, is the third cause of tumor related death with more than 600 thousand deaths per year [1, 2]. Although surgical resection and liver transplantation are effective curative therapeutic strategies for early-stage HCC, the prognosis of advanced HCCs remains poor because of the high frequencies of vascular invasion, metastasis, frequent intrahepatic spread and resistance to chemotherapy [3, 4]. Therefore, it's important to find novel valuable therapeutic targets and prognostic biomarker for improving overall survival of HCC. Nevertheless, the mechanism of HCC remains unclear. Furthermore, the regulation of cell cycle views importantly and its aberrant may be considered as a fundamental feature in the development of HCC. Thus, the challenge of identifying critical carcinogenic mechanism underlying cell cycle is urgent.

Here we will study one of the nine leaguers of the SRC family, the hematopoietic cell kinase (HCK) [5]. The SRC family of cytoplasmic tyrosine kinases (SFKs) regulates multiple cell progresses including proliferation, differentiation, motility, adhesion and survival [6]. HCK is the only SFK which exists as p59HCK and p61HCK isoforms in human cells by whether they are in myeloid cells or B-lymphocyte lineages [7, 8]. The high expression of HCK is observed in various solid cancers such as human gastric cancer [9], breast cancer [10], colorectal cancer [11] and non-small cell lung cancer [12]. Besides, SRC family tyrosine kinases engage many growth factor signalings [13] and intersetingly, HCK appears to the most important kinases. HCK not only mediates STAT5A activation but also involves in PI3K and AKT to facilitate cell growth and survival [14]. Furthermore, HCK attracts therapeutic targets to inhibit growth of tumor cells directly and depresses the source of changes in the

Table 1. Correlation of HCK and Ki67 expression with clinicopathological factors in 88 HCC specimens

Clinicopathological Factors	No. case	HCK		*P* Value	χ^2 Value
		Low score ≤5	High score >5		
Gender					
Female	24	11	13	0.813	0.121
Male	64	32	32		
Age (years)					
<45	35	15	20	0.391	0.839
≥45	53	28	25		
AFP (ng/ml)					
<50	51	24	27	0.829	0.158
≥50	37	19	18		
Cirrhosis					
Negative	50	23	27	0.667	0.38
Positive	38	20	18		
AJCC grade					
I-II	52	20	32	0.030*	5.504
III-IV	36	23	13		
Tumor size (cm)					
<5	27	19	8	0.011*	7.21
≥5	61	24	37		
No. of tumor nodes					
Single	53	33	20	0.002*	9.577
Multiple	35	19	25		
Capsular formation					
Negative	28	15	13	0.649	0.364
Positive	60	28	32		
Metastasis					
Negative	29	11	18	0.178	2.069
Positive	59	32	27		
Vein invasion					
Negative	35	12	23	0.031*	4.943
Positive	53	31	22		
Ki-67 expression					
Low expression	37	24	13	0.017*	6.542
High expression	51	19	32		

Statistical analyses were carried out using Pearson χ^2 test. *P<0.05 was considered significant.

tumor promoting microenvironment indirectly [5]. What's more, excessive HCK activation can also reduce drug efficacy and contribute to chemo-resistance. All the findings showed that HCK might serve as a cell cycle regulator in tumors. However, whether HCK contributes to HCC development has not yet been clarified.

In this paper, we studied the biological and clinical significance of HCK in HCC. Our research showed that HCK involved in regulating cell cycle and cell proliferation of HCC cells and the repression of HCK critically decayed the level of Stat5. Results implied the reduction of HCK attenuated the proliferation of HCC cells. These findings showed HCK may be novel to mark prognosis and be potential for therapeutic target in HCC.

Materials and methods

Patients and tissue samples

8 paired fresh tissue specimens and 88 HCC and corresponding peritumoral sections were obtained from patients in the Affiliated Hospital of Nantong University between 2007 and 2010. All patients underwent hepatic surgical resection without postoperative systemic chemotherapy. Tissues with clinicopathological data were collected from these patients to be formalin-fixed and paraffin-embedded. The institute approved all the HCC tissues by the ethics committee in this research. The main pathological and clinical characteristics are presented in **Table 1**. The 88 patients whose average age was 47.88 years (range, 22-73) comprised 24 females and 64 males. Tumors were classified into well (grade I-II; n=52) and poorly (grade III-IV; n=36) differentiated HCC according to American Joint Committee on Cancer (AJCC) stage. The total follow-up time for samples was 5 years with a range of 1-90 months. Furthermore, eight paired fresh tissues were surgical removal and then frozen in liquid nitrogen. These samples were stored at -80°C for Western blot analysis.

Immunohistochemistry (IHC)

First, we coated HCC sections with 10% polylysine and mounted five micrometer thick of them on glass. These sections deparaffinized with xylene and proceeded to rehydrate with graded alcohol. The sections were soaked in EDTA (1 mmol/L, pH 8.0) and boiled in citrate buffer (0.01 M, pH 6.0) for high pressure thermal repair for 15 min to retrieve the antigen. Then we used 3% hydrogen peroxide to block their endogenous peroxidase activity. After rinsed by phosphate-buffered saline (PBS, pH 7.2), we incubated the sections with anti-HCK antibody (Sigma-Aldrich, MO, USA, diluted 1:200) overnight at 4°C and anti-Ki-67 antibody (Millipore, Bedford, MA, USA diluted 1:500). Non-specific immunoglobulin IgG (Sigma Chemical Co., St. Louis, MO, USA) were used for negative control at the same concentration as the primary antibody. After washed in PBS twice, slides were incubated with secondary antibodies (biotin-labeled; Santa Cruz) and counterstained with 3,3'-diaminobenzidine tetrahydrochloride (DAB) and 20% hematoxylin. Finally they were dehydrated and cover-slipped. The slides were examined with a Leica CTR5000 microscope (Leica Microsystems, Wetzlar, Germany).

Immunohistochemical evaluation

In order to assess HCK and Ki-67 expression, we chosed at least five high-power fields randomly. Every immunostained slides were evaluated without the knowledge of the patients' clinicopathological information. What's more, at least 500 cells were counted per field to determine the labeling index. In order to avoid possible technical errors, the cell staining was repeated twice. The cell staining was repeated twice to avoid possible technical errors. Immunostaining score was calculated by the radio of stained tumor cells and the intensity of the staining [15]. The intensity of staining was evaluated as follows: 0 (no stained), 1 (weakly stained), 2 (moderately stained), or 3 (strongly stained). The extent of staining was recorded: 0 (≤10%), 1 (11-30%), 2 (31-50%), 3 (51-70%), and 4 (≥71%) according to the percentage of positive tumor cells. The score was evaluated by percentage positive score × staining intensity score and ranged from 0 to 12 which 0-4 was regarded as low expression and 5-12 was regarded as high expression.

Western blot analysis

Fresh frozen tissues and cells were carried out for western blot experiments. Before immunoblotting, cell samples and tissues were promptly resuspended in a homogenization buffer (50 mM Tris HCl pH 7.5, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and complete protease inhibitor cocktail (Roche Diagnostics, Mannheim, Germany)), and then centrifuged at 12,000 rpm, 4°C for 30 min to collect the supernatant liquid. Bio-Rad protein assay (Bio-Rad, Hercules, CA, USA) was used to detect total protein concentrations. The supernatant was diluted in 2× sodium dodecyl sulfate (SDS) loading buffer to be boiled 15 min. Subsequently, the protein samples were subjected equally for 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) separ ation and then transferred to polyvinylidene difluoride filter (PVDF) membranes (Millipore, Bedford, MA, USA). The membranes were blocked with 5% dried skim milk in TBST (20 mM Tris, 150 mM NaCl, 0.05% Tween-20). After 2 hours, primary antibodies were used to incubate them overnight at 4°C. The membrane was washed with TBST three times for 5 min each. Then we used horseradish peroxidase-linked IgG (Pierce Biotechnology, Rockford, IL, USA) as the second antibody at a dilution of 1:5000 according to the manufacturer's instructions for 2 h at a room temperature. The detection of immunecomplexes was visualized by chemiluminescence (NEN Life Science Products, Boston, MA, USA). The experiments are responsible for at least three independent reactions. The primary antibodies used for Western blot were as follows: rabbit polyclonal anti-HCK antibody (Abgent, USA), mouse monoclonal anti-proliferating cell nuclear antigen (PCNA) antibody (Santa Cruz Biotechnology, USA), rabbit polyclonal anti-cyclin D1 antibody (Santa Cruz), rabbit polyclonal anti-Stat5 antibody (Santa Cruz), rabbit polyclonal anti-GAPDH antibody (Santa Cruz), rabbit polyclonal anti-cleaved-caspase-3 antibody (Santa Cruz). Secondary antibody incubation was performed using horseradish peroxidaselinked IgG (Pierce Biotechnology, Rockford, IL, USA).

Cell culture and transient transfection

The hepatocellular carcinoma cell lines (HepG2, Huh7, Hep3B and SMCC-7721) and L02 normal hepatocytes were purchased from the Shanghai

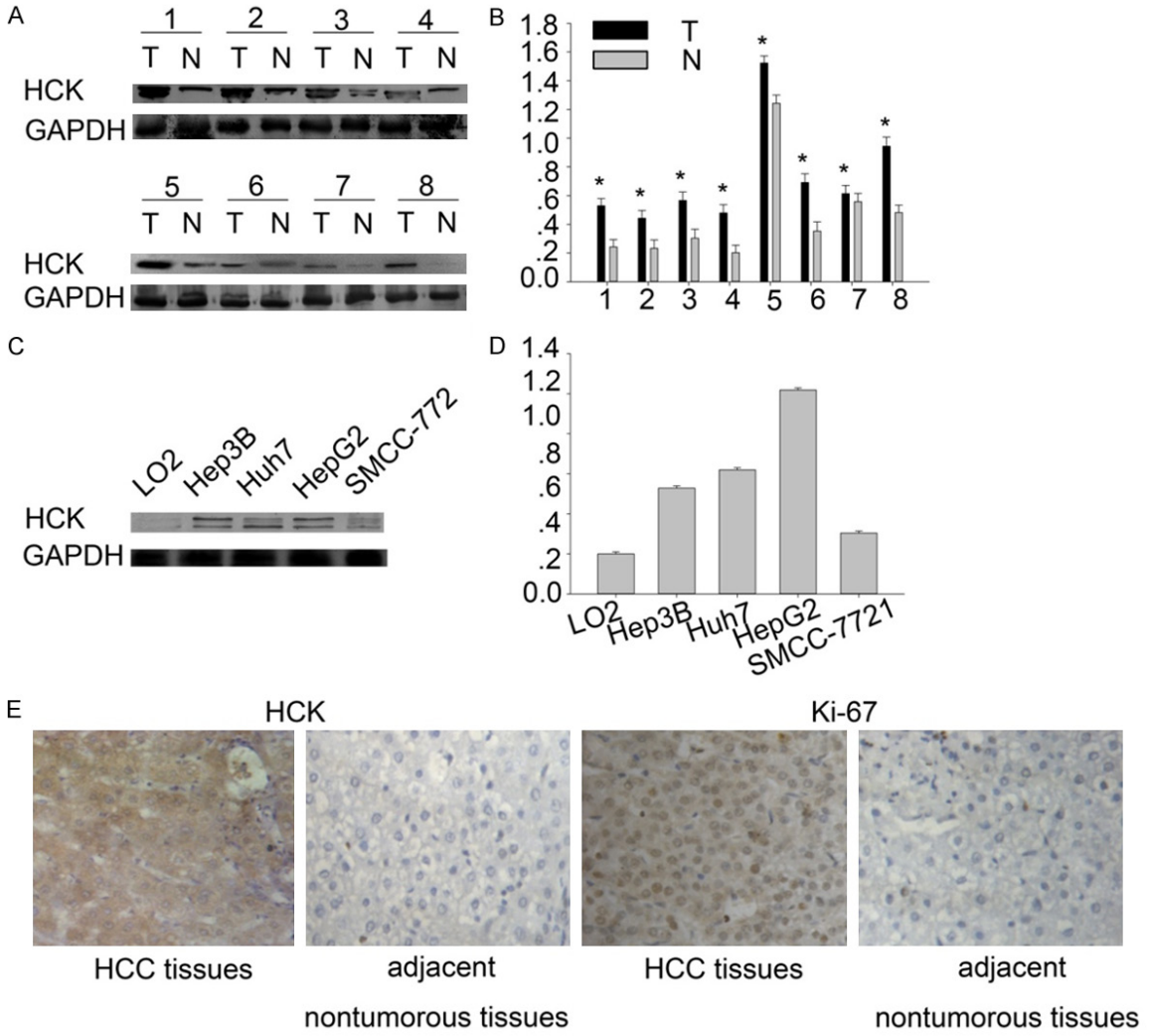

Figure 1. Expression of HCK in human HCC tissues and cell lines. (A, B) Western blot was used to analysis 8 representative paired samples of HCC tissue (T) and adjacent normal tissues (N). Western blot analysis indicates that HCK was overexpressed in HCC (A) and the quantification graph which demonstrates the ratio of HCK protein to GAPDH by densitometry (*P<0.05). (C, D) Western blot analysis shows that HCK protein expression is increased in HCC cells compared with the normal liver cell line (LO2). The bar chart demonstrates the ratio of HCK protein to GAPDH by densitometry. The data are mean ± SEM. GAPDH was used as a control for protein load and integrity. (E) Paraffin-embedded HCC tissue sections were stained with antibodies against HCK, Ki-67 and counterstained with hematoxylin. High HCK and Ki-67 expression was detected in HCC specimens (SP×400). Meanwhile, low levels of HCK expression were seen in the adjacent non-cancerous tissues, whereas low concentrations of Ki-67 were observed in the same adjacent non-cancerous tissues (SP×400).

Institute of Cell Biology. All cell lines were cultured by high-glucose Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO, USA) with 10% fetal bovine serum (FBS) (HyClone, Logan, UT) and 100 U/ml penicillin-streptomycin mixture in 5% CO_2 incubator at 37°C. Control siRNA and HCK siRNA oligos were synthesized by GenePharma. Cell transfection assays were performed with Lipofectamine 2000 transfection reagent (Invitrogen, Carlsbad, CA).

Cell cycle analysis and cell proliferation assay

For flow cytometrical analysis of cell cycle, cells were first harvested at a suitable time, washed twice with ice-cold 1 ml PBS, then fixed with 70% methanol for 24 h at 4°C. After three times washed by PBS, the cells were labeled with propidium iodide as described [16]. At last, the data were subected by the Becton-Dickinson BD FACScan flow cytometer (Becton Dickinson, San Jose, CA, USA) and Cell Quest acquisition

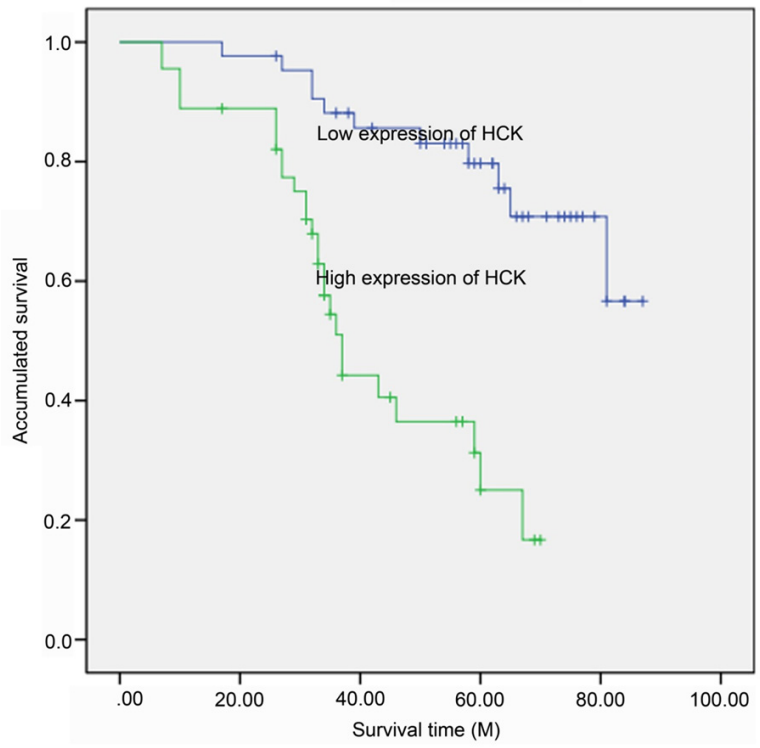

Figure 2. Kaplan-Meier survival curves for low versus high HCK expression in 88 patients with HCC. Based on the median HCK percentages, patients were divided into two groups: high HCK expressers and low HCK expressers. Low versus high Ring1 expression in 88 patients of HCC showed a highly significant separation (P<0.001, log rank test).

and analysis software. What's more, we use Cell Counting Kit-8 (CCK-8, Dojindo, Kumamoto, Japan) assay to evaluate cell viability. In accordance with the manufacturer's protocol, Huh7 and HepG2 cells were inoculated for 24 h into 96-well plate (Corning Inc., Corning NY, USA) to incubate at a density of 2×10^4/well. Then we added CCK-8 reagents to each well and the cells were incubated for another 2 h at 37°C in dark. The absorbance at the wavelength of 490 nm was measured by a microplate reader (Bio-Rad). These experiments were repeated at least three times.

Colony formation assays

Cells were inoculated at a density of 200 cells/well into 6-well plates. Followed by transfected with control-siRNA and HCK-siRNA transfection according to the manufacturer's instructions, we analyzed the clearly visible colonies (C50 cells/colony) using 0.5% crystal violet stain for 30 min after 14 days of the cells cultured.

Annexin-V/PI apoptotic assay

In order to evaluate the apoptosis, the HepG2 cells transfected with control-siRNA and HCK-siRNA were cultured for 48 h and harvested. Annexin V-fluorescein isothiocyanate (FITC) apoptosis detection kit (Dojindo, Kumamoto, Japan) was used in accordance with the manufacturer's protocol. After incubated in the dark at a room temperature for 20 min, the apoptosis assay was performed by flow cytometry (Becton Dickinson, San Jose, CA, USA) in line with the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed with the SPSS 21.0 statistical program. Every experiments were repeated at least three times and all values were presented as mean ± SEM. We used the χ^2 test to analyze the association between HCK and Ki-67 expression and other clinicopathological features. Survival analysis was undertaken with the Kaplan-Meier method and multivariate analysis. Cox's proportional hazards model was performed and P<0.05 was considered to be significant.

Results

The expression of HCK was frequently upregulated in HCC tissues and cells

To determine the aberrant expression of HCK in HCC tissues, Western blot analysis was used to investigate the level of HCK between eight paired fresh HCC and adjacent noncancerous samples. HCK was remarkably higher expressed in tumor tissues than in matched nontumorous ones as shown in **Figure 1A**, **1B**. Furthermore, the expression of HCK protein in several different HCC cell lines (Hep3B, Huh7, HepG2 and SMCC-7721) and a normal hepatocyte cell line (LO2) was also detected. Western blot showed a similar result with tissues that

Table 2. Survival status and clinicopathological parameters in 88 HCC specimens

	Hazard ratio	95% confidence interval	*p* value
Gender	0.684	0.306-1.530	0.355
Age (years)	1.636	0.750-3.571	0.216
AFP (ng/ml)	0.872	0.368-2.067	0.756
Cirrhosis	0.726	0.300-1.756	0.478
AJCC grade	2.271	0.847-6.089	0.103
Tumor size (cm)	1.900	0.686-5.257	0.217
No. of tumor nodes	2.129	0.886-5.117	0.091
Capsular formation	0.785	0.340-1.813	0.571
Metastasis	1.262	0.581-2.740	0.556
Vein invasion	0.913	0.421-1.982	0.818
HCK expression	7.183	2.139-24.114	0.001*
Ki-67 expression	0.156	0.064-0.375	<0.001*

Statistical analyses were carried out using log-rank test
*P<0.05 was considered significant.

the expression of HCK was increased in HCC cell lines, especially in HepG2 cells (**Figure 1C**, **1D**). All these findings implicated that HCK was up-regulated in HCC tissues and in HCC cells.

88 paraffin-embedded HCC clinical specimens were tested by immunohistochemistry (IHC) to verify this observation. Immunohistochemical staining confirmed that the expression of HCK and Ki-67 were higher in HCC tissues than adjacent non-tumorous liver tissues as shown in **Figure 1E**. What's more, we could also find that HCK was almost expressed in the cytoplasm. In addition, the statistical analysis of the HCK expression was summarized in **Table 1**. We divided all of the HCC specimens into 36 cases of poorly differentiated HCC (grade III-IV) and 52 cases of well-differentiated HCC (grade I-II). The positive expression of HCK was significantly correlated with tumor grade (P=0.030), tumor size (P=0.011), number of tumor nodes (P=0.002), vein invasion (P=0.031) and Ki-67 (P=0.017). Gender, age, capsular formation, metastasis and other prognostic factors were not statistical correlated with HCK (P>0.05). What's more, Kaplan-Meier analysis revealed that high expression of HCK was apparently associated with short overall survival (**Figure 2**). Besides, by using the Cox proportional hazards model, we proved that HCK was an independent prognostic indicator in HCC (**Table 2**).

Alteration of HCK expression correlated with the cell cycle progression in vitro

All above proved that HCK was associated with poor patients' survival and HCK. In **Figure 1C**, **1D**, we identified that HCK was obviously higher expressed in different proliferating statuses of HCC cells, especially in HepG2 cells. Then we choose HepG2 cells for the following work in this study. Based on these results, we further explored the potential role of HCK in HCC cell cycle progression. HepG2 cells were arrested in G1 phase after serum starvation for 72 h and then released them from G1 phase and reentered into S phase following serum stimulation. The flow cytometrical analysis showed that S phase were increased and western blot also indicated that the expression level of HCK was upregulated 8 h after serum-addition (**Figure 3A**). Besides, the expression of cell cycle protein (cyclin D1), proliferating cell nuclear antigen (PCNA) were also upregulated similarly (**Figure 3B**, **3C**). These results indicated that HCK played an important role in cell cycle of HCC.

HCK depletion inhibits HCC cell proliferation

Based on the results above, we conjectured that HCK might do important to HCC progression. Then we further explored the role of HCK in HCC progression. Several studies had been reported that Stat5 which was associated with cell growth interacted with HCK. Therefore, we analyzed the association between HCK and Cyclin D1 in HCC cells (**Figure 4A**). In addition, we transfected HepG2 cells with HCK siRNAs to interfere endogenous HCK expression. Con-siRNA were also used as negative control. After 48 h, we detected the expression of HCK and found that the expression of HCK was significantly downregulated in HepG2 cells when transfected HCK-siRNA3#2 as well as Stat5, cyclin D1 and PCNA (**Figure 4B-D**). Besides, CCK-8 assay and flow cytometry analysis proved that the depletion of HCK caused cell cycle arrest and had a role in the proliferation of HepG2 cells (**Figure 4E**, **4F**).

Interference of HCK expression sensitizes HCC cells to doxorubicin

Doxorubicin (DOX) served as an antibiotic with broad spectrum of antitumor activity and as

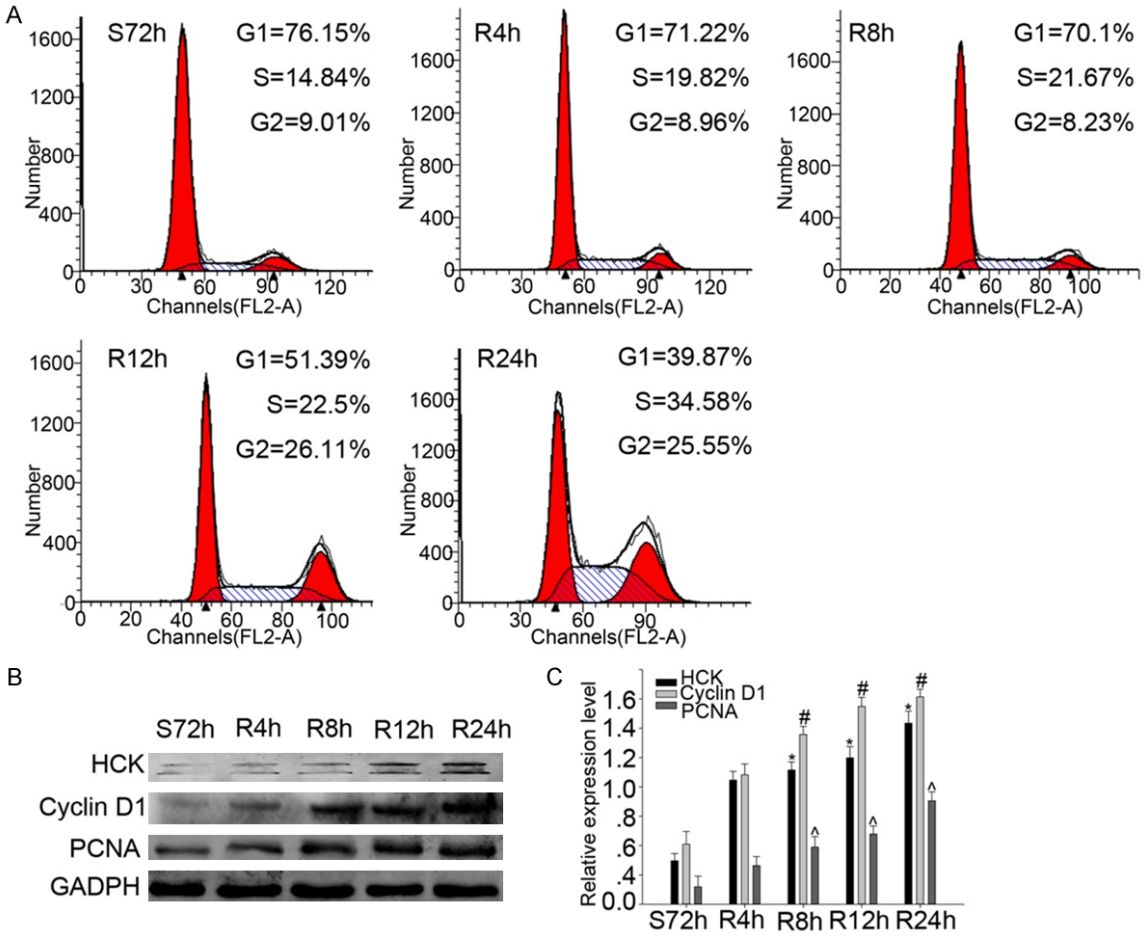

Figure 3. Expression of HCK in proliferating HCC cells. A. Flow cytometry quantitation of cell cycle progress in HepG2 cells. Cells were synchronized at G1 after serum starvation for 72 h and then progressed into cell cycle by adding medium containing 10% FBS for the indicated times (R4 h, R8 h, R12 h, R24 h). The data are mean ± SEM. B. After the same treatment, cell lysates were prepared and analyzed by Western blot with antibodies directed against HCK, Cyclin D1 and PCNA. GAPDH was used as a control for protein load and integrity. C. The bar chart below demonstrates the ratio of HCK, cyclin D1 and PCNA protein to GAPDH by densitometry.

one of the most potent antitumor agents in a variety of solid tumors [17]. After the addition of DOX in different concentrations, we analysed the cell proliferation in HepG2 cells to examinate the sensitivity of HCC cells to DOX. As shown in **Figure 5A**, the cell proliferation rate was decreased with the addition of DOX in a dose-dependent manner. The same method was performed to detect the cell growth rate following transfection of HCK-siRNA#2 and DOX exposure (**Figure 5B**). Moreover, we used Annexin V-FITC/PI double staining to detect the apoptosis rate of HCC cells with depetion of HCK and incubation with DOX (3 μmol/L). And we found that with the lessen of HCK, cell viaility obviously attenuated and the apoptosis cell ratio was dramatically increased. What's more, interference of HCK expression aggravated the cytotoxic effect of DOX on HCC cells (**Figure 5C**). In addition, western blot was used to detected the expression of HCK, Stat5 and cleaved caspase 3 (**Figure 5D**) and colony formation assays were used to prove these results (**Figure 5E**). To sum up, high expression of HCK could contribute to DOX resistance.

Discussion

Hepatocellular carcinoma (HCC) which is one of the most devastating cancer types worldwide, is a complex disease with high metastasis, recurrence [18]. Because the multiple molecular mechanisms of HCC has not yet been fully elucidated, the HCC parent's long-term survival

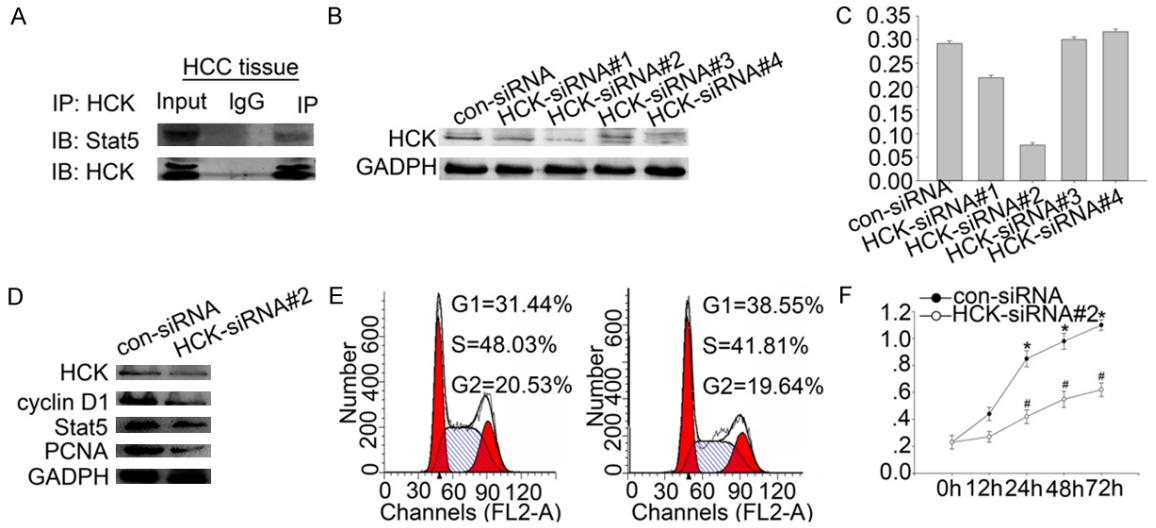

Figure 4. HCK knockdown suppress cellular proliferation and inhibit cell cycle progress. A. Western blot analysis detected the relationship between HCK and Stat5 in HCC tissues by immunoprecipitation. B, C. Western blotting and the bar chart showed that siRNA treatment of HCK in HepG2 cells. The expression level of the cells transfected by HCK-siRNA#2 displayed a more significant decrease. The data are mean ± SEM. D. After siRNA transfection, the level of Stat5, cyclin D1 and PCNA diminished. E. Flow cytometric assay analyzed the cell cycle progression of HepG2 cells that transfected with control-siRNA and HCK-siRNA#2. F. CCK-8 assay showed that HCK knockdown inhibited proliferation progress of HepG2 cells.

remains unsatisfactory. Therefore, the identification of effective targeted agents related with the prognosis of HCC is a great concern in the field of HCC research. HCK, also named hematopoietic cell kinase, was one of SRC family tyrosine kinases. It has be reported that HCK was implicated in many intracellular signaling pathway [19]. However, the role of HCK in HCC remains virtually obscure.

Here, we proved that HCK plays a direct role in HCC progression via regulating the G1/S transition. In the present datum, HCK was obviously upregulated in HCC tissues. Furthermore, we analyzed the relationship between the expression of HCK and clinicopathological factors and found that HCK expression was conspicuous correlated with clinicopathological parameters, such as tumor grade, tumor size, number of tumor nodes, vein invasion and Ki-67 expression. Besides, we used Kaplan-Meier survival analysis to demonstrate that HCK was obviously related to poor overall survival. What's more, we proved that HCK could be an independent prognostic indicator of the patients' overall survival via univariate and multivariate analyses. In particular, CCK-8 assay, flow cytometry analysis, and colony formation analysis indicated that the depetion of HCK in HepG2 cells was able to lead to cell growth arrest. In conclusion, all the datum above demonstrated that HCK could be an efficient molecular target for the treatment of HCC.

The proto-oncogene HCK is the only one member in the SRC family which encodes two forms of protein, -(the larger protein p61hck with molecular weights of 61,000 and the smaller protein p59hck with molecular weights of 59,000) in human cells [20]. Src-family are participated in the invasive capacity of tumor cells [21, 22]. The two proteins are distributed differently between the membranous organelles and cytoplasm. The association of p61hck with membranes is less complete than that of p59hck [23] and the p59hck is related to caveolae whether p61hck is neither associated with caveolae nor palmitoylation [24]. What's more, p61hck is related to the membrane of lysosomes and p59hck is related to the plasma membrane. Interestingly, podosome-like structure presents in tumor cells and is associated with invasion and metastasis in cancer cell [25]. Therefore HCK could be a pharmacologic target to inhibit macrophage tissue infiltration [26]. We will detect the more role of HCK in-depth study in HCC.

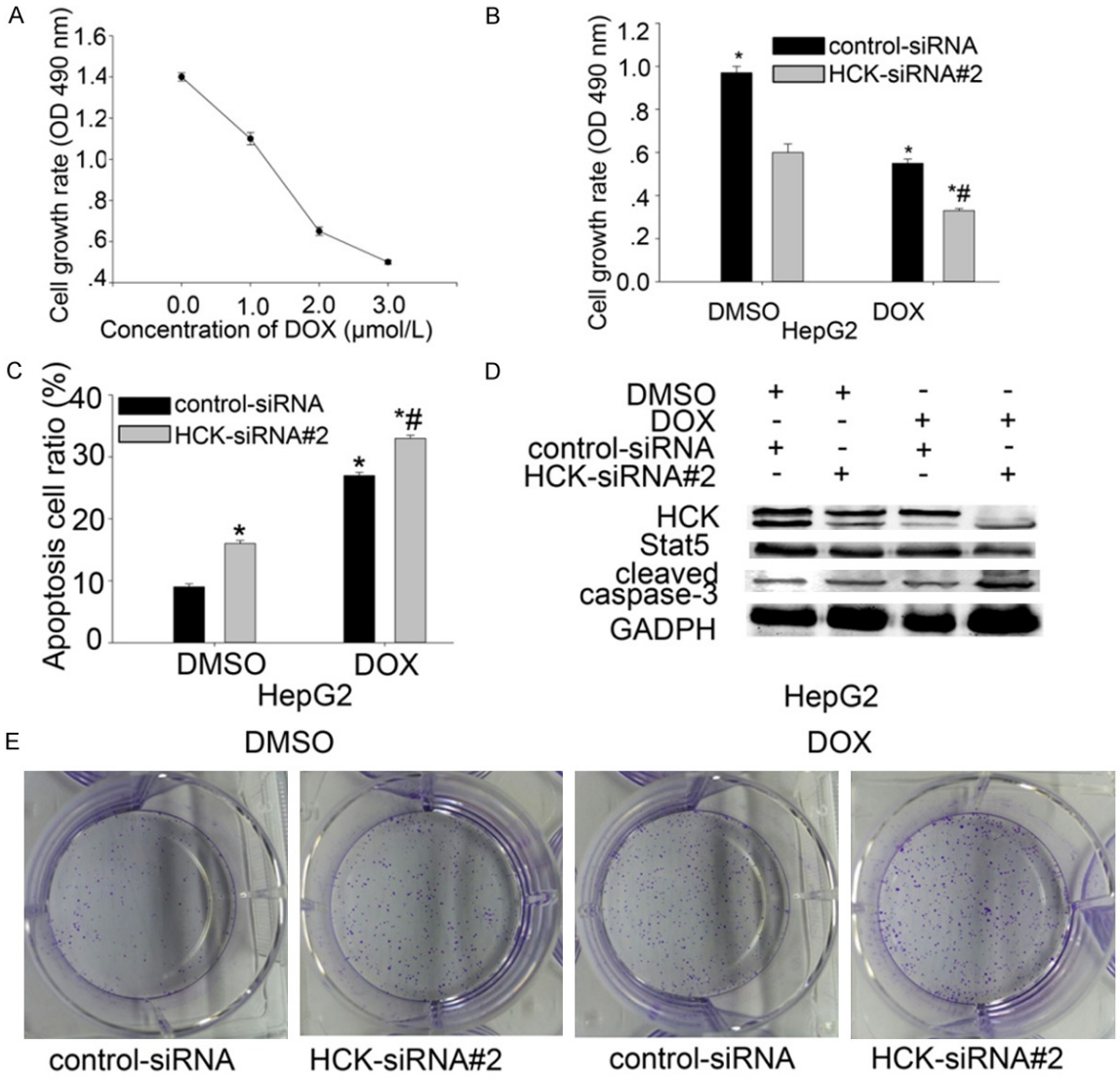

Figure 5. High expression of RBQ-3 in HepG2 and Huh7 cells resulted in doxorubicin resistance. A. CCK-8 assays domestrated the cell growth rate of HepG2 cells following addition of DOX for the indicated concentration. B. 48 h after HCK-3-siRNA#2 and control siRNA transfection, cells were treated with DOX stimulation (3 μmol/L) or not. CCK-8 assays were performed to compare cell viability of control siRNA group with HCK-siRNA#2 group. C. Tunnel assay shown cell apoptosis of HepG2 cells. D. Western blot detected HCK, Stat5 and cleaved caspase 3 expression in HepG2 cells. E. Colony formation analysis of HepG2 cells transfected with HCK-siRNA#2 or not and treated with DOX stimulation (3 μmol/L) or not.

To sum up, our research revealed that HCK was conspicuously overexpressed in HCC for the first time and was associated with Ki-67, tumor grade, tumor size, number of tumor nodes, vein invasion as well as poor prognosis. Furthermore, the expression of HCK was related to cell cycle progression and the reduction of HCK critically attenuated cell proliferation in HCC. It also enhanced sensitivity to doxorubicin in HCC cells. Thus, we believe that HCK do important to the diagnostic and therapeutic strategies of HCC.

Disclosure of conflict of interest

None.

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