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ORIGINAL ARTICLE

Basic Study

Cell division cyclin 25C knockdown inhibits hepatocellular carcinoma development by inducing endoplasmic reticulum stress

Yan-Fei Li, Fang-Yuan Zheng, Xin-Yu Miao, Hai-Long Liu, Yao-Yao Zhang, Nai-Xia Chao, Fa-Rong Mo

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Yan-Fei Li, Fang-Yuan Zheng, Xin-Yu Miao, Hai-Long Liu, Yao-Yao Zhang, Nai-Xia Chao, Fa-Rong Mo, School of Basic Medical Sciences, Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Nai-Xia Chao, Key Laboratory of Biological Molecular Medicine Research (Guangxi Medical University), Education Department of Guangxi Zhuang Autonomous Region, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Fa-Rong Mo, Key Laboratory of Human Development and Disease Research (Guangxi Medical University), Education Department of Guangxi Zhuang Autonomous Region, Nanning 530021, Guangxi Zhuang Autonomous Region, China

Co-first authors: Yan-Fei Li and Fang-Yuan Zheng.

Co-corresponding authors: Fa-Rong Mo and Nai-Xia Chao.

Corresponding author: Fa-Rong Mo, MD, Professor, School of Basic Medical Sciences, Guangxi Medical University, No. 22 Shuangyong Road, Nanning 530021, Guangxi Zhuang Autonomous Region, China. farongmo@126.com

Abstract

BACKGROUND

Cell division cyclin 25C (*CDC25C*) is a protein that plays a critical role in the cell cycle, specifically in the transition from the G2 phase to the M phase. Recent research has shown that *CDC25C* could be a potential therapeutic target for cancers, particularly for hepatocellular carcinoma (HCC). However, the specific regulatory mechanisms underlying the role of *CDC25C* in HCC tumorigenesis and development remain incompletely understood.

AIM

To explore the impact of *CDC25C* on cell proliferation and apoptosis, as well as its regulatory mechanisms in HCC development.

METHODS

Hepa1-6 and B16 cells were transduced with a lentiviral vector containing shRNA interference sequences (LV-*CDC25C* shRNA) to knock down *CDC25C*. Subsequently, a xenograft mouse model was established by subcutaneously injecting transduced Hepa1-6 cells into *C57BL/6* mice to assess the effects of *CDC25C*

knockdown on HCC development *in vivo*. Cell proliferation and migration were evaluated using a Cell Counting Kit-8 cell proliferation assays and wound healing assays, respectively. The expression of endoplasmic reticulum (ER) stress-related molecules (glucose-regulated protein 78, X-box binding protein-1, and C/EBP homologous protein) was measured in both cells and subcutaneous xenografts using quantitative real-time PCR (qRT-PCR) and western blotting. Additionally, apoptosis was investigated using flow cytometry, qRT-PCR, and western blotting.

RESULTS

CDC25C was stably suppressed in Hepa1-6 and B16 cells through LV-*CDC25C* shRNA transduction. A xenograft model with *CDC25C* knockdown was successfully established and that downregulation of *CDC25C* expression significantly inhibited HCC growth in mice. *CDC25C* knockdown not only inhibited cell proliferation and migration but also significantly increased the ER stress response, ultimately promoting ER stress-induced apoptosis in HCC cells.

CONCLUSION

The regulatory mechanism of *CDC25C* in HCC development may involve the activation of ER stress and the ER stress-induced apoptosis signaling pathway.

Key Words: Cell division cyclin 25C; Hepatocellular carcinoma; Endoplasmic reticulum stress; Proliferation; Apoptosis

Core Tip: In the current study, cell division cyclin 25C (*CDC25C*) is an important cell cycle regulatory protein and a potential target for cancer treatment. *CDC25C* knockdown not only inhibited cell proliferation and migration but also significantly increased the endoplasmic reticulum (ER) stress response. Furthermore, *CDC25C* knockdown promoted ER stress-induced apoptosis in hepatocellular carcinoma (HCC) cells. The regulatory mechanism of *CDC25C* in HCC development might be related to the activation of ER stress and the ER stress-induced apoptosis signaling pathway.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a prevalent form of cancer and is the third leading cause of cancer-related deaths worldwide[1]. The development of HCC is attributed primarily to oxidative stress or inflammation, and a majority of patients are diagnosed with advanced tumors[2]. Tumor cells exhibit uncontrolled proliferation, which is a key characteristic of an aberrant cell cycle. Cell cycle regulation is influenced by Cell division cyclin 25C (*CDC25C*), a crucial protein that plays an important role in the initiation, progression, and prognosis of cancer. Numerous studies have reported elevated levels of *CDC25C* expression in various malignancies[3]. Furthermore, *CDC25C* has been recognized as a potential gene associated with tumor antigens and has demonstrated efficacy in hepatoma immunotherapy[4]. However, the specific regulatory mechanisms underlying the role of *CDC25C* in HCC tumorigenesis and development remain incompletely understood.

Hepatocytes contain a very large amount of endoplasmic reticulum (ER), which is essential for various cellular processes, such as protein synthesis, storage, folding, assembly, and transport. Proper protein folding in the ER requires a highly oxidized environment and the presence of molecular chaperones. However, the tumor microenvironment, which is characterized by conditions such as hypoxia and low nutrient levels, can disrupt ER function and cause the accumulation of unfolded or misfolded proteins, leading to ER stress[5,6]. Glucose-regulated protein 78 (*GRP78*) is a key chaperone in the ER and is also a major inducer of the ER stress response[7]. When protein folding in the ER fails, unfolded proteins accumulate and compete for *GRP78*, triggering the unfolded protein response (UPR). Through the UPR, a transcriptional pathway is activated to restore normal ER function. The splicing of X-box binding protein-1 (*XBP-1*) mRNA is commonly used as a marker for ER stress/UPR activation[8]. Another important transcription factor involved in ER stress-induced apoptosis is C/EBP homologous protein (*CHOP*)[9]. Persistent ER stress is a characteristic of cancer cells. ER stress-induced apoptosis has been implicated in the progression of various cancers, including prostate cancer, lung cancer, and HCC[10-12]. Studies have shown that inhibiting *CDC25C* can induce cell cycle arrest and promote tumor cell apoptosis[13]. Therefore, *CDC25C* may play a role in the occurrence and development of HCC by activating ER stress signaling pathways.

In this study, we aimed to determine the roles of *CDC25C* in cell proliferation, migration, and apoptosis by conducting *CDC25C* knockdown in Hepa1-6 cells and subcutaneous xenografts established with these cells. Furthermore, we investigated the potential involvement of *CDC25C* in activating ER stress. The findings from our study will be beneficial for

future molecular research on the regulatory mechanism of *CDC25C* in the development of HCC.

MATERIALS AND METHODS

Cell lines and cell culture

The mouse HCC cell line Hepa1-6 and the mouse melanoma cell line B16, which were derived from *C57BL/6* mice, were obtained from the Cell Bank of the Chinese Academy of Medical Sciences in Beijing, China. Hepa1-6 cells were cultured in Dulbecco's modified Eagle's medium (HyClone, Los Angeles, CA, United States) supplemented with 10% fetal bovine serum (FBS) (Dojindo, Tokyo, Japan), 100 U/mL penicillin (Wisent, Nanjing, China), and 100 μg/mL streptomycin (Wisent). B16 cells were cultured in RPMI 1640 medium (Wisent) supplemented with 10% FBS, 100 U/mL penicillin, and 100 μg/mL streptomycin. The cells were incubated at 37 °C with 5% CO_2 and 95% humidity.

Plasmid construction and cell transduction

The lentiviral empty vector (LV3H1/GFP and Puro) and the lentiviral vector containing the *CDC25C* shRNA interference sequence (LV-*CDC25C* shRNA) were purchased from GenePharma (Suzhou, China). Hepa1-6 and B16 cells were seeded in 24-well plates at a density of 5 × 10^4 cells/well and cultured until they reached 70%-80% confluence. *CDC25C* expression was suppressed by transducing cells with LV-*CDC25C* shRNA, and these cells served as the experimental (shRNA-*CDC25C*) group. The empty lentiviral vector was transduced to establish the negative control (shRNA-NC) group, and uninfected cells were used as the blank control (MOCK) group. The transduction efficiency was monitored using fluorescence microscopy. Stably transduced cells were selected using 4 μg/mL puromycin (Solarbio, Beijing, China) and expanded for further experiments.

Establishment of a xenograft model in mice

C57BL/6 mice (age: 4 wk; weight: 14-16 g) were obtained from Changsha Tianqin Bioscience Co., Inc. (Changsha, China). The mice were housed in a specific pathogen-free animal room at a temperature of 22 °C-25 °C with ad libitum access to water and food. To establish the experimental group, infected Hepa1-6 cells (1 × 10^7) were subcutaneously injected into mice in the anterior right subaxillary region (N = 15). The body weight and tumor volume were measured every 5 d. Twenty days post-injection, the tumor tissues were harvested and weighed. All animal experiments were reviewed and approved by the Ethics Review Committee for Animal Experiments at Guangxi Medical University (Approval Number: 202201093).

RNA extraction and quantitative real-time PCR

Total RNA was isolated from cells/tissues using TRIzol reagent (TaKaRa, Dalian, China) following the manufacturer's protocol. Subsequently, the isolated RNAs were reverse transcribed into cDNAs using a PrimeScript RT Reagent Kit (TaKaRa). mRNA expression levels were measured using quantitative real-time PCR (qRT-PCR) with a SYBR Green Master Mix Kit (TaKaRa) in a Step One Real-time PCR System (Applied Biosystems, Waltham, MA, United States). The sequences of the primers used for amplification are provided in Table 1. To ensure accuracy, *GAPDH* was used as an internal control. The amplification reaction consisted of an initial denaturation step at 95 °C for 5 s followed by 40 cycles of denaturation at 95 °C for 5 s and annealing/extension at 60 °C for 30 s. Negative controls without template DNA were included, and each reaction was performed in triplicate. The relative expression levels of the target genes were calculated using the $2^{-\Delta\Delta Ct}$ method.

Cell proliferation assay

A Cell Counting Kit-8 (CCK-8) assay kit (Dojindo) was used to assess the proliferation of Hepa1-6 and B16 cells. Transduced cells were seeded in 96-well plates at a density of 2 × 10^3 cells per well. Then, 0, 24, 48, and 72 h post-transfection, the cells were incubated with 10 μL of CCK-8 solution for 2 h at 37 °C. The optical density at 450 nm was then measured using a microplate reader (BioTek Synergy H1, Winooski, United States).

Wound healing assay

The migration ability of Hepa1-6 and B16 cells was assessed using a wound healing assay. After transduction for 24 h, cells were seeded in 24-well plates at a density of 2 × 10^5 cells/well and cultured for an additional 24 h. Subsequently, a uniform scratch was made in the cell layer on the bottom of the plate, and the detached cells were removed by washing using 1 × phosphate-buffered saline (PBS) buffer. The cells were then cultured at 37 °C with 5% CO_2, and the scratch wounds were photographed at the 0, 12, and 24 h time points. The migration rate was determined by measuring the scratch width using ImageJ online software.

Western blot analysis

Total protein was isolated from cells/tissues using a total protein extraction kit (Vazyme, Nanjing, China) following the manufacturer's protocol. The proteins in the samples were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes (BD, United States). After completion of the blocking step, the membranes were incubated overnight at 4 °C with diluted primary antibodies (Table 2). *GAPDH* was used as a loading control. Subsequently, the membranes were incubated at room temperature for 2 h with the appropriate secondary antibody (rabbit, FDR007, Fdbio) at a 1:5000 dilution. Finally, the protein bands were visualized using an ECL kit (Fdbio, Hangzhou, China) in a FluorChem FC3

Table 1 Primers used in this study

Gene	Forward	Reverse
CDC25C	5′-CCTTGGATTCATCTGGACCTCTG-3′	5′-ATGTGCTACGCTTTGCAATCTTC-3′
GRP78	5′-GAAGAGCTGTGCTCGGACCT-3′	5′-CAGCAGCTTCTGCACCTTGG-3′
XBP-1	5′-CAGCAAGTGGTGGATTTGGAAG-3′	5′-TCTTAACTCCTGGTTCTCAACCACA-3′
CHOP	5′-GTCACACGCACATCCCAAA-3′	5′-GTTCTTCCTTGCTCTTCCTCCTC-3′
Caspase 9	5′-GGGACAGTGGCAGTAATGTCTTCA-3′	5′-AACTCAATGCCCAGTGTTCCAG-3′
Caspase 3	5′-TGAGCAGAGTCAAAGGCTGGAA-3′	5′-AGCTATAATGCATGCTATGGTGGTC-3′
GAPDH	5′-TGTGTCCGTCGTGGATCTGA-3′	5′-TTGCTGTTGAAGTCGCAGGAG-3′

CDC25C: Cell division cyclin 25C; *GRP78*: Glucose-regulated protein 78; *XBP-1*: X-box binding protein-1; *CHOP*: C/EBP homologous protein.

Table 2 Primary antibodies used in this study

Antibody	Species	Catalog	Company	Dilution
CDC25C	Rabbit	E302	Cambridge, MA	1:1000
GRP78	Rabbit	3177S	Cell Signaling Technology	1:500
XBP-1	Rabbit	AF5110	Affinity Biosciences	1:500
CHOP	Mouse	66741-1-lg	Proteintech	1:200
Caspase 9	Rabbit	abs145753	Absin Bioscience	1:2000
Caspase 3	Rabbit	ab184787	Abcam Cambridge, United Kingdom	1:500
GAPDH	Rabbit	FD0063	Fdbio	1:2000

CDC25C: Cell division cyclin 25C; *GRP78*: Glucose-regulated protein 78; *XBP-1*: X-box binding protein-1; *CHOP*: C/EBP homologous protein.

System (ProteinSimple, United States). Protein expression was quantified using ImageJ online software and is expressed as a ratio of target protein expression to *GAPDH* expression.

Cellular apoptosis assay

The apoptosis of Hepa1-6 cells was detected using a FITC Annexin V apoptosis detection kit (Keygen, Nanjing, China). After transduction, cells were fully detached with 0.25% EDTA-free trypsin and then washed twice with 1 × PBS at 4 °C. Subsequently, the cells were seeded in 6-well plates at a density of 1 × 10^6 cells/well and stained with 5 mL of FITC-Annexin V and 5 mL of PI for 15 minutes at room temperature in the dark, and each reaction was repeated three times. The apoptosis rate was analyzed using a flow cytometer (Tree Star, Ashland, OR, United States).

Statistical analysis

Statistical analyses were conducted using SPSS Statistics 22.0 (SPSS Inc., Chicago, IL, United States). Quantitative data are presented as the mean ± SD values. Student's *t* test was used for statistical analyses. A *P* value less than 0.05 was considered to indicate statistical significance.

RESULTS

Stable CDC25C knockdown in the Hepa1-6 and B16 cell lines

To investigate the role of *CDC25C* in HCC development, we established a *CDC25C* knockdown model in the Hepa1-6 cell line by transduction of the LV-*CDC25C* shRNA plasmid. The B16 cell line was subjected to the same process and served as a positive control. After 72 h of transduction, we observed the Hepa1-6 and B16 cells under an inverted fluorescence microscope to assess the expression of green fluorescent protein (GFP). High levels of GFP were detected in the shRNA-*CDC25C* and shRNA-NC groups but not in the MOCK group (Figure 1A). After 10 d of puromycin selection, the transduction efficiency of the lentiviral vector in Hepa1-6 cells was found to exceed 90% (Figure 1A). Immunoblotting using an anti-*CDC25C* antibody confirmed the presence of a 55 kDa band in the total protein lysate, and the size of this band was reduced in the shRNA-*CDC25C* group (Figure 1B). *CDC25C* mRNA expression was further evaluated using qRT-PCR, which showed a significant decrease in the shRNA-*CDC25C* group ($P < 0.001$) (Figure 1C). These results

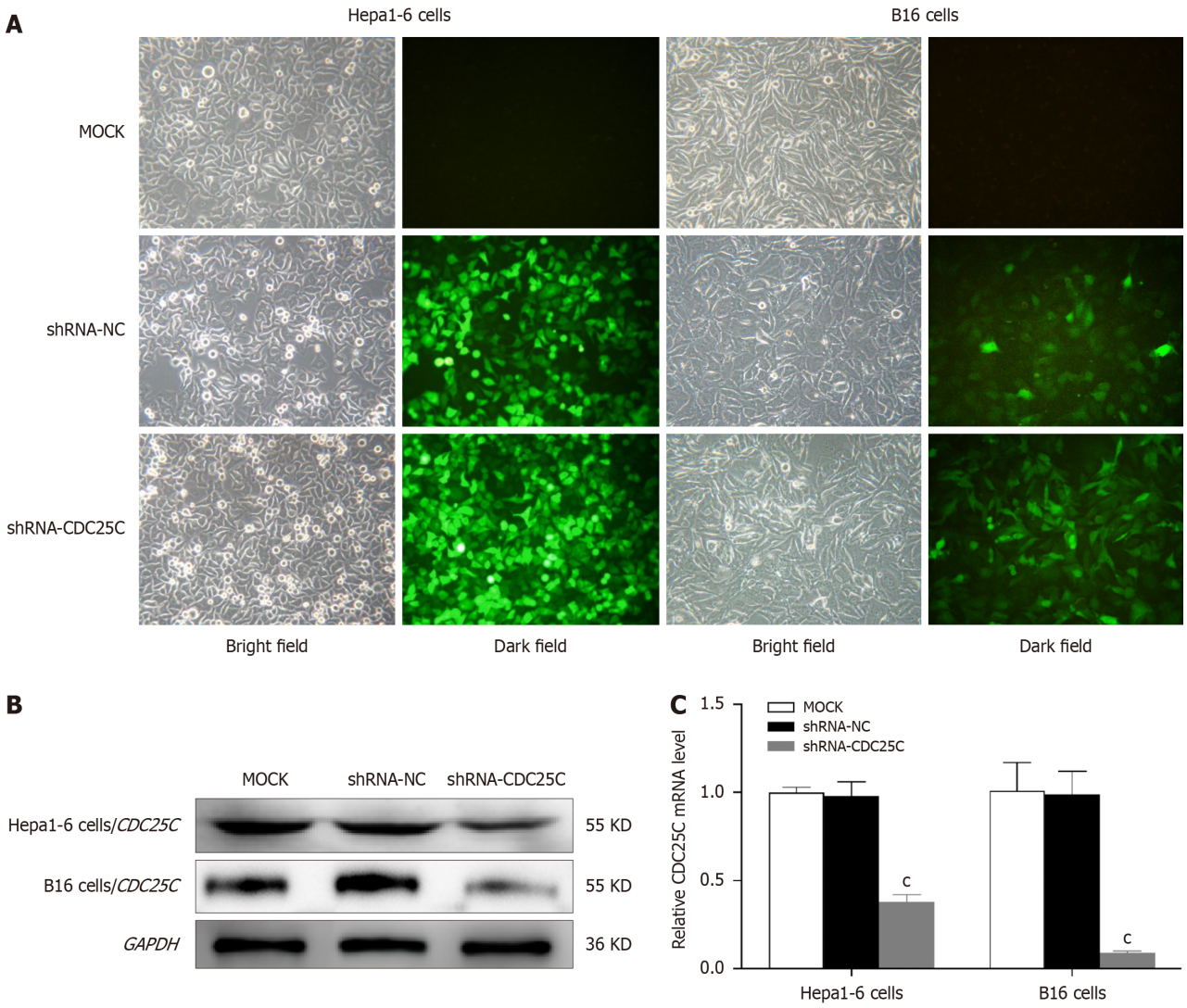


Figure 1 Cell division cyclin 25C down-regulated expression in Hepa1-6 and B16 cells. A: The green fluorescent protein expression in Hepa1-6 and B16 cells was observed at a magnification of × 200; B: The *CDC25C* expression in Hepa1-6 and B16 cells was detected using western blotting, with *GAPDH* serving as the internal control; C: The relative expression of *CDC25C* was assessed at the transcriptional level using quantitative real-time PCR. The data represents the mean ± SD of three independent experiments. $^{c}P < 0.001$. *CDC25C*: Cell division cyclin 25C.

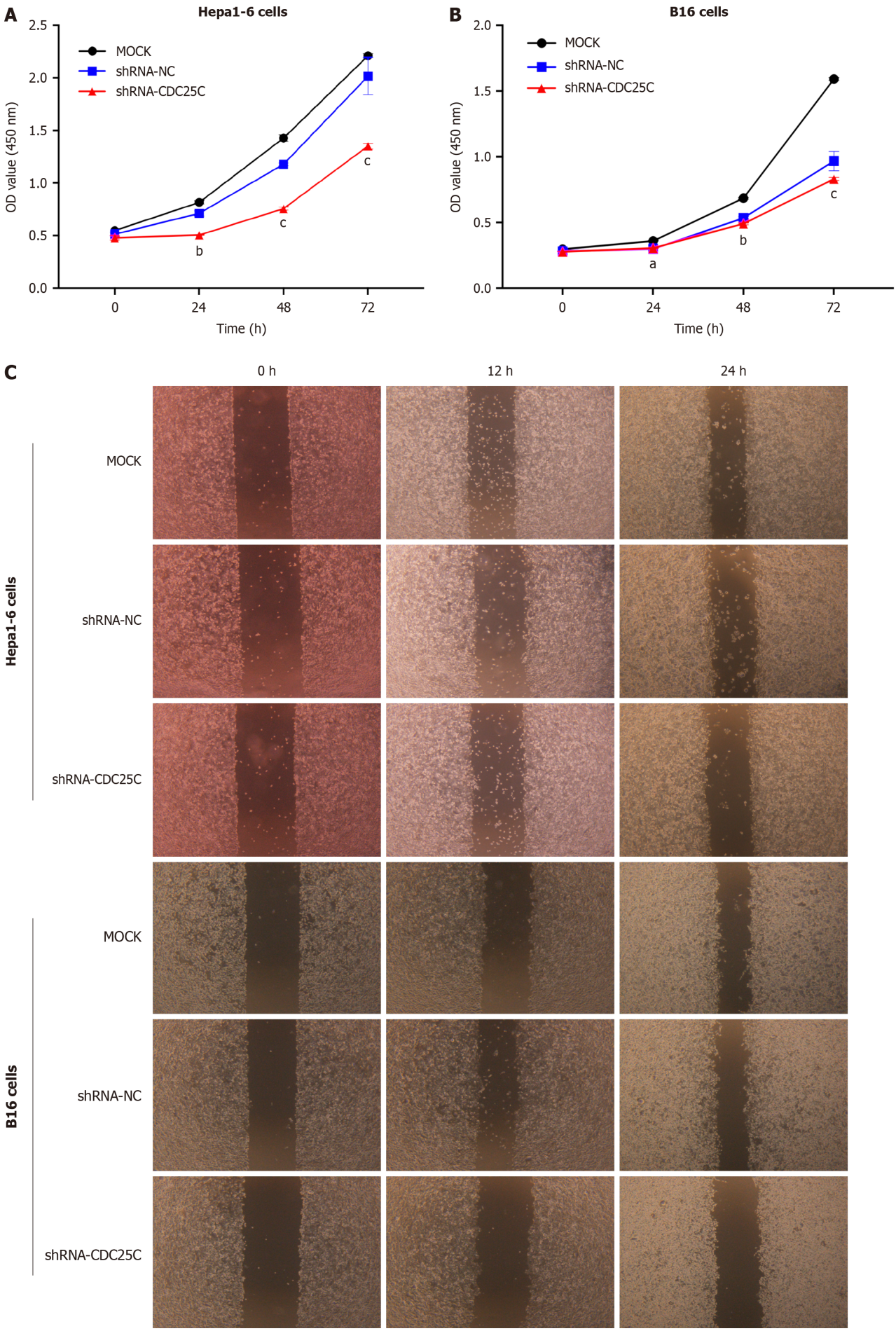
demonstrated that *CDC25C* was stably suppressed in Hepa1-6 and B16 cells through LV-*CDC25C* shRNA transduction.

CDC25C knockdown inhibited tumor cell proliferation and migration

After establishing *CDC25C* knockdown cells, we performed CCK-8 cell proliferation and wound healing assays to assess the proliferation and migration, respectively, of the Hepa1-6 and B16 cells. In the CCK-8 assay, we observed that the cells in the shRNA-*CDC25C* group had significantly attenuated proliferation compared to those in the MOCK and shRNA-NC groups at 24, 48, and 72 h post-transduction (Figure 2A and B). Additionally, the wound healing assay revealed that *CDC25C* knockdown reduced the migration ability of the cells. At both 12 and 24 h post-wounding, the migration rate in the shRNA-*CDC25C* group was significantly lower than that in either the MOCK or shRNA-NC group (Figure 2C). These findings collectively indicate that *CDC25C* knockdown inhibits the proliferation and migration of Hepa1-6 and B16 cells.

CDC25C knockdown inhibited HCC growth in xenograft mouse model

To further investigate the role of *CDC25C* knockdown in HCC development *in vivo*, we established a xenograft mouse model by subcutaneously injecting transduced Hepa1-6 cells into mice. The weights of the mice were recorded, and tumor growth curves were plotted every five days after xenografting. The body weight of mice in the shRNA-*CDC25C* group was significantly lower than that of mice in the MOCK group, but the difference in body weight between the mice in the shRNA-*CDC25C* group and those in the shRNA-NC group was not statistically significant (Figure 3A). Additionally, the volume and weight of the tumor tissues were noticeably reduced in the shRNA-*CDC25C* group at 20 d post-xenografting (Figure 3B). To confirm that *CDC25C* was knocked down in the experimental group, western blotting was performed and revealed a significant decrease in *CDC25C* protein expression in the shRNA-*CDC25C* group ($P < 0.001$) (Figure 3C). These findings indicated that a xenograft model with *CDC25C* knockdown was successfully established and that downregulation of *CDC25C* expression significantly inhibited HCC growth in *C57BL/6* mice.


A
Hepa1-6 cells
MOCK
shRNA-NC
shRNA-CDC25C
OD value (450 nm)
Time (h)
B
B16 cells
C
0 h
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24 h
Hepa1-6 cells
B16 cells

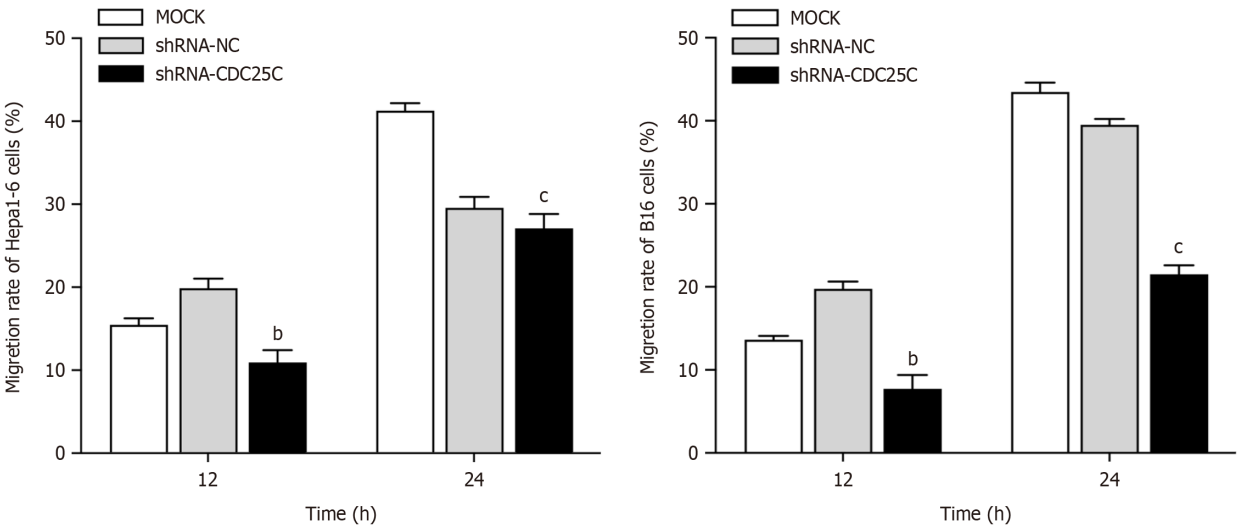


Figure 2 Cell division cyclin 25C knockdown inhibited proliferation and migration in Hepa1-6 and B16 cells. A and B: The proliferation of Hepa1-6 and B16 cells was measured using a Cell Counting Kit-8 assay, with the results reported as optical density 450 nm; C: The migration ability of Hepa1-6 and B16 cells was evaluated using a wound healing assay at × 100 magnification. The data are presented as means. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. OD: Optical density.

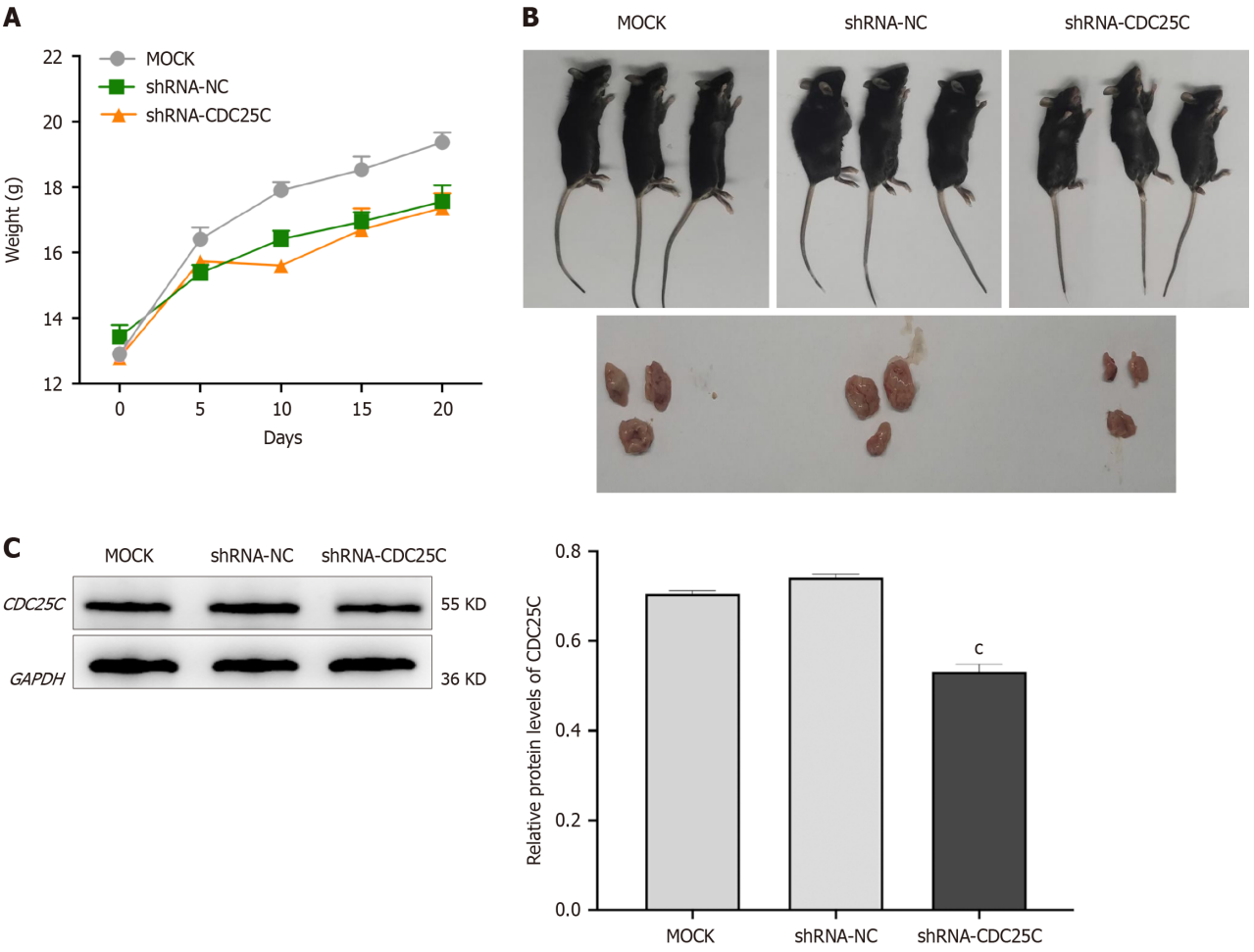


Figure 3 Cell division cyclin 25C knockdown inhibited hepatocellular carcinoma growth in xenograft mouse model. A: The growth curve of xenograft mice was plotted based on body weight measurements; B: The volume and weight of tumor tissues were measured at 20 d post-xenografting; C: The *CDC25C* protein expression was detected using western blotting, with *GAPDH* as the internal control. $^{c}P < 0.001$. *CDC25C*: Cell division cyclin 25C.

CDC25C knockdown induced ER stress and activated ER stress-induced apoptosis

To further investigate the regulatory mechanism of *CDC25C* in HCC development, we examined the expression of ER stress-related proteins in *CDC25C*-knockdown cells and subcutaneous xenografts formed from these cells using qRT-PCR and western blotting. The results revealed increased levels of *GRP78*, *XBP-1*/*XBP-1*s (spliced form in the western blot analysis), and *CHOP* in the shRNA-*CDC25C* group (Figure 4A and B). Among these ER-related molecules, *GRP78* exhibited the highest expression level, indicating its importance as an ER chaperone. Furthermore, the increase in *GRP78* expression after *CDC25C* downregulation was greater in Hepa1-6 cells than in the corresponding xenograft tumor tissues. As *CHOP* plays a crucial role in ER stress-induced apoptosis, we conducted flow cytometric analysis of apoptosis. The flow cytometric analysis results demonstrated a significantly greater number of apoptotic cells in the shRNA-*CDC25C* group (Figure 4C). The mean apoptosis rates of the MOCK, shRNA-NC and shRNA-*CDC25C* groups were 6.423 ± 3.376, 7.763 ± 1.762, and 14.953 ± 1.980, respectively ($P < 0.05$) (Figure 4D). We also examined the expression of apoptotic proteins at the transcriptional and translational levels and found increased expression of *Caspase 9* and *Caspase 3* after *CDC25C* knockdown (Figure 4A and B). In conclusion, these data suggest that *CDC25C* knockdown induces ER stress and apoptosis in HCC cells.

DISCUSSION

One of the key characteristics of tumor cells is their abnormal cell cycle, which is often accompanied by abnormal expression of cell cycle-related proteins such as *CDC25C*. In tumor cells, this abnormal expression has been linked to metastatic potential, drug resistance, and the ability to evade cell death[3]. In this study, our focus was on investigating the role of *CDC25C* in the development of HCC. To this end, we established a *CDC25C* knockdown model in Hepa1-6 cells and used a similarly established B16 cell model as a positive control. Previous research has shown that the B16 cell model is suitable for studying the antitumor effects of inducing cell cycle arrest[14]. Our results demonstrated that *CDC25C* knockdown significantly inhibited the proliferation and migration of the experimental cells. Furthermore, we observed that *CDC25C* knockdown also inhibited tumor growth in mice. These findings suggest that *CDC25C* could be a promising therapeutic target for HCC.

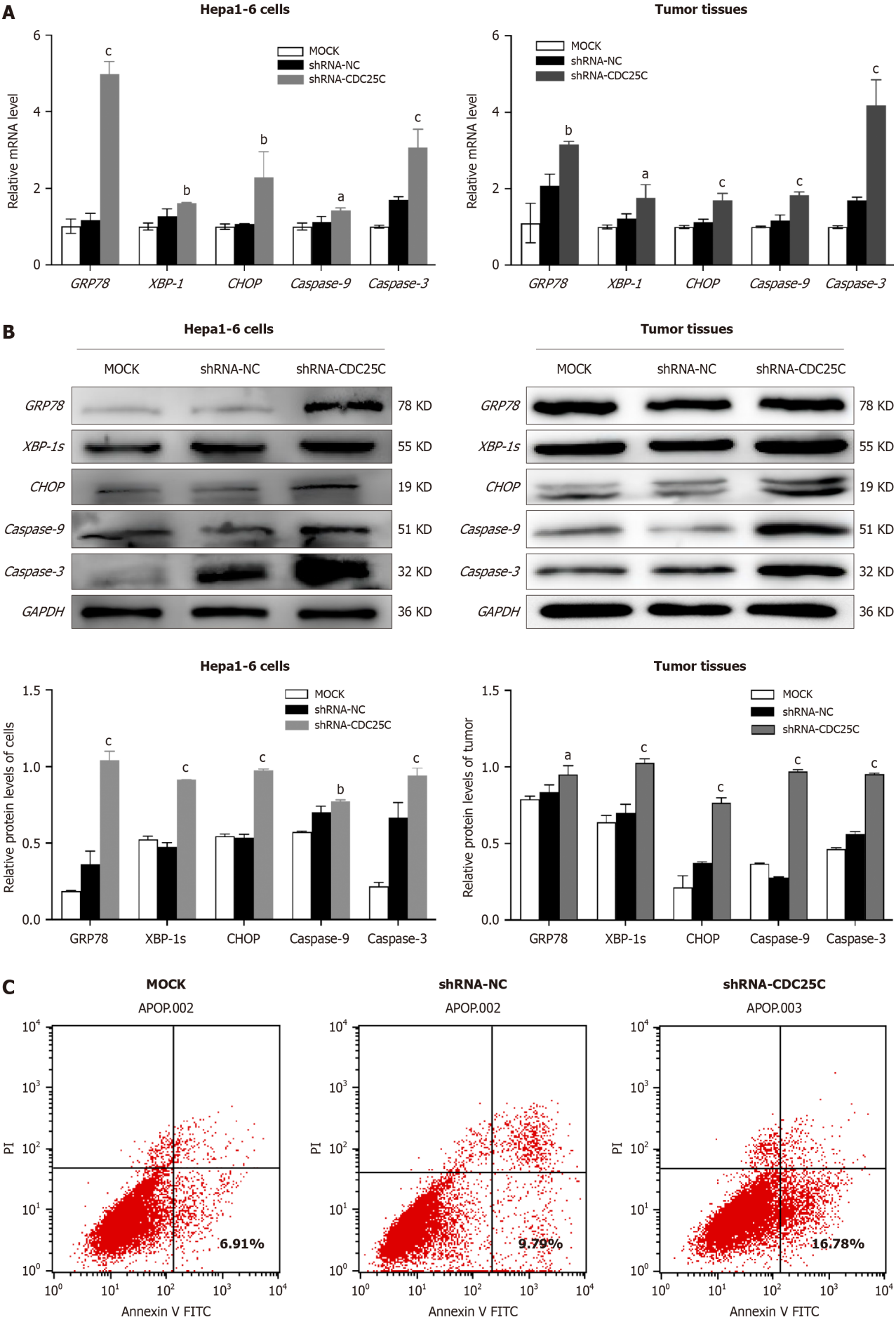
GRP78, the most important ER chaperone, is a major inducer of ER stress[7]. In recent years, numerous researchers have focused on therapeutic targeting of *GRP78*, particularly its role in tumor development[15]. For instance, polyphyllin I was found to effectively suppress the proliferation, invasion, and metastasis of HCC cells by modulating *GRP78* activity [16]. Furthermore, researchers discovered that a *GRP78*-targeted drug delivery system significantly suppressed HCC tumor growth and metastasis in mice[17]. Consistent with previous reports, our findings demonstrated increased *GRP78* expression in both cells and tissues after *CDC25C* knockdown, leading to inhibited HCC growth in mice. *XBP-1* is also commonly used as a marker for ER stress[8,18]. It has been observed that silymarin attenuates nonalcoholic fatty liver disease by regulating *GRP78* and *XBP-1* in mice[19]. In our study, we observed much greater expression of *GRP78* than of *XBP-1* in the *CDC25C* knockdown model. This finding aligns with existing literature suggesting that *GRP78* has a greater impact on inducing ER stress than does *XBP-1*. However, after downregulation of *CDC25C* expression, the increase in *GRP78* expression in Hepa1-6 cells was considerably greater than that in xenograft tumor tissues. We attribute this difference to the tumor microenvironment, which may lead to a decrease in *GRP78* stoichiometry in xenograft tumor tissues. This finding is similar to that in another report indicating that UPR signaling was downregulated in murine models of prostate cancer[20].

Upon high-intensity or prolonged ER stress, homeostasis is not restored, and apoptosis is induced through ER-related molecules. ER stress-induced apoptosis is generally activated through three primary pathways: The IRE1-JNK pathway, the caspase-12 kinase pathway, and the *CHOP* pathway[9]. *CHOP* plays a critical role in ER stress-induced apoptosis. Recent studies have shown that kaempferol can protect hepatocytes from ER stress-induced apoptosis by downregulating *CHOP* expression[21]. This finding suggested that both the overexpression of *CHOP* and ER stress can induce tumor cell apoptosis and inhibit tumor development. Our results indicated that the expression of *GRP78*, *XBP-1*, and *CHOP* was significantly elevated in the *CDC25C* knockdown model. These findings suggest that *CDC25C* knockdown may induce ER stress and lead to apoptosis through the *CHOP* pathway.

In addition, we also assessed the apoptosis rate of Hepa1-6 cells using flow cytometry. After *CDC25C* knockdown, the number of apoptotic cells in the shRNA-*CDC25C* group was significantly increased. Additionally, both the mRNA and protein levels of *Caspase 9* and *Caspase 3* were significantly elevated. These results align with a previous report suggesting that downregulating *CDC25C* expression can induce ER stress and mitochondrial damage, ultimately leading to autophagy and apoptosis in prostate cancer cells[22].

In conclusion, our data indicate that the activation of the ER stress response is involved in the apoptosis of hepatoma cells. The regulatory mechanism of *CDC25C* in HCC development might be associated with the activation of ER stress and the ER stress-induced apoptosis signaling pathway. Our findings are anticipated to provide a reference for further molecular studies on the regulatory mechanism of *CDC25C* in HCC development.

However, we verified the anti-hepatoma effect of *CDC25C* knockdown only in mouse HCC cells and xenograft tumors formed from these cells. Despite the high sequence homology between the mouse and human genomes, it remains unclear whether the effect of downregulating *CDC25C* expression in HCC differs due to species variation. Additionally, the ER stress-induced apoptosis signaling pathway is just one of the mechanisms underlying the role of *CDC25C* in HCC development. In the future, we plan to further investigate the effect of *CDC25C* on human HCC cells and its potential correlation with ER stress and to explore other molecular mechanisms involved.


A
Hepa1-6 cells
Tumor tissues
MOCK
shRNA-NC
shRNA-CDC25C
Relative mRNA level
GRP78
XBP-1
CHOP
Caspase-9
Caspase-3
B
Hepa1-6 cells
Tumor tissues
MOCK
shRNA-NC
shRNA-CDC25C
GRP78
XBP-1s
CHOP
Caspase-9
Caspase-3
GAPDH
78 KD
55 KD
19 KD
51 KD
32 KD
36 KD
Hepa1-6 cells
Tumor tissues
Relative protein levels of cells
Relative protein levels of tumor
GRP78
XBP-1s
CHOP
Caspase-9
Caspase-3
C
MOCK
shRNA-NC
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APOP.002
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PI
Annexin V FITC
6.91%
9.79%
16.78%

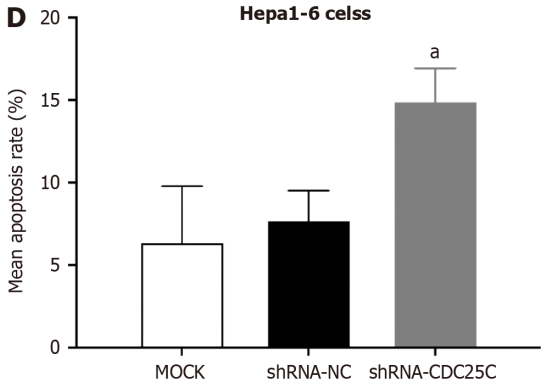

Figure 4 Cell division cyclin 25C knockdown induced endoplasmic reticulum stress and activated endoplasmic reticulum stress-induced apoptosis in Hepa1-6 cells and the corresponding xenograft tumors. A and B: The expression of *GRP78*, *XBP-1*/s, *CHOP*, *Caspase 9*, and *Caspase 3* in Hepa1-6 cells and the corresponding xenograft tumor tissues was analyzed at both the transcriptional and translational levels; C and D: The apoptosis rate of Hepa1-6 cells was assessed using flow cytometric analysis. The data represents the mean ± SD of three independent experiments. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$. *CDC25C*: Cell division cyclin 25C; *GRP78*: Glucose-regulated protein 78; *XBP-1*: X-box binding protein-1; *XBP-1*s: Splicing of X-box binding protein-1; *CHOP*: C/EBP homologous protein.

CONCLUSION

The regulatory mechanism of *CDC25C* in HCC development may involve the activation of ER stress and the ER stress-induced apoptosis signaling pathway. This finding suggests that targeting *CDC25C* and the associated ER stress response may be a potential strategy for cancer treatment, particularly in the context of HCC.

ACKNOWLEDGEMENTS

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FOOTNOTES

Author contributions: Li YF, Zheng FY, Miao XY, Liu HL, Zhang YY performed research. All authors contributed to the manuscript review. Li YF and Zheng FY contributed equally to this work as co-first authors. Because they made equal and significant contributions throughout the research process, such as research design, acquisition of data, statistical analysis, creation and improvement of tables and figures, and drafting of the manuscript. Mo FR and Chao NX are designated as co-corresponding authors in recognition of their roles as supervisors in the project. Mo FR was the project leader responsible for funding acquisition for this study. He mainly contributed to research design, supervision of the project, providing material support, and handling the submission and revision of the manuscript during this study. Chao NX focused on project administration tasks such as technical support for establishing a xenograft mouse model with *CDC25C* knockdown, enhancing the tables and figures, conducting data statistical analysis, and participating in manuscript revisions. Mo FR and Chao NX made equal contributions in terms of technical and material support, research design, study supervision, manuscript submission, and revision.

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Country of origin: China

ORCID number: Yan-Fei Li 0000-0002-1533-7373; Nai-Xia Chao 0000-0001-9470-4954; Fa-Rong Mo 0000-0003-2564-7460.

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REFERENCES

1 **Ji B**, Cai H, Yang Y, Peng F, Song M, Sun K, Yan F, Liu Y. Hybrid membrane camouflaged copper sulfide nanoparticles for photothermal-chemotherapy of hepatocellular carcinoma. *Acta Biomater* 2020; **111**: 363-372 [PMID: 32434082 DOI: 10.1016/j.actbio.2020.04.046]
2 **De Mattia E**, Cecchin E, Guardascione M, Foltran L, Di Raimo T, Angelini F, D'Andrea M, Toffoli G. Pharmacogenetics of the systemic treatment in advanced hepatocellular carcinoma. *World J Gastroenterol* 2019; **25**: 3870-3896 [PMID: 31413525 DOI: 10.3748/wjg.v25.i29.3870]
3 **Liu K**, Zheng M, Lu R, Du J, Zhao Q, Li Z, Li Y, Zhang S. The role of *CDC25C* in cell cycle regulation and clinical cancer therapy: a systematic review. *Cancer Cell Int* 2020; **20**: 213 [PMID: 32518522 DOI: 10.1186/s12935-020-01304-w]
4 **Li CM**, Li YF, Tian L, Zhang QH, Zheng FY, Mo FR. Anti-hepatoma Effect of DC2.4 Cells Transfected with Tumor-Associated Antigen Cdc25C In Vitro. *Curr Med Sci* 2022; **42**: 491-497 [PMID: 35292875 DOI: 10.1007/s11596-022-2556-x]
5 **Kim C**, Kim B. Anti-Cancer Natural Products and Their Bioactive Compounds Inducing ER Stress-Mediated Apoptosis: A Review. *Nutrients* 2018; **10** [PMID: 30081573 DOI: 10.3390/nu10081021]
6 **Wires ES**, Trychta KA, Kennedy LM, Harvey BK. The Function of KDEL Receptors as UPR Genes in Disease. *Int J Mol Sci* 2021; **22** [PMID: 34063979 DOI: 10.3390/ijms22115436]
7 **Dudek J**, Benedix J, Cappel S, Greiner M, Jalal C, Müller L, Zimmermann R. Functions and pathologies of BiP and its interaction partners. *Cell Mol Life Sci* 2009; **66**: 1556-1569 [PMID: 19151922 DOI: 10.1007/s00018-009-8745-y]
8 **van Schadewijk A**, van't Wout EF, Stolk J, Hiemstra PS. A quantitative method for detection of spliced X-box binding protein-1 (XBP1) mRNA as a measure of endoplasmic reticulum (ER) stress. *Cell Stress Chaperones* 2012; **17**: 275-279 [PMID: 22038282 DOI: 10.1007/s12192-011-0306-2]
9 **Hu H**, Tian M, Ding C, Yu S. The C/EBP Homologous Protein (*CHOP*) Transcription Factor Functions in Endoplasmic Reticulum Stress-Induced Apoptosis and Microbial Infection. *Front Immunol* 2018; **9**: 3083 [PMID: 30662442 DOI: 10.3389/fimmu.2018.03083]
10 **Nagesh PKB**, Hatami E, Chowdhury P, Kashyap VK, Khan S, Hafeez BB, Chauhan SC, Jaggi M, Yallapu MM. Tannic Acid Induces Endoplasmic Reticulum Stress-Mediated Apoptosis in Prostate Cancer. *Cancers (Basel)* 2018; **10** [PMID: 29518944 DOI: 10.3390/cancers10030068]
11 **Fang C**, Weng T, Hu S, Yuan Z, Xiong H, Huang B, Cai Y, Li L, Fu X. IFN-γ-induced ER stress impairs autophagy and triggers apoptosis in lung cancer cells. *Oncoimmunology* 2021; **10**: 1962591 [PMID: 34408924 DOI: 10.1080/2162402X.2021.1962591]
12 **Wang L**, Hu T, Shen Z, Zheng Y, Geng Q, Li L, Sha B, Li M, Sun Y, Guo Y, Xue W, Xuan D, Chen P, Zhao J. Inhibition of USP1 activates ER stress through Ubi-protein aggregation to induce autophagy and apoptosis in HCC. *Cell Death Dis* 2022; **13**: 951 [PMID: 36357365 DOI: 10.1038/s41419-022-05341-3]
13 **Hsiao YP**, Tsai CH, Wu PP, Hsu SC, Liu HC, Huang YP, Yang JH, Chung JG. Cantharidin induces G2/M phase arrest by inhibition of Cdc25c and Cyclin A and triggers apoptosis through reactive oxygen species and the mitochondria-dependent pathways of A375.S2 human melanoma cells. *Int J Oncol* 2014; **45**: 2393-2402 [PMID: 25340978 DOI: 10.3892/ijo.2014.2689]
14 **Pan Z**, Zhang X, Yu P, Chen X, Lu P, Li M, Liu X, Li Z, Wei F, Wang K, Zheng Q, Li D. Cinobufagin Induces Cell Cycle Arrest at the G2/M Phase and Promotes Apoptosis in Malignant Melanoma Cells. *Front Oncol* 2019; **9**: 853 [PMID: 31552178 DOI: 10.3389/fonc.2019.00853]
15 **Lu G**, Luo H, Zhu X. Targeting the *GRP78* Pathway for Cancer Therapy. *Front Med (Lausanne)* 2020; **7**: 351 [PMID: 32850882 DOI: 10.3389/fmed.2020.00351]
16 **Du H**, Wu H, Kang Q, Liao M, Qin M, Chen N, Huang H, Huang D, Wang P, Tong G. Polyphyllin I attenuates the invasion and metastasis *via* downregulating *GRP78* in drug-resistant hepatocellular carcinoma cells. *Aging (Albany NY)* 2023; **15**: 12251-12263 [PMID: 37934581 DOI: 10.18632/aging.205176]
17 **Jiang B**, Zhang R, Zhang J, Hou Y, Chen X, Zhou M, Tian X, Hao C, Fan K, Yan X. *GRP78*-targeted ferritin nanocaged ultra-high dose of doxorubicin for hepatocellular carcinoma therapy. *Theranostics* 2019; **9**: 2167-2182 [PMID: 31149036 DOI: 10.7150/thno.30867]
18 **Barua D**, Gupta A, Gupta S. Targeting the IRE1-XBP1 axis to overcome endocrine resistance in breast cancer: Opportunities and challenges. *Cancer Lett* 2020; **486**: 29-37 [PMID: 32446861 DOI: 10.1016/j.canlet.2020.05.020]
19 **Sahin E**, Bagci R, Bektur Aykanat NE, Kacar S, Sahinturk V. Silymarin attenuated nonalcoholic fatty liver disease through the regulation of endoplasmic reticulum stress proteins *GRP78* and *XBP-1* in mice. *J Food Biochem* 2020; **44**: e13194 [PMID: 32189355 DOI: 10.1111/jfbc.13194]
20 **Ramirez MU**, Hernandez SR, Soto-Pantoja DR, Cook KL. Endoplasmic Reticulum Stress Pathway, the Unfolded Protein Response, Modulates Immune Function in the Tumor Microenvironment to Impact Tumor Progression and Therapeutic Response. *Int J Mol Sci* 2019; **21** [PMID: 31881743 DOI: 10.3390/ijms21010169]
21 **Wang H**, Chen L, Zhang X, Xu L, Xie B, Shi H, Duan Z, Zhang H, Ren F. Kaempferol protects mice from d-GalN/LPS-induced acute liver failure by regulating the ER stress-Grp78-CHOP signaling pathway. *Biomed Pharmacother* 2019; **111**: 468-475 [PMID: 30594786 DOI: 10.1016/j.biopha.2018.12.105]
22 **Chan ML**, Liang JW, Hsu LC, Chang WL, Lee SS, Guh JH. Zerumbone, a ginger sesquiterpene, induces apoptosis and autophagy in human hormone-refractory prostate cancers through tubulin binding and crosstalk between endoplasmic reticulum stress and mitochondrial insult. *Naunyn Schmiedebergs Arch Pharmacol* 2015; **388**: 1223-1236 [PMID: 26246051 DOI: 10.1007/s00210-015-1152-z]

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