Downregulation of p57 accelerates the growth and invasion of hepatocellular carcinoma

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Introduction  
As a cyclin-dependent kinase inhibitor, p57 belongs to the Cip/Kip family, which includes p21, p27 and p57. The p57 gene encodes a 316 amino acid protein that is maternally expressed. p57 expression differs spatially and temporally as well as displays an organ- and tissue-specific pattern (1,2). p57 was initially considered a candidate tumor suppressor because the human p57 gene is located on chromosome 11p15.5, a region implicated in sporadic cancers and in the familial cancer syndrome Beckwith–Wiedemann syndrome; however, an increasing number of studies have shown that p57 is a multifunctional protein that is involved in the regulation of proliferation, transcription, apoptosis, differentiation and motility (3–5). In particular, p57 has been suggested to be involved in the co-ordinated control of cell proliferation and cell migration in tumors (6).  

Materials and methods  
Patients and samples  
The 45 pairs of HCC specimens and adjacent non-cancerous specimens were obtained from surgical resections performed at the First Affiliated Hospital, College of Medicine of Xi’an Jiaotong University. These samples were obtained from 36 males and 9 females with a mean age of 48.33 ± 10.55 years (range, 29–77 years). The other clinicopathological data are presented in Table I. The pathological types of all of the specimens were confirmed by independent pathologists. No patients received chemotherapy or radiotherapy before surgery.  

Histopathology and immunohistochemistry  
Paraffin-embedded sections were deparaffinized and rehydrated. For histopathology, the sections were stained with hematoxylin/eosin. For immunohistochemistry, the antigens were retrieved in citrate buffer, and the sections were incubated with an isotype-matched secondary antibody conjugated to horseradish peroxidase. The reaction was visualized with diaminobenzidine and hematoxylin.  

Abbreviations: DMEM, Dulbecco’s modified Eagle’s medium; FBS, fetal bovine serum; HCC, hepatocellular carcinoma; MTT, 3-(4,5-dimethylthiazo-l-2-yl)-2,5-diphenyltetrazolium bromide; mRNA, messenger RNA.
control antibody. Next, the sections were incubated with biotin-conjugated secondary antibodies for 30 min and streptavidin peroxidase for 30 min. Immunoreactive products were stained with 3,3'-diaminobenzidine and subsequently counterstained with hematoxylin. Finally, the sections were examined and analyzed with a microscope (Q550CW; Leica, Manheim, Germany). For the evaluation of p57 protein expression, the staining intensity was graded and scored as follows: 0, no staining; 1, weak staining; 2, moderate staining or 3, strong staining. The extent of staining was evaluated by assigning samples scores based on the percentage of positively stained cells as follows: 1, ≤25%; 2, 26–50%; 3, 51–75% or 4, ≥76%. The number of positive cells was assessed by counting 10 random fields at ×400 magnification. The final immunohistochemical staining score was obtained by multiplying the staining intensity score and the extent of staining score. Samples with staining scores of 0–2 were defined as having negative expression, samples with score of 3–5 were defined as having weak expression, samples with scores of 6–9 were defined as having moderate expression and samples with scores of 10–12 were defined as having strong expression. Samples with scores of 6–12 were defined as displaying positive staining, whereas samples with scores of 0–5 were defined as displaying markedly reduced staining or loss of p57 expression.

Real-time reverse transcription–polymerase chain reaction analysis

Total mRNA was extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA) and reverse transcription was performed using an RT–PCR kit (Takara, Dalian, China). Complementary DNA synthesis was conducted using the SYBR ExScript RT–PCR kit (Takara) according to the manufacturer’s instructions. Real-time PCR was conducted using the iQ5 Multi-color Real-Time PCR Detection System (Bio-Rad, Hercules, CA) and SYBR Premix Ex TaqTM II (Takara). The primer sequences used to amplify p57 were 5'-GGCGGCGAT-CAAGAAGCTGT-3' and 5'-ATGCAGCGACGATCTTCATCA-3'. The primer sequences used to amplify glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were 5'-ACCCGATGTCATGCTCATAC-3' and 5'-TCCAC-CACCCGTGTTGGTGA-3'. Each measurement was performed in triplicate. For each qPCR, a dissociation curve analysis was conducted. GAPDH was used as the internal housekeeping gene control.

Western blot

Tissues and cells were lysed using cell lysis buffer as described previously (21). Equivalent amounts of protein were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (8–12%) and transferred onto polyvinylidene fluoride membranes (Millipore, Danvers, MA). The membranes were blocked and subsequently incubated with the following primary antibodies: anti-p57 antibody (1:500), anti-cyclin D1 antibody (1:200), sc-8396; Santa Cruz Biotechnology), anti-cyclin E antibody (1:200, sc-8396; Santa Cruz Biotechnology), anti-CDK4 antibody (1:500, sc-16873; Santa Cruz Biotechnology), anti-CDK2 antibody (1:500, sc-136191; Santa Cruz Biotechnology), anti-RhoA antibody (1:300, 10749-1-AP; ProteinTech Group, Chicago, IL), anti-ROCK1 antibody (1:300, 1761-1, Epitomics, San Francisco, CA, USA), anti-LIMK1 antibody (1:300, sc-28370, Santa Cruz Biotechnology), anti-cofilin antibody (1:500, sc-33779, Santa Cruz Biotechnology), anti-phospho-LIMK1 (Thr508) antibody (1:250, PAB10997; Abnova, Taiwan, China), anti-phospho-collin (Ser-3) antibody (1:300, 11139, Signalway Antibody, Pearlhand, TX) or anti-ß-actin antibody (1:1000, sc-13031; Santa Cruz Biotechnology). The blots were visualized with a horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnology) and an ECL detection system (Millipore). Western blots were repeated three times for each protein.

Plasminogen

The p57 shRNA plasmids (pGPU6/GFP/Neo-shp57) were designed and synthesized by GenePharma Co. (Shanghai, China). The oligonucleotide sequences were 5'-CAGCGTTTAAAGAAGCTTTATCAAGGATATAATTAGCTCCTAAAGCTTTTAGT-3' and 5'-GATCAGAAAAAGCTTTAGAGCTTTATATCCTTGAATATATGACTTCTTATAGCCG-3'. pGPU6/GFP/Neo was not found in human genome databases, was used as a negative control.

Cell culture, construction of stable transfectants and treatments

The human hepatoma cell lines, BEL7402 and SMMC7721, were selected for our study. These two cell lines are characterized by low proliferative and metastatic capacity and can form tumor xenografts in vivo. BEL7402 cells and SMMC7721 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (FBS; Invitrogen), penicillin (100 IU/ml) and streptomycin (0.1 mg/ml) in 5% CO2 at 37°C. The cells were transfected with plasmids expressing shp57 and shNC using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s instructions. Twenty-four hours after transfection, the cells were diluted and selected for 1 month by culturing the cells in G418 (Invitrogen) at a concentration of 600 µg/ml for BEL7402 cells or 800 µg/ml for SMMC7721 cells. Stable BEL7402 and SMMC7721 transfectants were confirmed by western blot and maintained in medium containing 300 or 400 µg/ml G418, respectively. The stable transfectants expressing p57-specific shRNA were referred to as BEL7402-shp57 and SMMC7721-shp57. The control HCC cells transfected with shNC were referred to as BEL7402-shNC and SMMC7721-shNC.

For the pharmacological inhibition assays, cells were seeded into a six-well plate and treated with 2 ml DMEM containing 1% FBS and the ROCK1 inhibitor Y27632 (10 µM; Enzo, Farmingdale, NY). After 48 h of incubation, the cell extracts were prepared as described below.

Proliferation inhibition assay

Cells (5 × 104 per well) were seeded into 1% gelatin-coated 96-well plates. Relative cell numbers were quantified every day using the 3-(4,5-dimethylthiazo-l-2,5-diphenyltetrazolium bromide (MTT) assay. The absorbance was measured at 492 nm using a Multifunction Microplate Reader (POLARstar OPTIMA; BMG, Offenburg, Germany).

Cell cycle assay

Cells (2 × 105 per well) were cultured in 1% gelatin-coated six-well plates. After 24 h of incubation, the cells were collected, fixed and stained with 150 µl propidium iodide and 150 µl RNAase A (Sigma–Aldrich, St Louis, MO). After incubation for 30 min, the samples were examined by flow cytometry (FACS–Calibur, Franklin Lakes, NJ), and the data were analyzed using Cell Quest software.

Kinase activity assay

The kinase activities of CDKs/cyclins were measured using kinase activity assay kits according to the manufacturer’s instructions (Genmed Sciences, Arlington, MA). In this assay, changes in the absorption peak are used to detect kinase activity. Briefly, 500 µl cell lysate samples were obtained by suspending 1 × 106 cells in lysis buffer. The samples were then incubated for 30 min on ice and centrifuged at 13 000 r.p.m. for 5 min at 4°C. The supernatants were collected to assess enzyme activity. Subsequently, 65 µl buffer solution was incubated with 10 µl substrate, 10 µl reacting solution and 10 µl enzymatic solution in 96-well plates at 30°C for 5 min. Immediately after the addition of 5 µl supernatant to the reagent mixture, the optical density was measured at 340 nm (POLARstar OPTIMA) every 1 min for 5 min. The activity was measured by calculating the difference between the absorbance value at 0 min and the absorbance value at 5 min. The assay was repeated three times for each sample.

Coimmunoprecipitation and immunoblotting

Cells were harvested and lysed in 0.1% NP-40 lysis buffer (50 mM Tris, pH 7.4, 250 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 50 mM NaF, 1 mM Na2VO4, 0.1% NP-40, 0.02% NaN3, 1 mM phenylmethylsulfonyl fluoride). For immunoprecipitation, 2 µg anti-cyclin D1 antibody and 2 µg anti-cyclin E antibody (sc-198; Santa Cruz Biotechnology) were incubated with 50 µl Dynabeads/protein G (Invitrogen) for 10 min at room temperature and subsequently

### Table I. Relationship between the level of p57 protein and clinicopathologic factors

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<th>Variable</th>
<th>N</th>
<th>p57 protein score(±SD)</th>
<th>P</th>
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<tr>
<td>Tumor size</td>
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<td>II</td>
<td>9</td>
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<tr>
<td>Negative</td>
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N, Number of patients.

*P < 0.05.
incubated with 500 l cell lysate samples for 10 min at room temperature with gentle rotation to form Dynabead-Ab-Ag complexes. These complexes were then washed extensively with phosphate-buffered saline, resolved using 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis and immunoblotted.

Matrigel invasion assay
An invasion assay was performed using a Millicell invasion chamber (Millipore). The 8 μm pore inserts were coated with Matrigel (Sigma–Aldrich). Confluent cells were trypsinized and resuspended in DMEM containing 1% FBS and 5 μg/ml mitomycin C (Sigma–Aldrich). Cell suspensions (100 μl, 5 × 10^5 cells) were seeded into the tops of the inserts. Simultaneously, 500 μl of DMEM containing 10% FBS was placed in the lower chamber. After 24 h of migration, non-invading cells were removed with a cotton-tipped swab. Invading cells on the bottom surface of filter were stained with 0.1% crystal violet, and the number of invading cells per field was assessed by counting 10 random fields at ×200 magnification.

Subcutaneous and subrenal capsule xenografts
Four- to six-week-old male BALB/c nude mice were purchased from the Shanghai Experimental Animal Center and were housed in a pathogen-free facility of the Animal Center of Xi’an Jiaotong University. Mice received food and water ad libitum. The experimental protocols were evaluated and approved by the Animal Care and Use Committee of College of Medicine, Xi’an Jiaotong University.

The mice were injected subcutaneously with 2 × 10^6 cells in a 50% Matrigel mixture. The tumor dimensions were measured with vernier calipers every

Fig. 1. Association of p57 expression levels with HCC. (A) Immunohistochemical staining of p57 in adjacent non-cancerous tissues (A1) and HCC tissues (A2–A4) (×200); (A1) strong expression (scores 10–12), (A2) negative expression (scores 0–2), (A3) weak expression (scores 3–5), and (A4) moderate expression (scores 6–9). Each inset shows images taken at ×400 magnification. (B) Kaplan–Meier curve showing that the median survival times of the p57-positive group (n = 18) and p57-negative group (n = 27) were 21.0 months (95% CI, 18.23–23.77) and 13.0 months (95% CI, 7.94–18.06), respectively. (C) The comparative CT method was used to quantitate the relative levels of p57 mRNA. Columns, mean (n = 40); bars, SD; **P < 0.01. Compared with adjacent non-cancerous tissues, 26 of 40 (65%) HCC tissues displayed decreased expression of p57 mRNA. Student’s t-test was used for statistical analyses. (D) Representative western blot analyses show p57 expression in 15 pairs of HCC tissues (HCC) and matched adjacent non-cancerous tissues (NC).
3 days, and tumor volume was calculated using the equation 
\( l 	imes w^2 \times 1/2 \), in which \( l \) and \( w \) refer to the length and width of the tumors, respectively.

The mice were euthanized 6 weeks after the subcutaneous injection. The tumor xenografts were removed, and the net weights were immediately measured.

For subrenal capsule xenograft experiments, the mice were anaesthetized by intraperitoneal injection with 1% pentobarbital sodium (50 mg/kg). After induction of deep anesthesia, an oblique incision (<1 cm) was made on the skin parallel and adjacent to the long axis of the left kidney of the mouse and 6 × 10⁵ cells were injected under the left kidney capsule of each mouse. Subsequently, the left kidney was returned to the retroperitoneal space, and the incision was closed with surgical staples. Mice were euthanized 3 weeks after the injection. The kidneys were removed, and the subrenal capsule xenografts were measured. The xenografts were then fixed in 4% paraformaldehyde and analyzed by hematoxylin/eosin staining. The invasion index was calculated using the equation \( l \times w \times d \). In this equation, \( l \) and \( w \) refer to the length and width of the xenograft, respectively, and \( d \) represents the diameter between the innermost tumor in the kidney and the outermost tumor in the subrenal capsule (22).

**Statistical analysis**

Descriptive data were assessed using the Pearson’s chi-square test (two sided). Measurement data were assessed using Student’s t-test. Survival curves were plotted using the Kaplan–Meier method, and the log-rank test was used to compute the differences between the curves. \( P \leq 0.05 \) were considered statistically significant. All statistical analyses were performed using SPSS 13.0 for Windows software (Chicago, IL).

**Fig. 2.** Downregulation of p57 accelerates the growth of HCC cells in vitro and in vivo. (A) MTT assays were performed to quantify relative cell numbers at the indicated time points (1–7 days), **P < 0.01. (B)** The proportion of cells at different cell cycle phases was assessed by flow cytometric analysis. (C) Quantitative analysis of the different cell cycle phases. Columns, mean (n = 3); bars, SD; **P < 0.01 compared with the corresponding shNC cells. (D) Tumor volumes were determined by measuring the width and length of the tumors every 3 days. Mean (n = 5); bars, SD; **P < 0.01 compared with the corresponding groups with shNC. (E) The weights of the tumor xenografts. The mice were euthanized 6 weeks after implantation, and the tumors were removed and weighed. Columns, mean (n = 5); bars, SD; **P < 0.01 compared with shNC controls.
Results

Association of p57 expression level with HCC

To assess the expression of p57 protein, immunohistochemistry was performed on 45 pairs of HCC tissues and adjacent non-cancerous tissues. The results showed that 18 of 45 HCC tissues and 32 of 45 corresponding adjacent non-cancerous tissues expressed p57. The levels of p57 protein in the HCC tissues were lower than those in the adjacent non-cancerous tissues (P < 0.05) (Figure 1A). In HCC tissues, we further analyzed whether p57 downregulation was linked to the clinical progression of cancer. Our findings showed that the expression of p57 was reduced in specimens from patients with a larger tumor size, a more advanced TNM stage or the presence of capsule invasion and extrahepatic metastasis (P < 0.05) (Figure 1B). Notably, p57 expression was observed in both the nucleus and the cytoplasm in HCC tissues and the adjacent non-cancerous tissues (Figure 1A). The 5-year follow-up of 45 HCC patients indicated that the median survival time of the p57-positive group and the p57-negative group were 21.0 and 13.0 months, respectively, supporting the utility of p57 as a prognostic factor in HCC progression.

To assess p57 mRNA levels, real-time PCR analysis was performed on 40 HCC tissues and adjacent non-cancerous tissues. The relative amount of p57 mRNA was decreased in HCC tissues when compared with adjacent non-cancerous tissues (P < 0.01) (Figure 1C). Using this comparison, 26 of 40 (65%) HCC tissues showed a decreased level of p57 mRNA. To further confirm these results, western blot analysis was performed on 15 pairs of HCC tissues and the corresponding adjacent non-cancerous tissues. Of these pairs, 12 of 15 (80%) displayed decreased levels of p57 protein in HCC tissues when compared with the adjacent non-cancerous tissues (P < 0.05). Moreover, western blot analysis demonstrated that p57 expression was decreased in samples from patients with a larger tumor size, a more advanced TNM stage or the presence of capsule invasion (Figure 1D).

These data convinced us that p57 downregulation may play a role in HCC progression.

Downregulation of p57 accelerates the growth of HCC cells in vitro and in vivo

Although p57 is a well-known cyclin-dependent kinase inhibitor, the role of p57 in the proliferation of HCC cells remains unknown. MTT assays showed that BEL7402-shp57 cells and SMMC7721-shp57 cells grew faster than BEL7402-shNC cells and SMMC7721-shNC cells, respectively (P < 0.05) (Figure 2A). Moreover, cell cycle analysis using flow cytometry showed that BEL7402-shp57 and SMMC7721-shp57 cells displayed a significant decrease in the G1/G0 phase and an increase in the S phase when compared with BEL7402-shNC cells and SMMC7721-shNC cells, respectively (P < 0.05) (Figure 2B, C). This finding suggests that the downregulation of p57 significantly accelerated the proliferation of HCC cells.

To verify these in vitro findings, we performed subcutaneous xenografts. The results of these experiments showed that the average volumes of the tumor xenografts derived from BEL7402-shp57 cells and SMMC7721-shp57 cells were greater than those of the tumor xenografts derived from BEL7402-shNC cells and SMMC7721-shNC cells, respectively (P < 0.05) (Figure 2D). Similarly, the weights of the tumor xenografts derived from cells transfected with shp57 were markedly increased when compared with tumor xenografts derived from cells transfected with shNC (P < 0.05) (Figure 2E). These data support a role for p57 downregulation in promoting the formation and growth of tumor xenografts in vivo.

p57 regulates the expression of cyclin D1 and CDK2 and the kinase activity of cyclin D1-CDK4 and cyclin E-CDK2 complexes

To elucidate the mechanism by which p57 regulates HCC cell proliferation and cell cycle, cells transfected with shp57 and shNC were analyzed by western blot. The results showed that p57
downregulation in BEL7402-shp57 cells and SMMC7721-shp57 cells increased cyclin D1 and CDK2 protein expression (Figure 3A). Furthermore, p57 could specifically coprecipitate with the complexes of CDK2, CDK4 and cyclins E, A and D1 at G1/S phase (3,4). Thus, we further explored the effect of p57 downregulation on the activity of CDK/cyclin complexes. Compared with control cells, the kinase activity of CDK4/cyclin D1 and CDK2/cyclin E were increased in BEL7402-shp57 cells and SMMC7721-shp57 cells (P < 0.05) (Figure 3B, C); however, CDK6/cyclin D1 and CDK2/cyclin A did not exhibit any significant changes in kinase activity (Figure 3D, E). In addition, p57 downregulation promoted the assembly of CDK4/cyclin D1 and CDK2/cyclin E complexes, indicating that p57 could regulate kinase activity by inhibiting the formation of active kinase complexes (Figure 3F). These findings suggest that the ability of p57 to inhibit the proliferation of HCC cells and arrest the cell cycle at G0-G1 stage may be mediated by affecting the expression of cyclin D1 and CDK2 and the kinase activity of cyclin D1-CDK4 and cyclin E-CDK2 complexes.

Downregulation of p57 enhances HCC cell invasion in vitro and in vivo
Because the immunohistochemical findings indicated that p57 downregulation was associated with capsule invasion and extrahepatic metastasis in HCC tissues, we next assessed the invasive capacity of HCC cells transfected with shp57 and shNC in a transwell assay. To ensure that any observed increases in invasion were not due to increased proliferation, we treated the HCC cells with mitomycin C. The results showed that the numbers of invading BEL7402-shp57...
cells and SMMC7721-shp57 cells were more than those of BEL7402-shNC cells and SMMC7721-shNC cells, respectively (P < 0.05) (Figure 4A, B), suggesting that the downregulation of p57 significantly accelerated the invasion of HCC cells in vitro.

To assess the impact of p57 downregulation on the invasive capacity of HCC cells in vivo, we performed subrenal capsule xenografts. The results of these experiments indicated that the range of invasion was higher in xenografts of BEL7402-shp57 cells and SMMC7721-shp57 cells when compared with xenografts of control cells (Figure 4C). Hematoxylin-eosin staining showed that the depth of invasion was deeper in the xenografts of cells transplanted with shp57; in these samples, the HCC cells invaded the renal parenchyma (Figure 4D). The average invasion indexes of the xenografts of BEL7402-shp57 cells and SMMC7721-shp57 cells were higher than those of the xenografts of BEL7402-shNC cells and SMMC7721-shNC cells, respectively (P < 0.05) (Figure 4E), indicating that p57 downregulation results in increased HCC cell invasion in vivo.

**p57 regulates the activity of LIMK1**

The Cip/Kip proteins have been reported to interact with the RhoA/ROCK1/LIMK1/cofilin pathway, which plays important roles in modulating HCC invasion and metastasis. In this pathway, RhoA-GTP activates ROCK1, which in turn activates LIMK1 by phosphorylating Thr508. Subsequently, LIMK1 phosphorylates cofilin at Ser-3 and blocks the ability of cofilin to depolymerize actin filaments during cell migration (12). Thus, to clarify the potential mechanism by which p57 regulates invasion in HCC cells, we investigated the association of p57 with the RhoA/ROCK1/LIMK1/cofilin pathway, which plays important roles in modulating HCC invasion and metastasis.

**Discussion**

In tumors, the absence of p57 largely occurs through transcriptional and posttranslational silencing due to the loss of imprinting or heterozygosity, promoter methylation, histone deacetylation, microRNAs, ubiquitylation or the regulation of upstream signaling (2). p57 knockout mice have been shown to develop prostatic intraepithelial neoplasia and adenocarcinoma (8). Moreover, the microRNA221-mediated downregulation of p57 contributes to hepatocarcinogenesis, and the ubiquitin-mediated degradation of p57 by Cks1-Skp2 ligase increases susceptibility to developing HCC (23,24). These findings indicate that the possibility that the downregulation of p57 might be an important molecular event in tumor development. Here, we showed that p57 mRNA and protein levels were decreased in HCC tissues when compared with adjacent non-cancerous tissues. Moreover, reduced p57 expression was associated with a larger tumor size, a more advanced TNM stage, the presence of capsule invasion and metastasis and decreased overall patient survival time in HCC. These findings indicate that similar to its role in other malignancies, including breast, lung, bladder and pancreatic cancers, p57 is a key regulator of the development of HCC and an important prognostic marker (25-29).

p57 is characterized as a strict inhibitor of all cyclin–CDK complexes at the G1/S transition and the G2/M transition; however, p57 displayed stronger inhibition of cyclin–CDK complexes at the G1–S transition and decreased affinity for the cyclin B–CDK1 complex at the G2/M transition. Numerous studies have indicated that p57 principally regulates the expression level and kinase activity of cyclins and CDKs at the G1/S transition to inhibit the proliferation of tumor cells (23,31). High levels of cyclin D1 and CDK2 have been reported to be a common event in human hepatocarcinogenesis, and p57 can regulate the levels of cyclin D1 and CDK2 in other human tumors (8,32,33). Consistent with these results, our results demonstrate that p57 downregulation in HCC cells significantly accelerates cell proliferation and growth of xenografts by inducing cells to transition from the G0/G1 phase into S phase. Additionally, p57 downregulation may increase cell proliferation by regulating the levels of cyclin D1 and CDK2 as well as the kinase activity of cyclin D1–CDK4 and cyclin E–CDK2 complexes in HCC cells. These results demonstrate that p57 can serve as an important tumor suppressor and that its downregulation accelerates HCC growth.

Several clinical studies have shown that p57 is associated with biologically aggressive tumor phenotypes and that reduced p57 expression is correlated with lymph node metastasis (1,34). Furthermore, the inducible expression of p57 reduced the motility and invasiveness of glioma cells (35). In human prostate cancer, the expression of p57 is significantly decreased, and the overexpression of p57 considerably reduced invasive ability (8). These findings demonstrate that p57 plays an inhibitory role in tumor invasion. In the current study, we observed that p57 downregulation enhanced the invasive capacity of HCC cells in vitro. Moreover, using a subrenal capsule xenograft model, we showed that xenografts derived from cells in which p57 was downregulated were more aggressive.
demonstrating that p57 functions as a tumor invasion suppressor in HCC. Vlachos et al. (16) showed that human p57 interacts with and enhances the activity of LIMK1 in the cytoplasm, resulting in increased levels of phospho-cofilin and the inhibition of motility in human cervical carcinoma cells. In addition, downregulation of p57 promotes cell invasion via the LIMK/cofilin pathway and might contribute to tumor cell metastasis in human nasopharyngeal carcinoma cells (36). Consistent with these studies, our data demonstrate that the downregulation of p57 decreases the activity of LIMK1 and the level of phospho-cofilin, thereby promoting the invasion of HCC cells.

Notably, p57 was localized to both the nucleus and the cytoplasm of HCC cells in our study. p57 is frequently considered to be a nuclear protein, but cytoplasmic expression of p57 has been found in various tissues, tumors and cell lines (28,37–39). The mechanism underlying the nuclear cytoplasmic shuttling of p57 remains unknown. It is conceivable that nuclear localization of p57 might regulate cellular proliferation and that cytoplasmic localization of p57 might regulate cell motility (5,6). We are thus interested in further investigating the role of the subcellular distribution of p57 in the development of HCC.

In conclusion, our data indicate that p57 expression was significantly decreased in HCC tissues and that this protein is a key marker of the biological behavior of HCC tumors and HCC patient prognosis. Furthermore, p57 downregulation may accelerate the growth and invasion of HCC, suggesting that p57 may be an important tumor suppressor in HCC. Importantly, p57 may be a promising target for HCC prevention and therapy in the future.

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Conflict of Interest Statement: None declared.

References


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