=== **CELL MOLECULAR BIOLOGY** ===

Silencing of the S-Phase Kinase-Associated Protein 2 Gene (*SKP2*) Inhibits Proliferation and Migration of Hepatocellular Carcinoma Cells

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Abstract—*SKP2* gene is an independent prognostic factor in some diseases and a potential oncogene. The molecular mechanism underlying the occurrence and development of hepatoma, and the involvement of the *SKP2* in this process remain unclear. Here, in order to study the effect of SKP2 on proliferation, apoptosis and migration of hepatoma cells, we utilized lentivirus-mediated RNA interference technology using short hairpin RNAs (shRNAs) specific for *SKP2*. It was demonstrated that *SKP2* expression was significantly upregulated in 809 hepatocarcinoma tissues compared to 379 normal liver tissues. The survival time of patients with high levels of SKP2 mRNA was shorter than those with low levels, and *SKP2* expression was maximal in stage III hepatocellular carcinoma tissues. The effects of *SKP2* silencing on proliferation, apoptosis, cell cycle, migration, and the expression of apoptosis proteins in Huh7 and HepG2 cells were evaluated by MTT assay, flow cytometry, colony formation assay, Transwell, and Western blot analysis. *SKP2* expression was significantly reduced in stably transfected Huh7 and HepG2 cells, with knockout efficiencies of 95.7 and 85.8%, respectively. The viability, proliferation, and migration of transfected cancer cells were reduced. In these cells, the apoptosis rate was increased, and the cell cycle was arrested in the G2/M phase. The expression of the apoptosis-associated BCL-2/BAX proteins was decreased, while p53 was upregulated. Thus, we have shown that inhibiting the expression of *SKP2* can significantly impede cancer cell proliferation and migration, halt the cell cycle, and induce apoptosis.

Keywords: *SKP2*, hepatocellular carcinoma, cell proliferation, apoptosis, small interfering RNA

DOI: 10.1134/S0026893324700651

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, with its mortality rate ranking third [1]. Most HCC patients are diagnosed in the middle to late stage. Due to a series of reasons, such as rapid development, extremely high malignancy, insensitivity to chemotherapy, and cancer cell proliferation, HCC treatment is extremely difficult, and the treatment effect and prognosis are not ideal [2]. Studies have shown that multiple signaling factors play a crucial role in the occurrence and development of HCC [3−5]. Thus, searching for reliable biomarkers to diagnose HCC and developing new treatment strategies is necessary for patients with HCC.

The ubiquitin-proteasome system (UPS) is a primary pathway for protein degradation in cells [6]. The ubiquitin-proteasome system (UPS) activity also plays a crucial role in regulating various life processes, such as the cell cycle, signaling, DNA repair, etc. [7−9]. The F-box protein S-phase kinase-associated protein 2 (SKP2) is a key member of the UPS, forming the SCF complex with SKP1, CUL1, and RBX1 [10, 11]. In the SCF complex, SKP2 serves as a substrate recognition factor involved in ubiquitination, cell cycle regulation, and signal transduction [12−14]. SKP2 utilizes its substrate-specific adaptor connectors to recruit various substrate proteins onto the core ubiquitination complex, leading to the corresponding ubiquitination and degradation of the substrate. Current studies have shown that SKP2 can degrade a variety of cell cycle regulatory proteins, thereby regulating the cell cycle, cell metastasis, and apoptosis. These processes are closely related to tumorigenesis and pro-

gression and contribute to the enhancement of drug resistance in cancer cells [15−18].

CDK is a crucial regulator of the cell cycle and is controlled by its inhibitor CKIs [19]. CKIs consist of two families, INK4 and CIP/KIP. The CIP/Kip family consists of three proteins: p21, p27, and p57 [20]. This family is specifically recognized, ubiquitinated, and modified by SCF-SKP2, thereby targeting it for proteasomal degradation. The degradation of CKIs by the SCF-SKP2 ubiquitin ligase increases the activity of the cell cycle protein-CDK complex, promoting cell cycle progression and favoring the development of cancer [21]. SKP2 deletion has been shown to lead to the up-regulation of the CIP/Kip family and the down-regulation of the cell cycle proteins E and CDK2. Consequently, the cell cycle arrests in the G1/S phase [22]. In addition, relevant studies have shown that SKP2 deletion leads to reduced expression and activity of MMP-2 and MMP-9, which inhibits the metastasis of gastric cancer cells [23]. Apoptosis is the process of programmed cell death. The tumor suppressor p53 can combat cancer by promoting apoptosis, while the acetyltransferase p300 stimulates p53 transcription by binding to p53 [24]. However, SKP2 can antagonize the relationship between p300 and p53 by forming a complex with p300, thereby impairing the p300-p53 apoptosis signaling pathway and inhibiting apoptosis [25]. In one study, SKP2 was found to be an accurate predictor of the efficacy of adriamycin chemotherapy. 94% of patients with *SKP2*-overexpressing breast cancer responded poorly to adriamycin therapy [26].

Currently, the utilization of RNA interference technology to suppress *SKP2* expression for gene therapy in malignant tumors has emerged as a prominent area in anti-tumor research [27]. The expression of SKP2 protein was reduced in melanoma cell lines, human oral squamous cell carcinoma cell lines, lung cancer cell lines, and T98G malignant glioma cell lines transfected with the *SKP2* gene silencing vector [28−31]. The growth and invasiveness of tumor cells are inhibited, and apoptosis increases [27]. However, there are few studies on the effect of SKP2 on hepatocarcinoma and the molecular mechanism of *SKP2* gene silencing in regulating the biological behavior of hepatocarcinoma cells. In this study, the lentivirus-mediated RNA interference technique was used to silence the expression of *SKP2* in the human hepatocarcinoma cell lines Huh7 and HepG2. The study aimed to observe the effects of *SKP2* silencing on the proliferation, apoptosis, and migration of HCC Huh7 and HepG2 cells, thus providing a scientific basis for exploring new strategies for hepatocarcinoma gene therapy.

EXPERIMENTAL

Materials. Human embryonic kidney 293T cells and human hepatocarcinoma Huh7 and HepG2 cells were purchased from Xiamen Antihala Biotechnology Co., Ltd.; DMEM high-glucose culture medium, fetal bovine serum, and trypsin were purchased from Gibco Company; crystal violet dye solution and MTT were purchased from Shenggong Biotechnology Co., Ltd.; the qRT-PCR reagent kit was purchased from TaKaRa Company; and the apoptosis detection kit was purchased from Biyuntian Biotechnology Co., Ltd.

Data collection. The disparity in *SKP2* gene expression abundance between HCC tissue and normal liver tissue was extracted from the TNMplot database (https://tnmplot.com/analysis/). We opted to utilize non-paired tumors and normal tissues to acquire a more extensive sample dataset. Correlation data between the expression level of the *SKP2* gene and patient survival in HCC patients and normal hepatocarcinoma tissues were obtained from the Kaplan–Meier plotter (https://kmplot.com/analysis/). In order to enhance the resolution efficiency between two groups with high and low expression levels, we choose to automatically select the optimal demarcation value mode for grouping individuals with high and low expression. The default settings of the database are preset by others. The "StagePlots" module was utilized in Gepia (http://gepia.cancer-pku.cn/), with the retrieval condition set to "Use major stage" to acquire correlation data between the expression level of the SKP2 gene in HCC patients and normal liver tissues, and the patients' survival time.

Construction of stable transgenic cell lines. Three short hairpin RNA (shRNA) sequences targeting the *SKP2* gene were designed, with the most effective one being shSKP2-3. The primers are shown in Table 1 and were synthesized by Shanghai Bioengineering Company. The shRNA sequence was ligated into the vector pLKO.1-TRC-puro. Subsequently, the shS-KP2-plko.1 and packaging plasmids (psPAX2 and pMD2.G) were co-transfected into 293T cells using Lipofectamine 2000. After 48 hours post-transfection, the collected supernatants containing packaged lentivirus were utilized to infect Huh7 and HepG2 cells, designated as shSKP2-1, shSKP2-2, and shSKP2-3, respectively. Three days after infection, puromycin (10 μg/mL) was used for a two-week screening process to establish stable transgenic strains of Huh7-shSKP2 and HepG2-shSKP2.

qRT-PCR detection. Huh7 and HepG2 cells were collected, and total RNA was extracted from each group of cells using TRIzol. Subsequently, cDNA was synthesized with a reverse transcription kit following the instructions of the qRT-PCR kit (Vazyme, China). The mRNA level of *SKP2* in each group of cells was determined by fluorescence quantitative PCR using Thermo Fisher's ILM-EC100-1004 instrument model. The interference efficiency was calculated for each group of cells.

Western blot detection. Cells were incubated for 30 min with 100 μL of pre-cooled RIPA lysis buffer

Table 1. DNA oligo sequence primers

Name	Sequence (5'−3')
shSKP2-1	F:CCGGGCCTAAGCTAAATCGAGAGAACTCGAGTTCTCTCGATTTAGCTTAGGCTTTTTGGTACC R:AATTGGTACCAAAAAGCCTAAGCTAAATCGAGAGAACTCGAGTTCTCTCGATTTAGCTTAGGC
shSKP2-2	F:CCGGCCATTGTCAATACTCTCGCAACTCGAGTTGCGAGAGTATTGACAATGGTTTTTGGTACC R:AATTGGTACCAAAAACCATTGTCAATACTCTCGCAACTCGAGTTGCGAGAGTATTGACAATGG
shSKP2-3	F:CCGGGATAGTGTCATGCTAAAGAATCTCGAGATTCTTTAGCATGACACTATCTTTTTGGTACC R:AATTGGTACCAAAAAGATAGTGTCATGCTAAAGAATCTCGAGATTCTTTAGCATGACACTAT

(containing 1% PMSF). Afterward, the cells were centrifuged at 13000 rpm for 5 min, and the supernatant was collected. The protein concentration was measured using the Bradford reagent kit from Tiangen Biotech Corporation. After SDS-PAGE and applying a 100 V transmembrane voltage for 90 min, the membrane was blocked with 5% skim milk powder for 1 h. Subsequently, the primary antibody was added, and the membrane was incubated at 4°C overnight, followed by the addition of the secondary antibody. The image was captured using an ultrasensitive chemiluminescence imaging system. Antibodies used were as follows: SKP2 (1 : 1000, Abcam, ab124799), ACTIN (1 : 25000, Bioss, bs-10966R), p53 (1 : 500, Beyotime, AG3444), BCL-2 (1 : 200, Beyotime, AG1225), BAX (1 : 500, Beyotime, AF1270).

MTT assay. Huh7 and HepG2 cells in the logarithmic growth phase were inoculated at 2000 cells per well in a 96-well plate, with three wells in each group. After laying the board, it was placed in a 37°C, 5% CO_2 incubator for cultivation. Starting from the second day, 20 µL of MTT (5 mg/mL) was added to each well of the 96-well plate for 4 h. Subsequently, 150 µL of dimethyl sulfoxide (DMSO) was added, and the absorbance (OD) was measured at 490 nm.

Colony formation assay. HepG2 and Huh7 cells in the logarithmic growth phase were seeded into a 6-well plate at 2000 cells per well. Three wells were set up and placed in the incubator for further cultivation for 10 d. After washing twice with PBS, 1 mL of 4% paraformaldehyde was added to each well, and the cells were fixed for 30 min. Crystal violet staining was performed for 15 min. Finally, the staining solution was washed away, and the resulting clones were photographed and counted.

Flow cytometry detection. Collect cells, fix overnight with pre-cooled 70% ethanol, centrifuge at 1000 g for 5 min, wash twice with PBS, and add 500 µL of PBS and 10 µL of 10 mg/mL of RNase A. Incubate at 37°C for 30 min, then add 10 µL of PI (2.5 mg/mL) and incubate at 4°C for 30 min. Filter the sample with a 300 mesh filter, and analyze it using flow cytometry.

Annexin V-APC detection. According to the reagent kit, cells from each group were collected, washed with PBS twice, resuspended in 200 µL of 1× binding buffer, added to 5 µL of Annexin V-APC dye solution, incubated at room temperature in the dark for 15 min, and then detected by flow cytometry.

Transwell cell migration assay. The Transwell chamber was placed in a 24-well plate, and 600 µL of complete culture medium was added to the lower chamber. Cells (5×10^4) in the logarithmic growth phase per well were seeded into the Transwell chamber. After 48 h of cultivation, cells on the inner membrane of the chamber were wiped off with a cotton swab. Then, 500 µL of 4% paraformaldehyde was added to fix the cells for 30 min. Subsequently, they were stained with crystal violet for 15 min, and photographed for counting.

Statistical analysis. The data analysis was conducted using GraphPad Prism 8 software, and the measurement data were presented as using the mean ± standard deviation ($\bar{x} \pm s$). Component comparisons were conducted using one-way ANOVA, and multiple comparisons were conducted using a *t*-test. $P < 0.05$ represents statistical significance. All experiments were replicated three times.

RESULTS

Expression of SKP2 mRNA in HCC Cells

The expression levels of *SKP2* in 809 HCC tissues and 379 normal liver tissues were obtained through the TNMplot database. After statistical analysis, the difference in *SKP2* expression between normal human liver tissues and patients with HCC was determined. The results showed that the expression level of the *SKP2* gene in HCC tissue was significantly higher compared to normal liver tissue (Fig. 1, $p < 0.05$).

Relationship between SKP2 Expression and the Prognosis of HCC

The relationship between the expression of *SKP2* and the prognosis and survival of HCC patients was analyzed using the Kaplan−Meier plot database. Overall, the survival rate of hepatocarcinoma patients in the low *SKP2* expression group was better than that in the high *SKP2* expression group (Fig. 2a). The hazard ratio (HR) value is 1.75, which is significantly greater than 1 ($p < 0.05$), indicating that high expression of *SKP2* significantly increases the risk of death in HCC patients and reduces the survival rate, making it
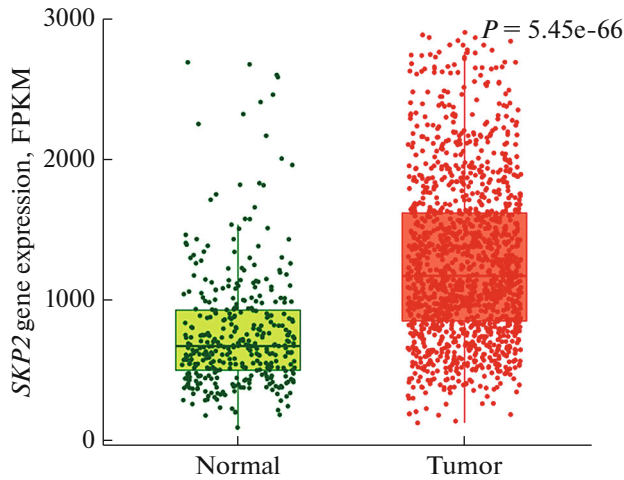
Fig. 1. Expression of the *SKP2* gene in normal liver and hepatocellular carcinoma tissues.

an unfavorable factor. The GEPIA database was used to analyze the expression of the *SKP2* gene at various stages of HCC. The results showed that *SKP2* had the highest expression abundance in stage III hepatocarcinoma (Fig. 2b).

Expression of SKP2 mRNA and Protein in Huh7 and HepG2 CELLS

HepG2 and Huh7 cells were infected with SKP2-shRNA and SKP2-shCtrl lentiviruses. After 14 d of puromycin screening, stably transformed cell lines of HepG2 and Huh7 were obtained. As shown in Fig. 3a,

the expression of *SKP2* mRNA in HepG2 and Huh7 stably transformed cell lines was measured by qRT-PCR. Compared with the shCtrl group, the expression levels of *SKP2* in Huh7 and HepG2 cells were significantly reduced when infected with *shSKP2-1*, *shSKP2-2*, and *shSKP2-3* lentiviruses, particularly in Huh7-*shSKP2-3* and HepG2-*shSKP2-3* cells. In the transformed cell line, the interference efficiency of the *SKP2* gene was 95.7% and 85.8%, respectively, and its protein expression was also significantly inhibited (Figs. 3b, 3c). Thus, *shSKP2-3* was selected as a stable transgenic strain for subsequent experiments.

The Effect of SKP2 Gene Silencing on the Growth of Huh7 and HepG2 Cells

Previous studies have found that the downregulation of the *SKP2* gene inhibits the proliferation ability of human tumor cells. To investigate the role of *SKP2* in regulating the proliferation of HCC cells, we utilized the MTT method was used to assess the viability of HCC cells infected with lentivirus *shSKP2* for 5 days. As illustrated in Fig. 4, the knockdown of *SKP2* markedly suppressed the proliferation of Huh7 and HepG2 cells, with inhibition rates of cell proliferation reaching 41.34 and 34.51% respectively on the fifth day.

The Effect of SKP2 Gene Silencing on the Clonogenic Ability of Huh7 and HepG2 Cells

After silencing the *SKP2* gene, the number of clones formed by Huh7 and HepG2 cells was significantly lower than that of the *shCtrl* group. The inhibi-
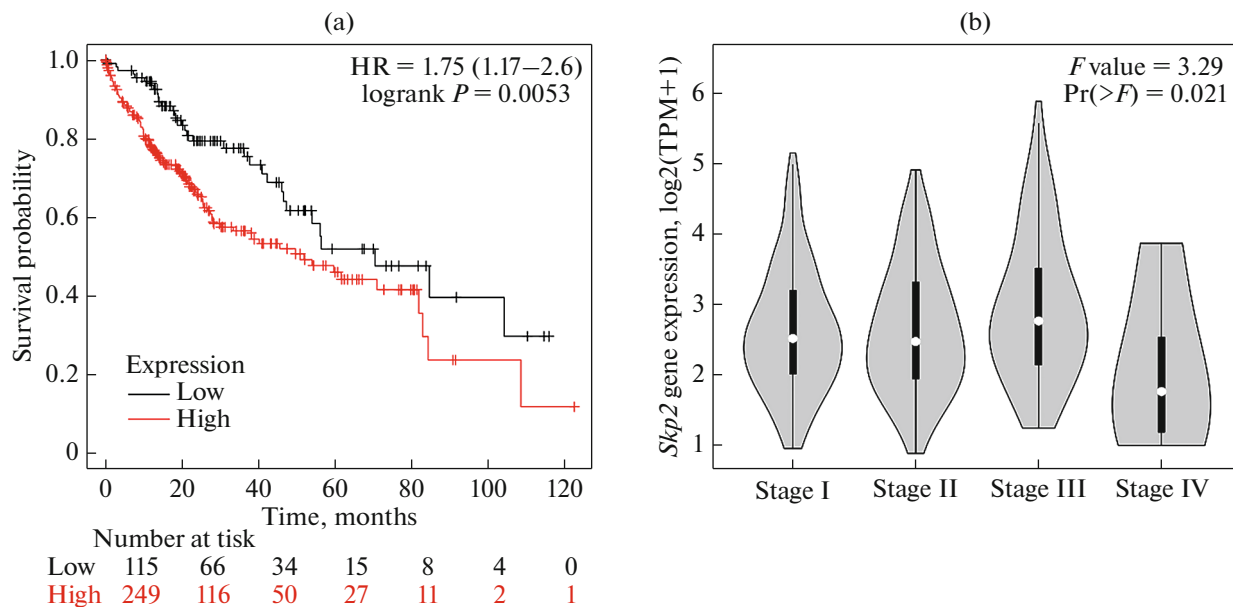

Fig. 2. Correlation between *SKP2* expression, survival curves, and staging of HCC patients. (a) Correlation between *SKP2* expression and the survival of HCC patients. (b) Correlation between *SKP2* expression and the staging of HCC patients.
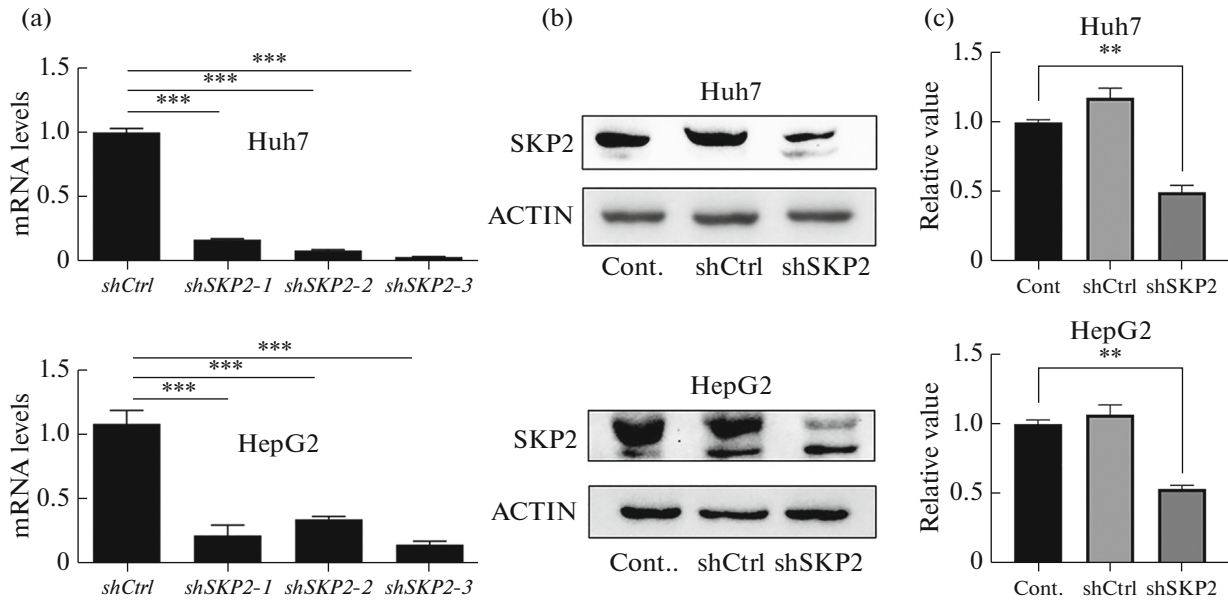
Fig. 3. The mRNA expression and protein levels of *SKP2* in Huh7 and HepG2 cells. (a) Expression of *SKP2* mRNA in Huh7 and HepG2 cells was measured by qRT-PCR. (b) Expression of SKP2 protein in Huh7 and HepG2 cells. (c) The relative gray value of SKP2 protein expression. ** $p < 0.01$, *** $p < 0.001$.
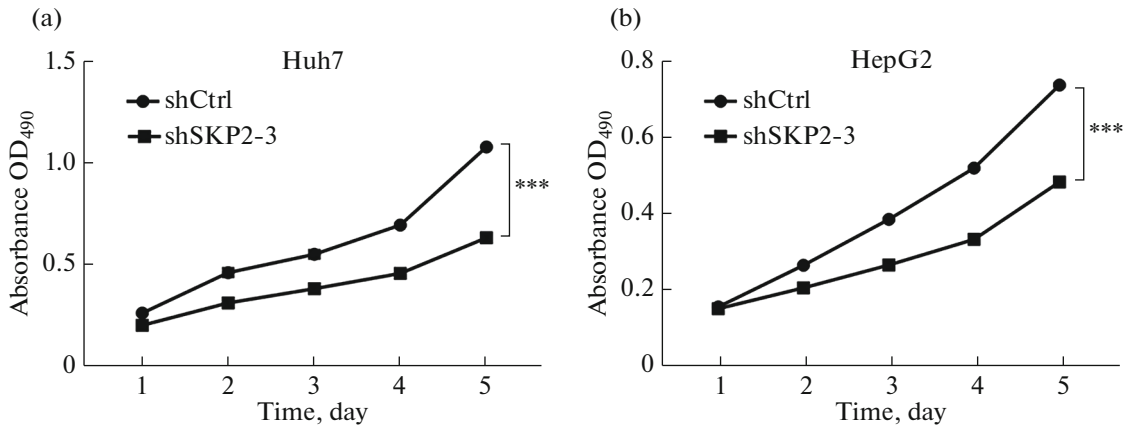

Fig. 4. Effect of *SKP2* knockdown on the proliferation of Huh7 and HepG2 cells. (a) MTT assay was used to detect the proliferative activity of Huh7 cells. (b) MTT assay was used to detect the proliferative activity of HepG2 cells. *** $p < 0.001$.

tion rates were 79.68 and 83.62%, respectively, indicating a decrease in the proliferation ability of hepatocarcinoma cells after knocking down the *SKP2* gene (Fig. 5).

The effect of SKP2 Gene Silencing on the Cell Cycle of Huh7 and HepG2 Cells

As depicted in Fig. 6, the percentages of Huh7-*shCtrl* and HepG2-*shCtrl* cells entering the G2/M phase were 15.92 ± 0.35 and $25.44 \pm 0.73\%$, respectively. In contrast, those in the *shSKP2* group were 26.67 ± 0.70 and $34.55 \pm 0.46\%$, respectively. Compared with the *shCtrl* group, there was a significant

increase in cells entering the G2/M phase, indicating that silencing the *SKP2* gene blocked the cell cycle of HCC cells in the G2/M phase.

The Effect of SKP2 Gene Silencing on Apoptosis in Huh7 and HepG2 Cells

After silencing the *SKP2* gene, the apoptosis rate of the two HCC cell lines significantly increased. The apoptosis rate of Huh7-*shSKP2* cells was 9 times higher than that of the Huh7-*shCtrl* group [$(27.22 \pm 0.7)\%$ vs. $(2.99 \pm 0.03)\%$] (Fig. 7). The apoptosis rate of HepG2-*shSKP2* cells was five times higher than that of the HepG2-*shCtrl* group [$(17.13 \pm 1.12)\%$ vs.
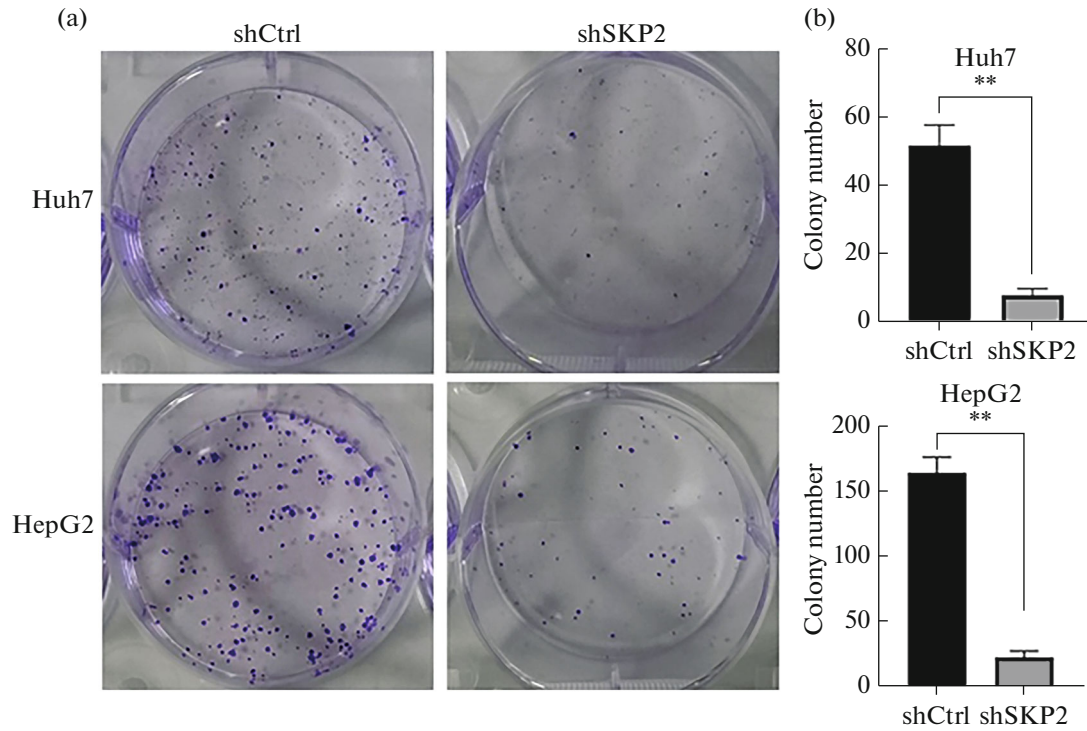
Fig. 5. The effect of *SKP2* gene knockdown on the colony formation ability of Huh7 and HepG2 cells. (a) The proliferative capacity of cells was assessed using a clonogenic assay. (b) Statistical analysis of the colony formation results. **$p < 0.01$, ***$p < 0.001$.
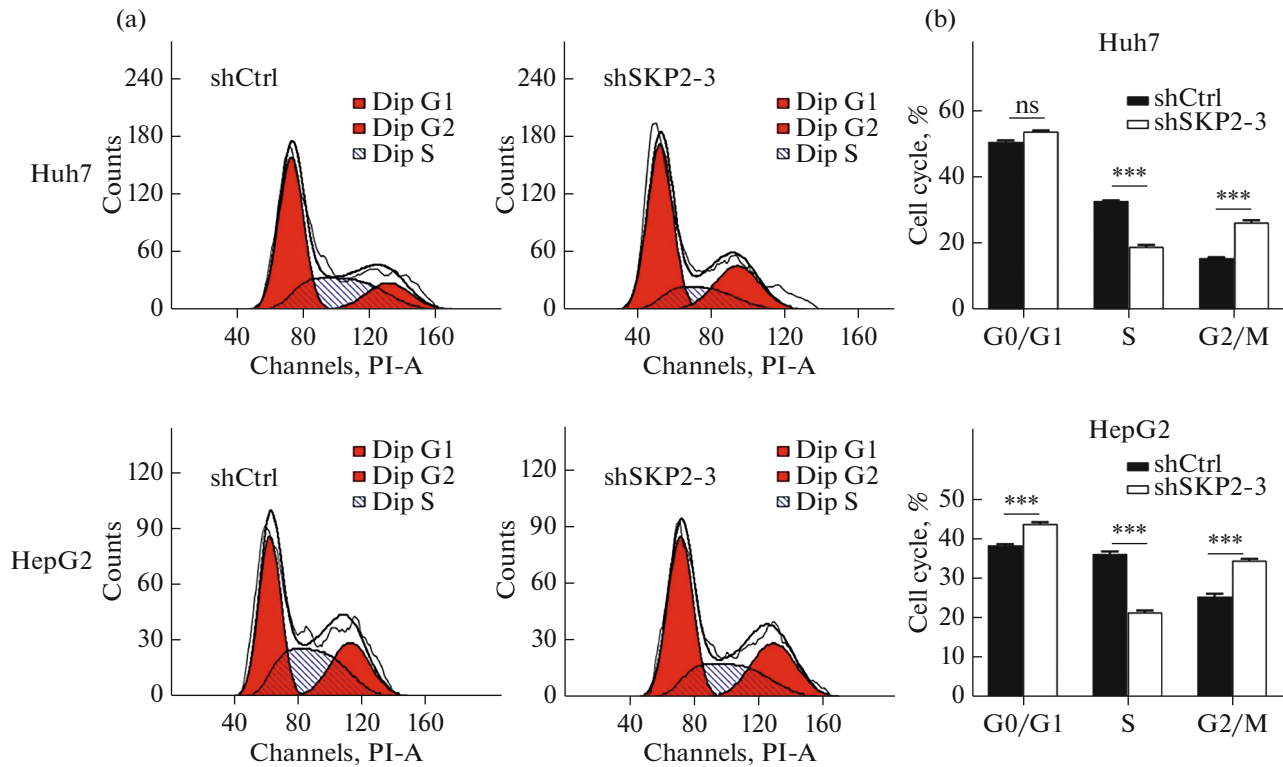

Fig. 6. Effect of *SKP2* gene downregulation on the cell cycle of Huh7 and HepG2 cells. (a) The effect of downregulating the *SKP2* gene on the cell cycle was detected using flow cytometry. (b) Statistical analysis of the cell cycle results. ***$p < 0.001$.
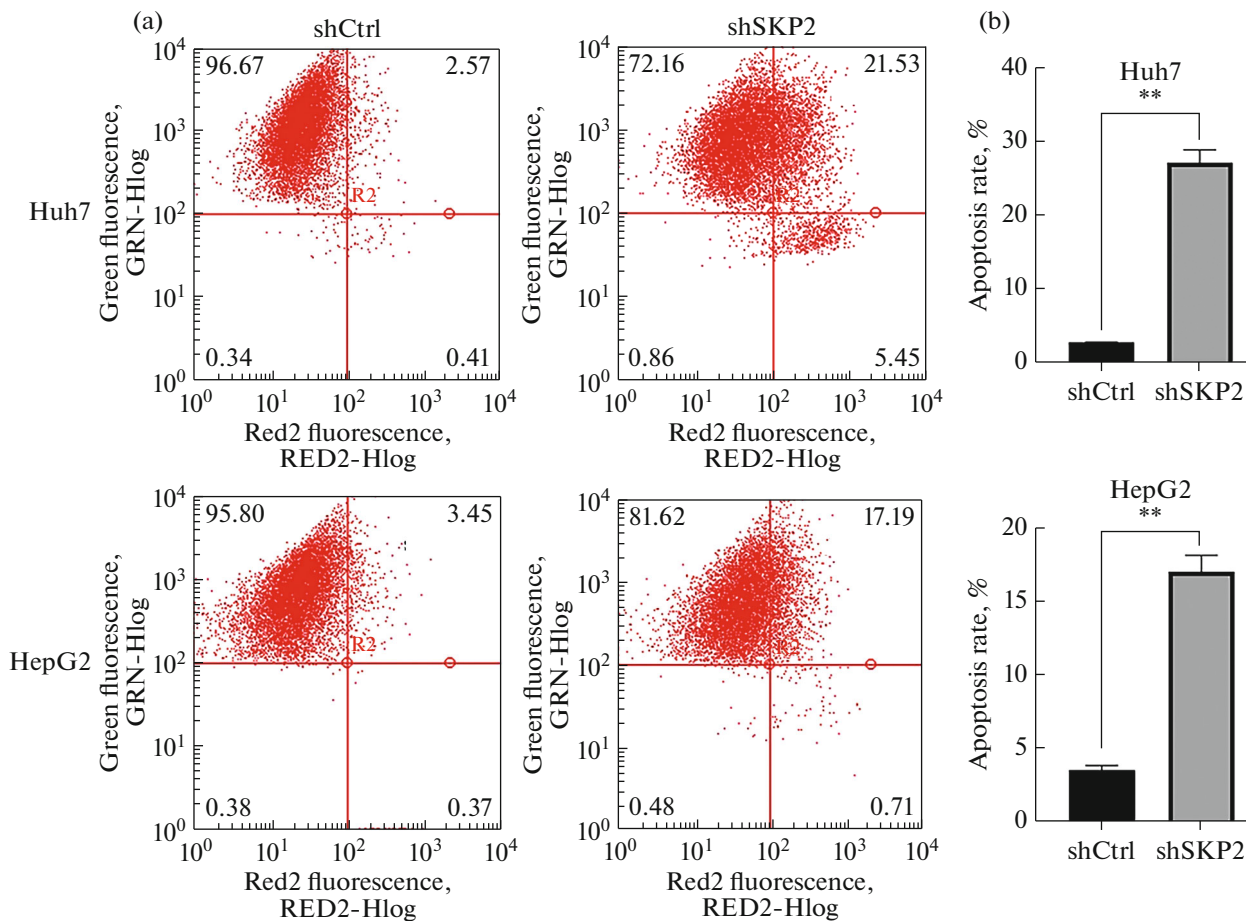
Fig. 7. Effect of *SKP2* gene downregulation on apoptosis in Huh7 and HepG2 cells. (a) The effect of *SKP2* gene downregulation on cell apoptosis was detected using flow cytometry. (b) Statistical analysis of the apoptosis rate results. **$p < 0.01$, ***$p < 0.001$.

$(3.62 \pm 0.31)\%$] (Fig. 7). These data suggest that the absence of *SKP2* inhibits cell growth in both HCC cell lines, possibly by inducing apoptosis.

The Effect of SKP2 Gene Silencing on the Migration Ability of Huh7 and HepG2 Cells

To investigate whether *SKP2* deficiency inhibits the migration ability of hepatocarcinoma cells, a transwell assay was used to evaluate the migration of Huh7 and HepG2 cells after *shSKP2* lentivirus infection. As shown in Fig. 8, the knockdown of *SKP2* significantly inhibited the migration ability of the two hepatocarcinoma cell lines. In Huh7 cells, the migration rate of the *shSKP2-3* group was only 26% of that of the *shCtrl* group. In HepG2 cells, the migration rate of the *shSKP2-3* group was 30% of that of the *shCtrl* group.

Changes in Several Apoptosis Proteins after SKP2 Gene Silencing

As shown in Fig. 7, the apoptosis detection results indicate that silencing the *SKP2* gene induces apopto-sis in Huh7 and HepG2 cells. To further investigate the mechanism by which *SKP2* gene silencing induces apoptosis in HCC cells, proteins from HepG2 cells were collected, and the changes in expression of apop-tosis-related proteins were detected using western blotting. As shown in Fig. 9, compared with the con-trol group cells, the expression of the apoptotic protein p53 was upregulated in HepG2 cells after *SKP2* gene silencing compared to the control group cells. In con-trast, the expression of the anti-apoptotic protein BCL-2 was downregulated, leading to a 58% decrease in the BCL-2/BAX ratio.

DISCUSSION

Hepatocellular carcinoma (HCC) is a rapidly developing cancer with high invasion and metastasis abilities. Unfortunately, nearly 60−80% of patients with HCC are diagnosed at an advanced stage, thereby missing the chance for surgical intervention. Targeted therapy may inhibit important molecules related to tumor proliferation, invasion, metastasis, and drug resistance, preventing the development and spread of
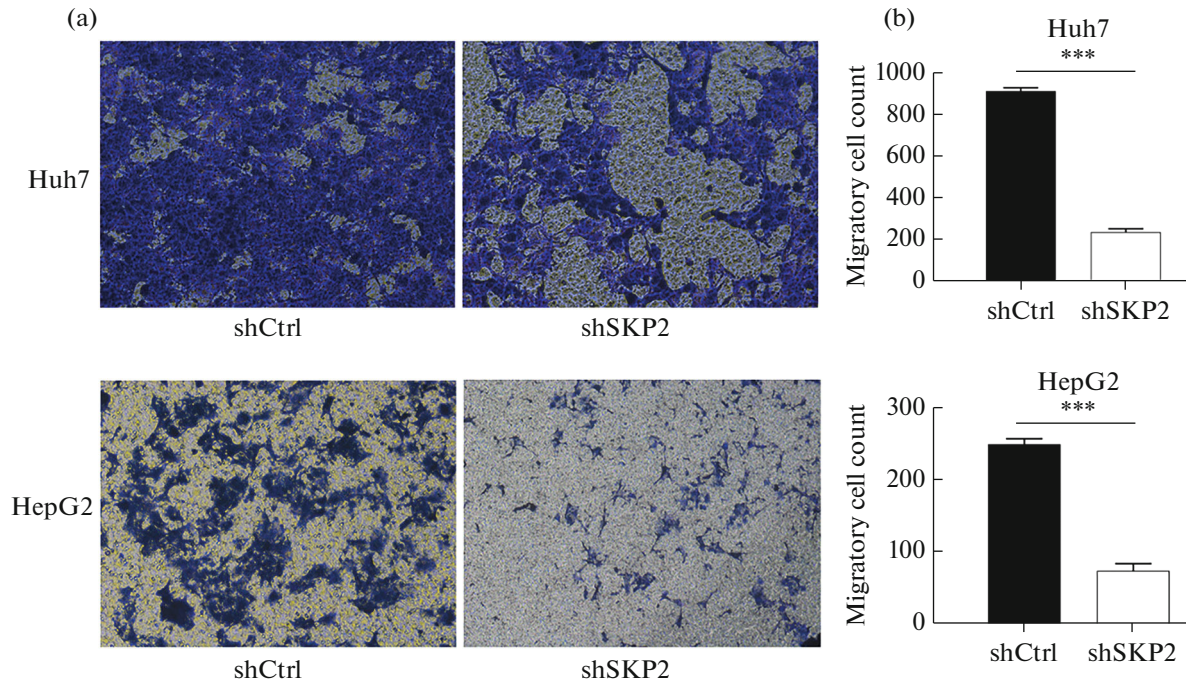
(a)

(b)

Huh7

HepG2

Fig. 8. *SKP2* knockdown inhibits the migration of hepatocarcinoma cells. (a) The effect of downregulating the *SKP2* gene on the invasion of Huh7 and HepG2 cells. (b) Statistical analysis of the migratory cell count. **$p < 0.01$.
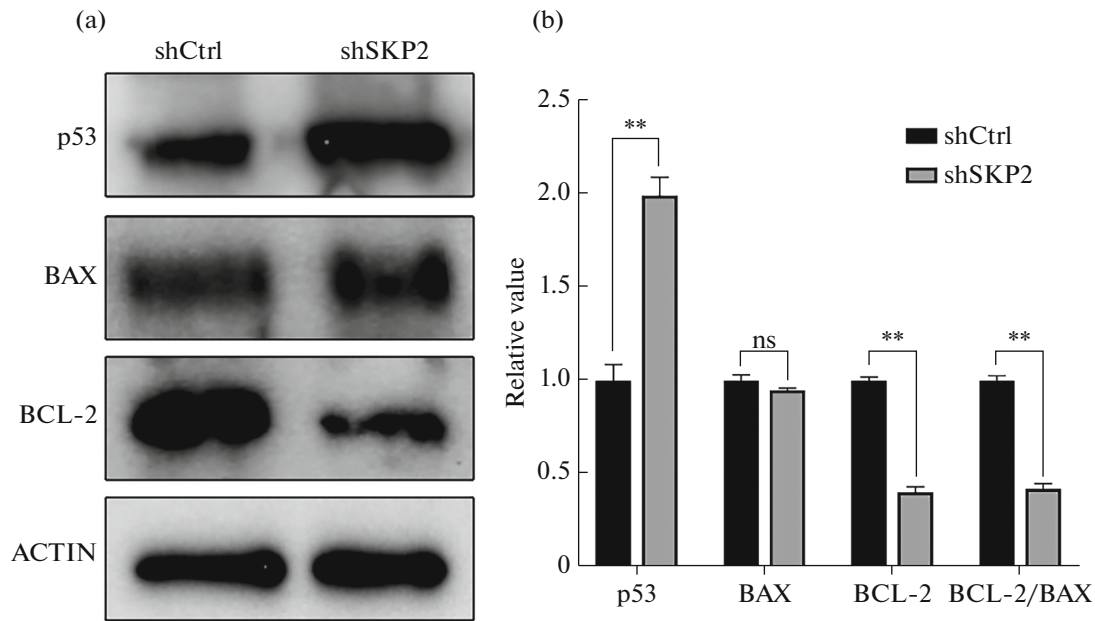
(a)

(b)

Fig. 9. The effect of *SKP2* gene silencing on apoptosis proteins. (a) Western blotting was used to detect the effect of *SKP2* gene silencing on the expression of apoptosis-related proteins. (b) The relative gray value of protein expression. **$p < 0.01$, * $p < 0.05$.

cancer, and paving the way for personalized drugs [32]. As an important E3 ligase, SKP2 functions to recognize substrates and mediate their ubiquitination-mediated degradation. *SKP2* is highly expressed in various types of tumors and plays a crucial role in the onset and progression of tumors, particularly in cell proliferation and drug sensitivity. Therefore, it may be an effective target for treating tumors.

SKP2 plays an important role in cell cycle progression and cell proliferation regulation. It is an essential

factor for cells to enter the S phase. *SKP2* is expressed at a high level during the S phase and plays an important role in cell cycle transition [33]. In this study, the stable transgenic strain was successfully constructed. After silencing the *SKP2* gene, the proliferation and metastasis abilities of Huh7 and HepG2 cells were significantly reduced, leading to cell apoptosis, which is consistent with previous research findings [15, 18, 34]. The proper functioning of the cell cycle is essential for maintaining the proliferation required for embryonic development and tissue repair [35]. However, unplanned cell cycle entry can induce replication stress, DNA damage, and tumorigenesis [36, 37]. SKP2 has previously been reported to play an important role in coordinating the G1/S transition and S phase progression in various mammalian cells [38]. Samuel *et al.* found that DNA damage induced p53-dependent transcription inhibition of NUCKS1, which led to the down-regulation of *SKP2* and cell cycle arrest in the G1 phase [37]. Li *et al.* found that LXR activation significantly inhibited the expression and protein levels of the *SKP2* gene, induced G1/S arrest, and suppressed the proliferation of pancreatic beta cells [39]. Xu *et al.* found that *SKP2* knockdown induced cell cycle arrest in the G1 phase by promoting the accumulation of p27 and p16, thus inhibiting the growth of colorectal cancer [40]. The impact of *SKP2* silencing on HCC cells is rarely reported. Qi et al. found that after transfection with *SKP2*-specific siRNA, endogenous p27 levels increased in HepG2 and SSMC-7721 cells [41]. The interference of *SKP2* significantly induced apoptosis and inhibited the proliferation of SSMC-7721 cells. However, the impact of *SKP2* silencing on HepG2 cell cycle alterations and its effect on the cancer cell line Huh7 have not been reported. Therefore, this study found that silencing *SKP2* can lead to G2/M phase arrest in Huh7 and HepG2 cell cycles, instead of the G1/S phase arrest commonly induced by *SKP2* silencing in other tumor cells. Indicating that the silencing *SKP2* in various tumor cells regulates different cell cycle regulatory factors, thereby activating their respective apoptotic signal transductions.

SKP2 plays two key roles in tumor development. First, as a component of the SCF-SKP2 ubiquitin ligase, SKP2 drives the cell cycle by facilitating the degradation of cyclin. In addition, SKP2 can bind to the transcriptional activator p300 with acetyltransferase activity, blocking its interaction with the tumour suppressor protein p53, thereby preventing p300-mediated p53 acetylation [42]. The downregulation of *SKP2* expression weakens the interaction between SKP2 and P300, promoting p300-mediated p53 acetylation. This process induces p53-mediated cancer cell apoptosis and inhibits cell growth. In this study, the expression of p53 was up-regulated, and the ratio of BCL-2/BAX decreased by 58% after *SKP2* gene silencing. These findings that apoptosis of HepG2 cells might be induced by this mechanism.

CONCLUSIONS

In summary, this study found that silencing the *SKP2* gene inhibits the proliferation and migration of hepatocarcinoma Huh7 and HepG2 cells, induces apoptosis in these cells, and arrests the hepatocarcinoma cell cycle at G2/M. These findings offer potential targets for the treatment of HCC.

AUTHOR CONTRIBUTION

Yaying Zhao: data curation, formal analysis, investigation, methodology, resources, writing—original draft. **Zixi Gao**: data curation, investigation, methodology, resources, writing—original draft. **Shudong Wei**: data curation, investigation, formal analysis, project administration, methodology, resources, supervision. **Wei Song:** conceptualization, project administration, methodology, resources, supervision, and validation.

FUNDING

DATA AVAILABILITY

All data supporting the findings of this study are available within the paper.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This work does not contain any studies involving human and animal subjects.

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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