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Original Article

RAB18 promotes proliferation and metastasis in hepatocellular carcinoma

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Abstract: RAB18, a member of the Ras family, has been suggested to play a critical role in multiple biological process. However, its functions in the development of hepatocellular carcinoma (HCC) remain unknown. In the present study, the expression and biological role of RAB18 in HCC were investigated. Results showed that the expression level of RAB18 was significantly increased in HCC tissue specimens and HCC cell lines. Kaplan-Meier survival analysis showed that high RAB18 expression was correlated with poor overall survival compared to those with low RAB18 expression. These results were further confirmed by analyses in the Cancer Genome Atlas (TCGA) database. Specific knockdown of RAB18 expression inhibited proliferation and clone formation of HCC *in vitro*. Western blot analyses showed that CCND1 was suppressed, and p21 and p27 were substantially upregulated in RAB18 knockdown HCC cells. Furthermore, we also observed that knockdown of RAB18 expression suppressed the migration and invasion of HCC cells and reversed expression of epithelial-mesenchymal transition (EMT)-related markers. Interestingly, the primary and xenograft tumor mouse models showed that RAB18 knockdown significantly reduced *in vivo* tumorigenesis and metastasis in nude mice. These results revealed that RAB18 was correlated with poor clinical outcomes and facilitated HCC progression via promotion of HCC cell proliferation and metastasis. These findings suggest that RAB18 may be a prognostic biomarker and potential therapeutic target in patients with HCC.

Keywords: Hepatocellular carcinoma, RAB18, metastasis proliferation, epithelial-to-mesenchymal transition

Introduction

Hepatocellular carcinoma (HCC) is the third most common malignant tumor, and due to high morbidity and mortality, poses a serious threat to human health worldwide [1]. Increasing evidence indicates that viral hepatitis, nonalcoholic fatty liver disease (NAFLD), and alcohol are potential risk factors for the progression of HCC [2]. Although surgical resection and liver transplantation are optimal therapeutic approaches for early-stage HCC, most patients with advanced HCC are not eligible for these curative treatments [3]. Other treatment options, including radiotherapy, systemic chemotherapy, and molecular-targeted therapies were proven to achieve minimal efficacy. The prognosis for patients diagnosed with HCC is poor, with a 3-year survival rate of 12.7% and a median survival of 9 months [4]. Therefore, identification of new molecular actors that contribute to the progression of HCC is urgently needed.

Rab guanosine triphosphatases (GTPases) belong to the Ras oncogene superfamily of small guanosine triphosphatases, which are essential for vesicular transport and cargo sorting in eukaryotic cells [5, 6]. In recent decades, evidence from human and animal studies revealed that Rab guanosine triphosphatases are involved in the pathogenesis of various human diseases such as infection with invading pathogens and cancer progression [7-9].

Table 1. Correlation of RAB18 expression with clinicopathological parameters

Characteristics	Cases (*n*)	RAB18 expression		*P** value
		Negative	Positive	
Age (years)				
≤ 50	33	19 (57.6%)	14 (42.4%)	0.372
> 50	60	38 (63.3%)	22 (36.7%)	
Gender				
Male	83	51 (61.4%)	32 (38.6%)	0.592
Female	10	6 (60%)	4 (40%)	
Tumor size (cm)				
≤ 5	43	26 (60.5%)	17 (39.5%)	0.475
> 5	49	31 (63.3%)	18 (36.7%)	
Tumor number				
Solitary	87	55 (63.2%)	32 (36.8%)	0.280
Multiple	5	2 (40%)	3 (60%)	
Clinical stage				
I-II	46	28 (60.9%)	18 (39.1%)	0.552
III-IV	47	29 (61.7%)	18 (38.3%)	
Liver cirrhosis				
No	57	34 (59.6%)	23 (40.4%)	0.426
Yes	36	23 (63.9%)	13(36.1%)	
Histological grade				
I-II	61	32 (52.5%)	29 (47.5%)	**0.013**
III	32	25 (78.1%)	7 (21.9%)	

*X^2 test was applied to access the associations between RAB18 expression and the clinicopathological parameters. ($P < 0.05$) is marked in boldface.

Rab5 is one of the most extensively studied Rab GTPase members, and it was found that mycobacteria manipulate Rab5 to facilitate fusion with endocytic vesicles for its replication in host cells [10]. Rab5 has also been associated with cancer development. Several studies reported that overexpression of Rab5A promoted the metastatic potential of lung adenocarcinoma, breast cancer, and ovarian cancer [11-13]. Lymphatic invasion of breast cancer has also been correlated with the increased expression of Rab25 [14]. Recently, Rab18 has gained attention in the field of cancer research due to its role in the tumor development of non-small cell lung and breast cancer [15, 16]. However, there are few data available on the functions of Rab18 and its impact on the progression of HCC.

In this study, we explored the regulatory mechanism and clinical significance of Rab18 in HCC. Upregulated Rab18 was an independent prognostic marker for reduced survival in HCC patients. *In vitro* and *in vivo* studies demonstrated that Rab18 knockdown markedly attenuated the proliferation, migration, and invasion of HCC. These findings are the first to reveal the function of RAB18 in HCC and suggest that Rab18 may be a suitable therapeutic target for HCC.

Materials and methods

Specimens

Patient tissue microarray slides of HCC including 93 tumor tissues and matched adjacent non-tumor tissues were purchased from Shanghai Outdo Biotech Co., Ltd (Product number: HLivH180Su10). The detailed clinical information of HCC patients is shown in **Table 1**.

Patients in the TCGA database

Expression of RAB18 in HCC data was analyzed based on The Cancer Genome Atlas (TCGA). Patients were divided into high- and low-expression groups, and the prognostic values of RAB18 regarding differential expression and survival analysis were analyzed.

Cell culture

HCC cell lines (Hep-3B, SMMC7721, Huh7, BEL-7402, and LO2) were obtained from the Cancer Research Institute of Southern Medical University in Guangzhou, China. The cells were routinely maintained in high-glucose DMEM (Life Technologies, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS, Life Technologies) at 37°C with 5% CO_2.

Lentivirus production and infection

Lentiviral particles expressing shRNA against RAB18 and the flanking control sequence were constructed by Genechem (Shanghai Genechem Co., LTD). SMMC-7721 and Huh7 cells were transfected with lentiviral vectors, and polyclonal cells with green fluorescent protein signals were selected using fluorescence-activated cell sorting for further experiments. RAB18 expression was confirmed by qPCR, and the levels of RAB18 protein were measured by western blotting.

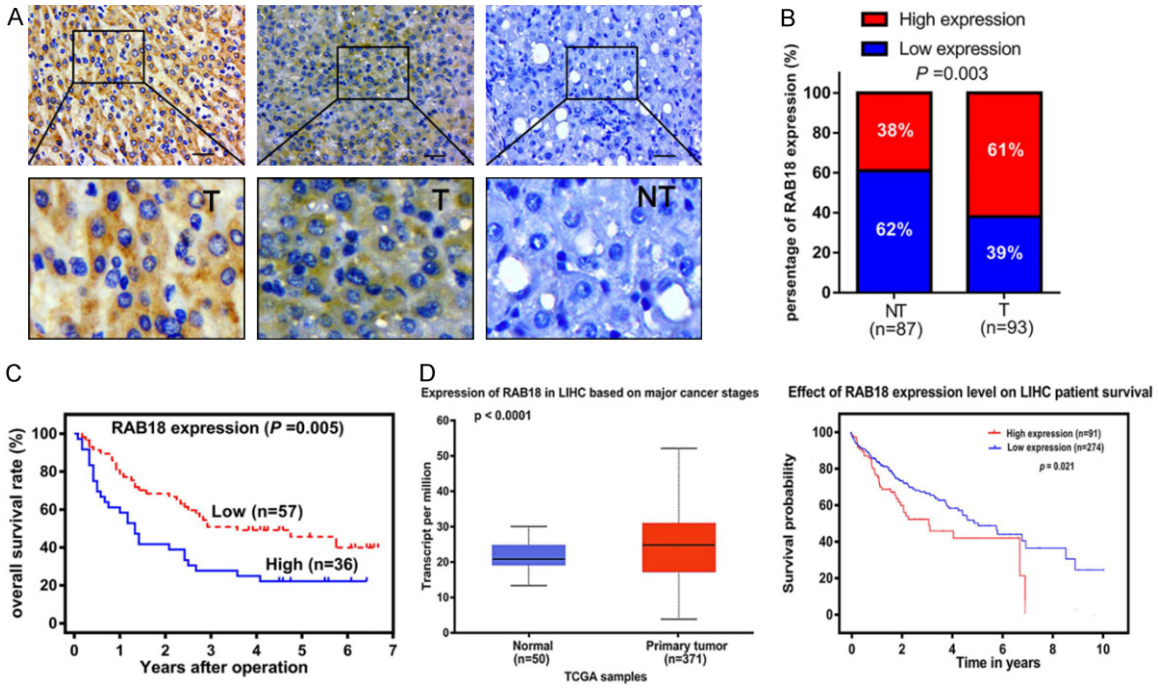

Figure 1. RAB18 expression increased in HCC and indicated poor prognosis. A. Representative IHC images of RAB18 expression in HCC tissues and matched adjacent non-tumor tissues. Scale bar: 100 μm. B. RAB18 expression levels were compared among HCC and adjacent non-tumor tissue specimens, $P = 0.003$. C. Kaplan-Meier curves of 93 HCC patients according to RAB18 expression level. D. HCC and survival of HCC patients were correlated with RAB18 expression based on the TCGA database.

MTT assay

Cell viability was determined by MTT assay. Cells were seeded into 96-well plates at a density of 1,000 cells/well and cultured in 100 μl of DMEM containing 10% FBS for 4 days. At each indicated time point, 20 μl of MTT (Sigma, St Louis, MO) solution was added to each corresponding well and incubated for 4 h. Formazan crystals were then solubilized with 150 μl dimethyl sulfoxide (Sigma) and the absorbance value (OD) was measured at 490 nm using a microplate reader.

EdU labeling

EdU incorporation assay using the Cell-Light EdU Apollo 567 *in vitro* Imaging Kit (RiboBio, Guangzhou, China) was performed to examine the proliferating cells. Cells were seeded in 96-well plates and labeled with 10 mM EdU for 2 h followed by 4% paraformaldehyde fixation and 0.3% Triton X-100 permeabilization. Afterward, cells were stained with Apollo fluorescent dyes and cell nuclei were stained with 50 μg/ml DAPI for 10 min. The number of EdU-positive cells was counted in five random fields using an inverted fluorescent microscope (OLYMPUS IX71).

Colony formation assay

Cells were plated in six-well culture plates at 100 cells/well. After incubation for 2 weeks at 37°C, the cells were washed twice with PBS and stained with 0.1% crystal violet solution. The number of colonies containing ≥ 50 cells was counted under a microscope. The colony formation efficiency was calculated as (number of colonies/number of cells inoculated) × 100%.

Wound-healing assay

In the wound-healing assay, cells seeded in six-well plates were grown to full confluence. Then, cells were wounded across the center of the well to form a clean, straight edge using 200 μl pipette tips and washed three times with PBS to remove detached cells and debris. The ability of the cells to migrate into the wound area was assessed every 24 h after scratching. The

Table 2. Univariate analysis of factors associated with survival

Variables	OS	*P** value
	Hazard ratio (95% CI)	
Age (years)	0.986 (0.575-1.692)	0.960
Gender	0.688 (0.275-1.721)	0.424
Tumor number	1.384 (0.500-3.830)	0.531
Tumor size (cm)	0.536 (0.313-0.916)	**0.022**
Liver cirrhosis	1.003 (0.594-1.696)	0.990
Clinical stage	2.346 (1.374-4.008)	**0.002**
Histological grade	1.258 (0.742-2.134)	0.394
RAB18 expression	0.489 (0.293-0.818)	**0.006**

*Variables were adopted for their prognostic significance by univariate analysis. ($P < 0.05$) is marked in boldface.

result of wound closure is expressed as a percentage of the initial scratch area.

Cell migration and invasive assays

Transwell chambers with or without Matrigel (BD, Biosciences, CA) were employed to assess cancer cell migration and invasion. Cells were resuspended in serum-free medium at density of 1×10^5 cells/well (100 μl/well) and added to the top chamber. Meanwhile, medium containing 10% FBS culture medium was added to the lower chamber. After 15 h, the migrated cells that attached to the lower surface of the membrane insert were fixed, stained using Giemsa (Jiancheng, Jiangsu, China), and photographed under a microscope.

Immunohistochemical analysis

The slides were dewaxed with xylene and rehydrated using graded alcohols after being dried for 2 h at 62°C. Then, the slides were immersed in 3% hydrogen peroxide for 15 min to block endogenous peroxidase activity. For antigen retrieval, sections were placed in sodium citrate buffer (0.01 M, pH 6.0) and microwaved for 20 min, followed by incubation with 10% goat serum for 30 min to prevent nonspecific binding. The slides were then incubated overnight at 4°C with a rabbit polyclonal antibody to RAB18, followed by incubation with an HRP-labeled anti-mouse/rabbit secondary antibody at 37°C for 1 h, and stained with DAB. Finally, the sections were counterstained with hematoxylin, dehydrated, and mounted.

Two pathologists independently evaluated the immunohistochemistry (IHC) for RAB18 and categorized the samples according to immunoreactive score (IRS): IRS = SI (staining intensity) × PP (percentage of positively stained cells). SI was scored as 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). PP was scored as 0 (negative), 1 (< 25% of the cells), 2 (25%-50% of the cells), 3 (50%-75% of the cells), or 4 (> 75% of the cells). Protein expression was classified as high expression if the IRS was higher than 6, and low expression if the score was 6 or less. The antibodies used were as follows: rabbit anti-RAB18 (1:200, Abcam, Cambridge, UK), anti-Ki67 (1:100, Abcam, Cambridge, UK), E-cadherin (1:500, CST, Boston, MA) and Vimentin (1:500, CST, Boston, MA).

Western blotting

Total proteins were extracted with RIPA lysis buffer supplemented with protease inhibitors, separated by SDSPAGE, and transferred to a PVDF membrane (Millipore, Billerica, MA). The membrane was blocked with 5% non-fat milk at room temperature for 1 h and incubated with the appropriate antibody. Protein bands were visualized using the chemiluminescent HRP detection system (Millipore). The antibodies are as follows: RAB18 (1:500, Abcam, Cambridge, UK), CCND1 (1:1000, CST, Boston, MA), p21 (1:1000, CST, Boston, MA), p27 (1:1000, CST, Boston, MA), E-cadherin (1:1000, CST, Boston, MA), Vimentin (1:1000, CST, Boston, MA), GAPDH (1:1000, CST, Boston, MA).

Real-time quantitative PCR

Real-time (RT) PCR was employed to quantify the genes of interest. Total RNA (1 μg) was reverse transcribed into cDNA and then subjected to real-time (RT) PCR for analysis of the genes of interest using specific primers and SYBR-Green as the fluorescent dye (TaKaRa Biotech). Real-time (RT) PCR was performed using a Bio-Rad Real-Time PCR system with the specific primers as follows: RAB18, Forward: CAGGGAAGAAGGCCAAGGAG, Reverse: CCCGGGGTCGATGGAGT. GAPDH, Forward: GAAGGTGAAGGTCGGAGTC, Reverse: GAAGATGGTGATGGGATTTC. The GADPH gene was used as the internal control. Relative gene expression was calculated using the $2^{-\Delta\Delta ct}$ method [17].

In vivo tumorigenesis assay

All animal studies were conducted in accordance with the principles and procedures outlined in the Southern Medical University Guide

Table 3. Multivariate analysis of factors associated with survival

Variables	OS	*P** value
	Hazard ratio (95% CI)	
Tumor size (cm)	0.811 (0.408-1.610)	0.549
Clinical stage	2.099 (1.057-4.167)	**0.034**
RAB18 expression	0.478 (0.284-0.803)	**0.005**

*Variables were adopted for their prognostic significance by multivariate analysis. ($P < 0.05$) is marked in boldface.

for the Care and Use of Animals under assurance number SCXK (Guangdong) 2008-0002. For *in vivo* tumorigenesis, 2×10^6 SMMC7721 cells logarithmically growing in 0.1 ml PBS medium and transfected with shRAB18, lentiviral vectors, or a negative control vector were used in the subcutaneous model. The cells were injected into the right leg of 4- to 5-week-old female BALB/c nude mice. After tumor transplantation, subcutaneous tumor size was calculated and recorded every 3 days. After 19 days, the mice were killed, and the tumor tissues were surgically excised, weighed, and stained with hematoxylin and eosin (H&E). For the metastatic lung tumor mouse model, SMMC7721 cells (1×10^6 in 200 μl PBS) were intravenously (i.v.) injected into the tail vein of nude mice and allowed to inoculate the lung (n = 10). Transplanted cells were allowed to grow for up to 6 weeks. After the mice were killed, the lungs were dissected, fixed, and paraffin embedded.

Statistical analysis

All data analyses were performed with the SPSS 19.0 statistical software package (SPSS, IBM, Armonk, NY). Survival curves were constructed using the Kaplan-Meier method and analyzed by the log-rank test. Significant prognostic factors identified by univariate analysis were entered into a multivariate analysis using the Cox proportional hazard regression model. The Pearson's chi-square test was used to analyze the association of RAB18 expression with various clinicopathologic characteristics. Data were expressed as mean ± SD from at least three independent experiments. Differences were statistically significant at a $P < 0.05$ as determined by Student's *t* test for two groups, one-way ANOVA analysis for multiple groups, and parametric generalized linear model with random effects for the MTT assay. All statistical tests were two-sided, and asterisks indicate statistical significance, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

Results

Expression level of RAB18 was significantly increased in HCC tissues and associated with poor prognosis

The expression level of RAB18 in HCC tissue specimens (n = 93) and matched adjacent non-tumor liver tissues (n = 87) was analyzed by immunohistochemical assays. Results showed that the expression level of RAB18 in HCC tissues was significantly higher than in the adjacent non-tumor liver tissues (**Figure 1A**). RAB18 expression was upregulated in 61.3% (57/93) of the HCC tissue specimens, compared with only 37.9% (33/87) in the adjacent non-tumor liver tissues (**Figure 1B**). To better understand the clinical relevance of RAB18 expression in HCC, the relationship of RAB18 expression in 93 HCC patients with the clinicopathological parameters was analyzed. High RAB18 expression correlated with histological grade ($P = 0.013$) (**Table 1**). Moreover, in a multivariate Cox model, univariate analysis revealed that tumor size, clinical stage, and RAB18 expression were unfavorable predictors for 5-year overall survival (OS) in HCC (**Table 2**). Next, the correlation of RAB18 expression with the survival of 93 HCC patients was analyzed. Kaplan-Meier analysis results showed that the 5-year OS in the high RAB18 expression group was significantly shorter than in the low RAB18 expression group ($P = 0.005$) (**Figure 1C**). Moreover, we carried out analyses in TCGA database and obtained similar results as these findings (**Figure 1D**). Further multivariate analysis revealed that RAB18 expression was an independent poor prognostic factor for the 5-year OS of HCC patients (**Table 3**). In summation, these data show that RAB18 is frequently upregulated in HCC tissues.

Knockdown of RAB18 inhibited the proliferation of HCC cells

The expression level of RAB18 was measured in the hepatic cell lines: Huh7, SMMC7721, Hep-3B, BEL-7402, and LO2. Compared with the LO2 normal human liver cell line, the protein and mRNA expression levels of RAB18 were upregulated in Hep-3B, BEL-7402, Huh7, and SMMC7721 cells, particularly in the latter

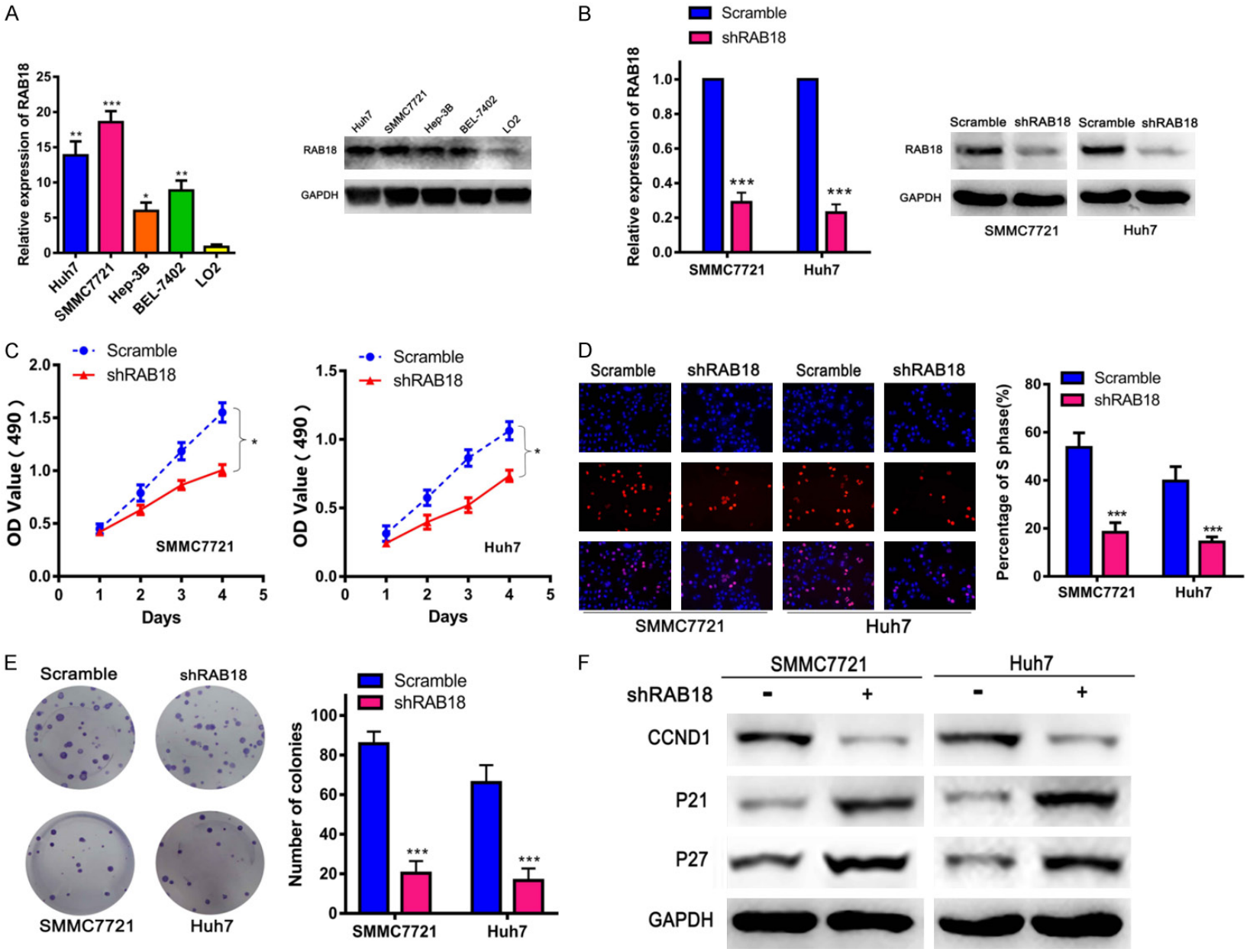
A
Relative expression of RAB18
Huh7
SMMC7721
Hep-3B
BEL-7402
LO2
RAB18
GAPDH
B
Scramble
shRAB18
Relative expression of RAB18
SMMC7721
Huh7
C
OD Value (490)
Days
D
Percentage of S phase(%)
E
Number of colonies
F
shRAB18
CCND1
P21
P27
GAPDH

Figure 2. Knockdown of RAB18 significantly decreased HCC cell proliferation *in vitro*. (A) RAB18 expression level in hepatic cell lines (Huh7, SMMC7721, Hep-3B, BEL-7402, and LO2) was determined by qRT-PCR and western blot analyses. (B) Expression levels of RAB18 were measured by qRT-PCR and western blot in HCC cells treated with LV-shRAB18 or LV-Scramble. (C, D) The effect of RAB18 on *in vitro* proliferation was analyzed by MTT (C), EdU incorporation assay (D), and colony-forming assay (E). (F) Western blot analysis was performed to detect the expression of CCND1, P21, and P27 in shRAB18 cells; GAPDH was used as a loading control.

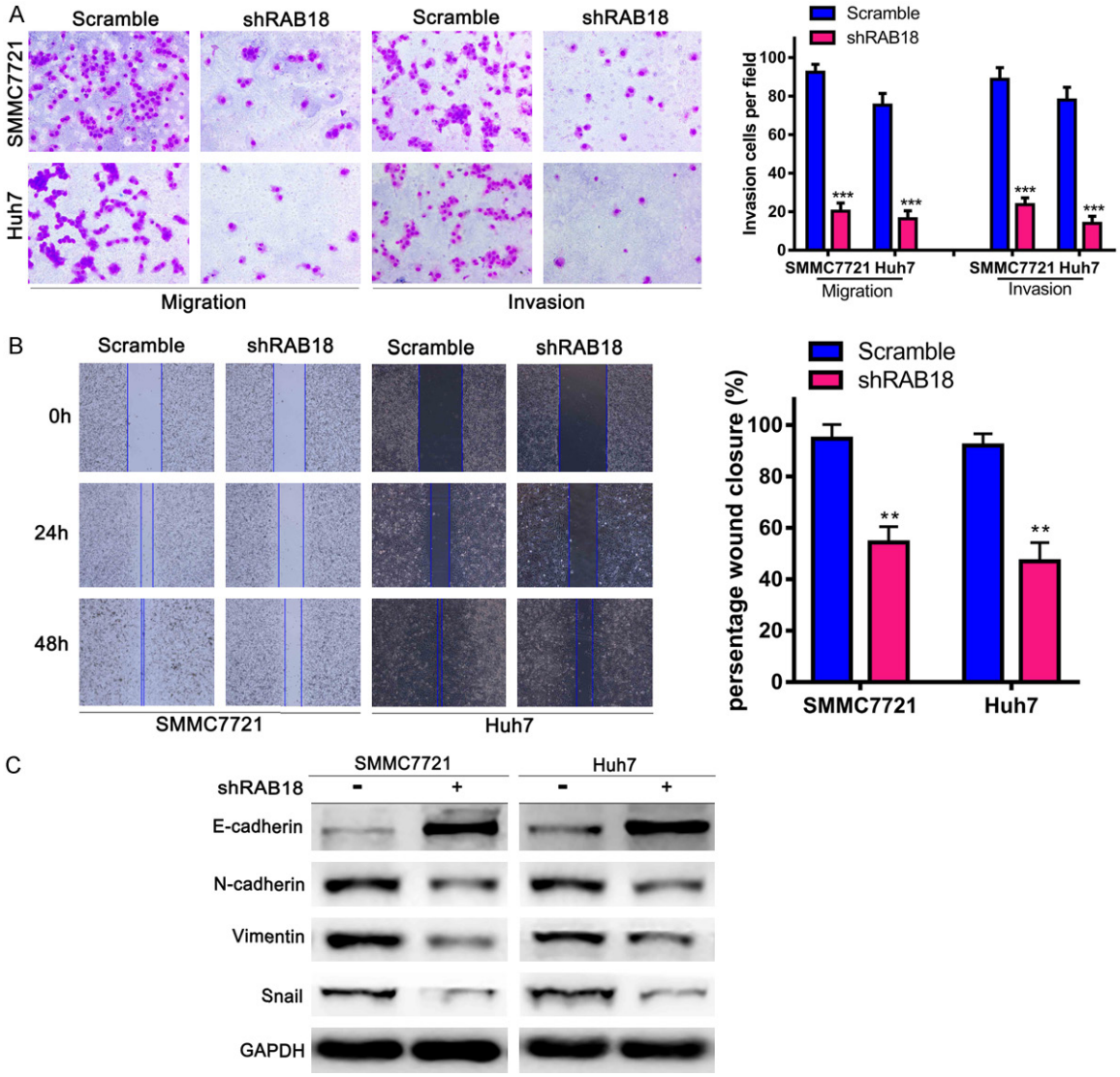

Figure 3. Decreased RAB18 expression inhibited the migration and invasion of HCC cells *in vitro*. A. Invasion capability of RAB18 siRNA-transfected cells was assessed by transwell assay. B. Migration of RAB18 siRNA-transfected cells was measured by wound-healing assays. C. Western blot analysis of the EMT-related markers E-cadherin, N-cadherin, vimentin, and snail after RAB18 siRNA transfection in HCC cells.

two HCC cell lines (**Figure 2A**). These results were similar to the observed RAB18 expression pattern in HCC tissue specimens and indicate that increased RAB18 expression could be involved in the tumorigenicity of HCC. To determine the functional role of RAB18 in HCC, we designed a short hairpin RNA (shRNA18) to silence RAB18 expression. More than a threefold decrease in RAB18 expression was observed in SMMC7721 and Huh7 cells treated with the shRNA compared with the control group as determined by qRT-PCR and western

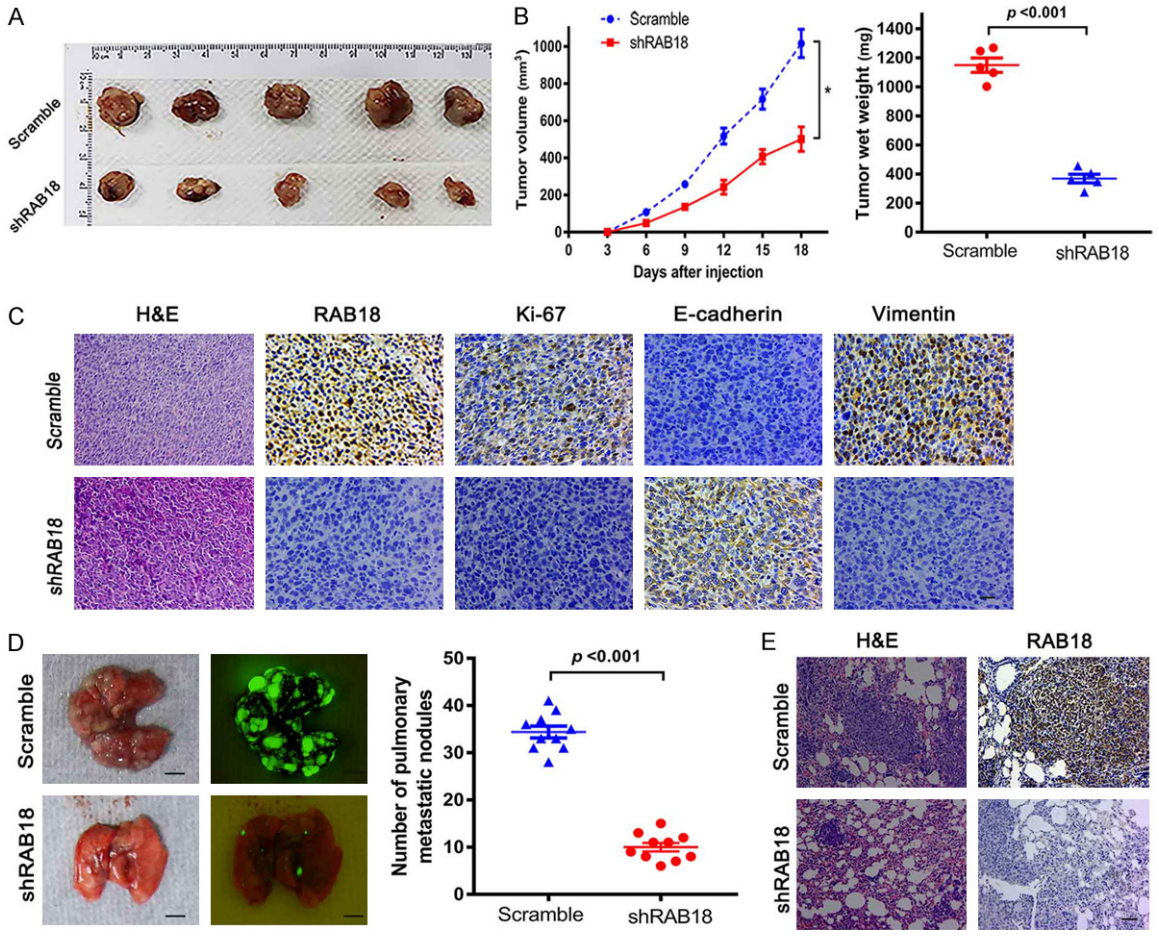

Figure 4. RAB18 augmented the proliferation and metastasis of HCC cells *in vivo*. A. The image of dissected tumors from nude mice with subcutaneous injection of SMMC7721-shRAB18 and control cells. B. The growth curves and weights of tumors derived from nude mice with subcutaneous injection of SMMC7721-shRAB18 and control cells. Tumor volume was measured with Vernier calipers every 3 days for 2 weeks, and the tumor wet weight was determined on day 18. Mean ± SD (n = 5). *$P < 0.05$. C. Tumors tissues were stained with H&E or antibodies against RAB18, Ki67, E-cadherin, and vimentin, Scale bar: 100 μm (bottom). D. Representative fluorescence imaging of the lungs. After 6 weeks, mice were sacrificed, and lungs were collected, *$P < 0.05$ based on Student's *t* test. Scale bar: 5 mm. E. Lung metastases were confirmed by H&E staining, scale bar: 100 μm.

blotting (Student's *t* test, $P < 0.05$), and the shRNA was chosen for further study (**Figure 2B**). Then, MTT assay and Edu incorporation assays were performed to investigate the effect of shRAB18 on the proliferation of HCC cells. As shown in **Figure 2C** and **2D**, introduction of shRAB18 into the cells resulted in a significant inhibition of proliferation. Similarly, colony formation was suppressed by RAB18 knockdown (**Figure 2E**). Of note, western blotting results demonstrated that RAB18 knockdown led to markedly reduced CCND1 expression and elevated expression of the cyclin-dependent kinase inhibitors p21 and p27 (**Figure 2F**). These results demonstrate the role of RAB18 in promoting HCC progression *in vitro*.

Knockdown of RAB18 inhibited migration and invasion of HCC cells

Cell migration and invasion are key factors in the process of HCC development and progression. Therefore, the effects of RAB18 knockdown on the migration and invasion potential of HCC cells were assessed. As expected, RAB18 knockdown markedly attenuated cell migration and invasion compared with control cells (**Figure 3A**). In addition, wound-healing assay results showed that RAB18 knockdown decreased the migration of HCC cells. Activation of the epithelial-mesenchymal transition (EMT) is important for HCC cell metastasis. Therefore, it was hypothesized that the migration and

invasion suppressed by RAB18 knockdown was associated with reversal of EMT. As shown in **Figure 3C**, RAB18 knockdown increased expression of the epithelial marker E-cadherin and decreased expression of mesenchymal markers (N-cadherin, vimentin, and snail). These results suggested that inhibition of HCC cell metastasis by RAB18 knockdown might be through reversal of the EMT process.

Knockdown of RAB18 attenuated the proliferation and metastatic potential of HCC in vivo

Subcutaneous xenograft tumor and orthotopic xenograft tumor models in nude mice were utilized to determine the effects of RAB18 knockdown on the proliferation and metastasis of HCC *in vivo*. Mice bearing RAB18-silenced HCC subcutaneous tumors showed a reduction in tumor volume and growth rate (**Figure 4A** and **4B**). In the subcutaneous xenograft model, mice with tumors derived from RAB18-silenced HCC cells exhibited fewer metastatic lung nodules than the control group (**Figure 4D**). Histologic analysis showed a more heterogeneous cell population and more RAB-positive cells in the lungs of the control group compared to the RAB18-silenced group (**Figure 4E**). Moreover, immunohistochemistry was employed to analyze the effects of RAB18 knockdown on the expression of Ki67, E-cadherin, and vimentin in the xenograft tumor tissues. Results showed that RAB18 knockdown led to reduced expression of Ki67, vimentin, and increased expression of E-cadherin (**Figure 4C**). Taken together, these results suggest that RAB18 knockdown can suppress HCC growth and metastasis *in vivo*.

Discussion

In the present study, the results clearly showed that RAB18 was upregulated in HCC tissues, and was significantly associated with histological grade, tumor size, and an unfavorable prognosis in patients with HCC. Meanwhile, RAB18 promoted HCC cell proliferation and metastasis *in vitro* and *in vivo*.

Recent studies have provided emerging evidence that RAB18 is involved in tumor progression. Increased RAB18 protein expression was associated with decreased expression of the tumor suppressor miR-30 in human non-small cell lung cancer cell (NSCLC) tissues, and enhanced NSCLC proliferation [16]. Similarly, Rab18 was upregulated in breast cancer tissues and contributed to breast cancer cell proliferation and invasion [15]. Interestingly, Rab18 was also upregulated in clinical HCC tissues, and was believed to be increased by the hepatitis B virus X protein (HBx), resulting in HCC development [18]. However, the correlation between Rab18 expression and clinical parameters in HCC patients was not analyzed in this study. The current results also clearly demonstrated that high expression of RAB18 was detected in 61.3% of HCC patient tissues. Importantly, this finding revealed that high expression of RAB18 correlated with an unfavorable prognosis in HCC patients. Therefore, the results strongly suggest that RAB18 has the potential to be a useful prognostic biomarker for HCC.

Furthermore, RAB18 was significantly upregulated in HCC cell lines compared with the normal human liver cell line. As evidenced by this study, RAB18 promoted the proliferation of HCC cells *in vitro* as well as the growth of HCC cells *in vivo*, suggesting that abnormal RAB18 expression may be involved in hepatocarcinogenesis. Further, knockdown of RAB18 led to increased expression of P21 and P27 in HCC cells, which are well known as cyclin-dependent kinase inhibitors capable of mediating cell cycle arrest [19]. Several studies have demonstrated that reduced P27 and P21 expression is correlated with histological grade, metastasis, and the prognosis of HCC patients [20, 21]. Therefore, it can be hypothesized that RAB18 influences the prognosis of HCC via downregulation of P27 and P21. Further studies are needed to investigate the underlying mechanism of inactivation of P27 and P21 by RAB18.

Despite significant advances in the treatment of HCC, the prognosis of patients with extrahepatic metastasis remains poor [22]. The EMT plays a critical role in cancer cell metastasis by conferring an invasive phenotype [23]. Results of this study showed that RAB18 knockdown increased epithelial marker expression and decreased mesenchymal marker expression *in vitro*. Moreover, the relationship between RAB18 and EMT markers was confirmed in xenograft tissue specimens. These results illustrated that RAB18 promoted the EMT process. Additionally, RAB18 increased the metastasis of HCC *in vitro* and *in vivo*. Therefore, RAB18

enhanced the metastasis of HCC cells through induction of EMT, resulting in poor prognosis in HCC patients.

In conclusion, this study suggests that abnormal RAB18 expression promoted the proliferation and metastasis of HCC cells, thus contributing to poor prognosis. Identification of the detailed molecular mechanisms of RAB18 involvement in hepatocarcinogenesis and HCC progression is a future research direction. Nevertheless, RAB18 could serve as a predictive marker of HCC prognosis and a therapeutic target for the treatment of HCC.

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Disclosure of conflict of interest

None.

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