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Original Article

Reduced selenium-binding protein 1 correlates with a poor prognosis in intrahepatic cholangiocarcinoma and promotes the cell epithelial-mesenchymal transition

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Abstract: Recent studies have found that selenium-binding protein 1 (SBP1) is downregulated in various malignant tumors. Nevertheless, the role of SBP1 in intrahepatic cholangiocarcinoma (ICC) is largely unknown. In the present study, we aimed to explore the clinical significance and biological function of SBP1 in ICC. Western blotting and immunohistochemistry were performed to evaluate SBP1 expression in ICC tissues, and correlations between SBP1 and clinicopathological parameters were further assessed. The prognostic significance of SBP1 in ICC patients was evaluated via Kaplan-Meier and Cox regression analyses. Moreover, we used RBE, a human ICC cell line, to study the effects of SBP1 knockdown on ICC cell proliferation, migration and invasion. Finally, the expression levels of epithelial-mesenchymal transition-related markers, including snail, vimentin, and E-cadherin, were investigated via Western blotting and immunohistochemistry. The results showed that SBP1 expression was significantly downregulated in ICC tumor tissues, especially in tumor tissues from ICC patients with recurrence or tumor vascular invasion, compared with that in peritumoral tissues (all $P < 0.05$). In addition, the reduction in SBP1 expression was related to microvascular invasion, lymphatic metastasis, and tumor-node-metastasis (TNM) stage (all $P < 0.05$). Furthermore, the SBP1 expression level was an independent prognostic factor in ICC ($P < 0.05$). Knockdown of SBP1 resulted in decreased in vitro proliferation, migration and invasion ability. Low SBP1 expression also resulted in the upregulation of mesenchymal markers such as vimentin and snail. In conclusion, SBP1 may be a prognostic indicator for patients with ICC as well as a potential target for ICC treatment.

Keywords: Selenium-binding protein 1, intrahepatic cholangiocarcinoma, prognosis, epithelial-mesenchymal transition

Introduction

Characterized by a dismal prognosis with five-year overall survival (OS) rates of less than 5%, intrahepatic cholangiocarcinoma (ICC) is among the most common primary liver cancers worldwide [1, 2], and ICC incidence has increased in recent years [3, 4]. Despite various therapeutic methods such as surgery, gemcitabine-based chemotherapy and neoadjuvant approaches, the clinical outcome of ICC patients has not been greatly improved, partly due to the lack of effective early-diagnosis techniques and to early metastasis of the disease [5-7]. Thus, it is necessary to clarify the molecular mechanisms of ICC and define novel targets to improve early diagnosis and develop innovative therapeutic interventions.

Selenium is a trace element that is critically involved in various biological processes and has been reported to demonstrate anticancer activity in many epidemiologic studies [8, 9]. An increased incidence of many types of cancers has been related to a dietary deficiency of selenium, possibly due to its antioxidant and anti-inflammatory effects [10, 11]. The biological function of selenium depends on the expression of selenium-containing proteins, which are categorized into different types and form a complex system [12]. Among these proteins, selenium-binding protein 1 (SBP1, also SELE-

NBP1), a 56-kDa molecule whose mRNA is abundantly expressed in many tissue types, is considered to mediate the antitumor function of selenium [13, 14]. Decreased SBP1 has been found in various malignant tumors, including gastric cancer [15], colorectal cancer [16], hepatocellular carcinoma [17] and ovarian cancer [18], indicating its critical role in malignant transformation and cancer progression. Nevertheless, the expression and function of SBP1 during ICC progression and its clinical significance remain obscure.

In the present study, SBP1 expression in the tumor tissues of patients with ICC was determined. We further analyzed the relationships between SBP1 and the clinicopathological data of patients with ICC and assessed the prognostic significance of SBP1 in ICC patients. Ultimately, we found that decreased SBP1 could promote cell proliferation, migration and invasion in ICC cells, and the underlying mechanism might involve the inhibition of the epithelial-mesenchymal transition (EMT).

Materials and methods

Patient characteristics and sample collection

Freshly resected tumor samples were collected from 110 patients with ICC who underwent complete surgical resection at Zhongshan Hospital, Fudan University, between March 2000 and December 2007. Written informed consent was obtained from every patient before samples were taken according to the regulations of the Zhongshan Hospital Research Ethics Committee. The disease status of the tissue samples was confirmed by at least two experienced pathologists. Patient follow-up was conducted routinely until February 2009, when the follow-up data were recorded and analyzed as described previously [19]. The average follow-up time of the cohort was 24 months. OS was defined as the time from the date of clinical diagnosis to the date of death from any cause. Time to recurrence was defined as the duration between the resection date and the date of the clinical diagnosis of tumor recurrence.

Tissue microarrays and immunohistochemistry

Suitable tissues with complete clinical data were selected to construct the tissue microarrays (TMAs) as previously described [20]. Immunohistochemistry (IHC) was carried out as described in our earlier study [21]. The primary antibodies used in IHC were against SBP1 (1:500; MBL, USA), vimentin (1:500; Abcam, USA), and E-cadherin (1:250; Abcam, USA). Immunohistochemical scores were assessed independently and blindly by at least two experienced pathologists. For each immunostain, a consensus score was given and recorded according to a simple scale (-, -+, -++, +++) depending on the staining intensity and area; "-" was recorded for no staining, "-+" for weak staining (staining area < 30%), "-++" for moderate staining (staining area 30~60%), and "+++" for strong staining (staining area > 60%). The scores "-++" and "+++" were considered high expression, and the scores "-" and "-+" were considered low expression based on receiver operating characteristic (ROC) analysis.

Cell lines and transfection

The RBE cell line, derived from a 64-year-old Japanese woman with ICC in 1997, was purchased from the Chinese Academy of Sciences (Shanghai, China) [22]. The cells were cultured in RPMI 1640 (Gibco, USA) supplemented with 10% inactivated fetal bovine serum (FBS; Gibco, USA) and 100 μg/mL penicillin and streptomycin (Invitrogen, USA) and were maintained at 37°C with 5% CO_2. The pENTR/U6 vector (Invitrogen, USA) was used for constitutive expression of SBP1 shRNA using the BLOCK-iT U6 RNAi Entry Vector Kit (Invitrogen, USA) following the manufacturer's instructions. The two SBP1 shRNAs used were as follows: shRNA-1, GCTTCCACAGCTACGAAATGTCGAAAC-ATTTCGTAGCTGTGGAAGC; shRNA-2, GCCCTG-CATTTACCGAAACACCGAAGTGTTTCGGTAA ATG-CAGGGC.

Western blot analysis

Twenty-four paired tumor and peritumoral tissues from ICC patients as well as cells from three RBE cell lines (RBE-sh-nc, RBE-sh-1 and RBE-sh-2) were lysed, and 25-30 μg of protein was extracted from each sample. The primary antibodies used were against glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 1:5000; Proteintech, China), SBP1 (1:1000; MBL, USA), snail (1:1000; Novus Biologicals, USA), vimentin (1:1000; Cell Signaling Technology, USA), and E-cadherin (1:500; Abcam, USA). Quantitative analysis was performed using Image-Pro Plus software.

RNA isolation and quantitative real-time polymerase chain reaction

Total RNA was extracted using TRIzol RNA isolation reagent (Invitrogen, USA). Reverse transcription was performed using an RT Reagent Kit (Takara, China), and complementary DNA (cDNA) was generated. SBP1 mRNA expression levels were evaluated by real-time PCR and were then normalized to GAPDH expression with the $2^{-\Delta Ct}$ method ($\Delta Ct = Ct_{SBP1}-Ct_{GAPDH}$). All assays were performed in biological triplicates. The SBP1 and GAPDH primers for PCR were as follows: SBP1 forward, 5'-CTCCTCTCGCATCTATGTGGT-3'; SBP1 reverse, 5'-CCATGTCCCCTTCACCTCGAAC-3'; GAPDH forward, 5'-GGTATGACAACGAATTTGGC-3'; GAPDH reverse, 5'-GAGCACAGGGTACTTTATTG-3'.

Wound-healing assay, matrigel invasion assay, and clonogenic assay

For the wound-healing assay, RBE cells transfected with either SBP1 shRNA or control shRNA were densely seeded in six-well plates and incubated until 100% confluence. A straight scratch was made on the cell monolayer, followed by washing three times with PBS. The scratched cells were then cultured in serum-free medium for 24 hours. The distance of cell migration was calculated from digital images of predetermined locations taken by a microscope.

Migration assays were carried out as previously described [23]. Briefly, 24-well Transwell inserts were coated with Matrigel (Corning, USA). Then, 1×10^5 cells were seeded into the top compartment with serum-free DMEM, while medium supplemented with 10% FBS (v/v) was added to each bottom compartment. After incubation for 24 hours, the cells on the upper filter membrane were removed with cotton swabs, and the cells on the lower filter membrane were fixed with 4% paraformaldehyde followed by crystal violet staining. The migrated cells were counted in ten preselected visual fields under a microscope. Each assay was carried out in triplicate.

For the clonogenic assay, cells were fully digested into single cells, diluted to 250 cells/mL, and seeded into a 6-well culture plate (2 mL per well). After incubation in complete DMEM for one week, the cells were fixed with 4% paraformaldehyde and stained with crystal violet. The cell colony numbers were counted, and group differences were analyzed in triplicate.

Cell cycle assay and cell proliferation test

The cell cycle was analyzed by flow cytometry. RBE cells were first synchronized in G_0 by serum starvation and were incubated in fresh complete medium for 24 hours. Thereafter, 70% ethanol was used to fix the cells at 4°C overnight. Next, 40 μg/mL propidium iodide and RNase A were added to the fixed cells for a 30-minute incubation on ice. Finally, the cell samples were analyzed using a FACSCanto flow cytometer (BD Biosciences, USA). The cell cycle results were further analyzed using FlowJo software (TreeStar, Inc., USA).

For cell proliferation, RBE cells were fully digested into single cells and seeded into 96-well culture dishes (5×10^3 cells per well). Next, 10 μL of Cell Counting Kit-8 solution (Yeasen, China) was added to each well at the indicated time points. After incubation for 2 hours, the number of viable cells in each well was determined based on the absorbance at 450 nm.

Statistical analysis

Statistical analysis was performed using SPSS v16.0 software (SPSS Inc., USA). Comparisons between groups were analyzed using Student's t-test. Chi-squared test or Fisher's exact test was used for categorical clinicopathological data analysis. The Kaplan-Meier survival method and the log-rank test were used to statistically evaluate and compare patient survival and the probability of recurrence between subgroups. Univariate and multivariate analyses were performed for prognostic factor identification using the Cox proportional hazards model. The data are presented as the means ± standard deviation (SD). All tests were two-sided, and statistical significance was defined as a p value below 0.05.

Results

SBP1 was expressed at low levels in ICC tumor samples

We first performed a Western blot assay to evaluate the SBP1 protein levels in 12 pairs of tumor and peritumoral tissues. Semiquanti-

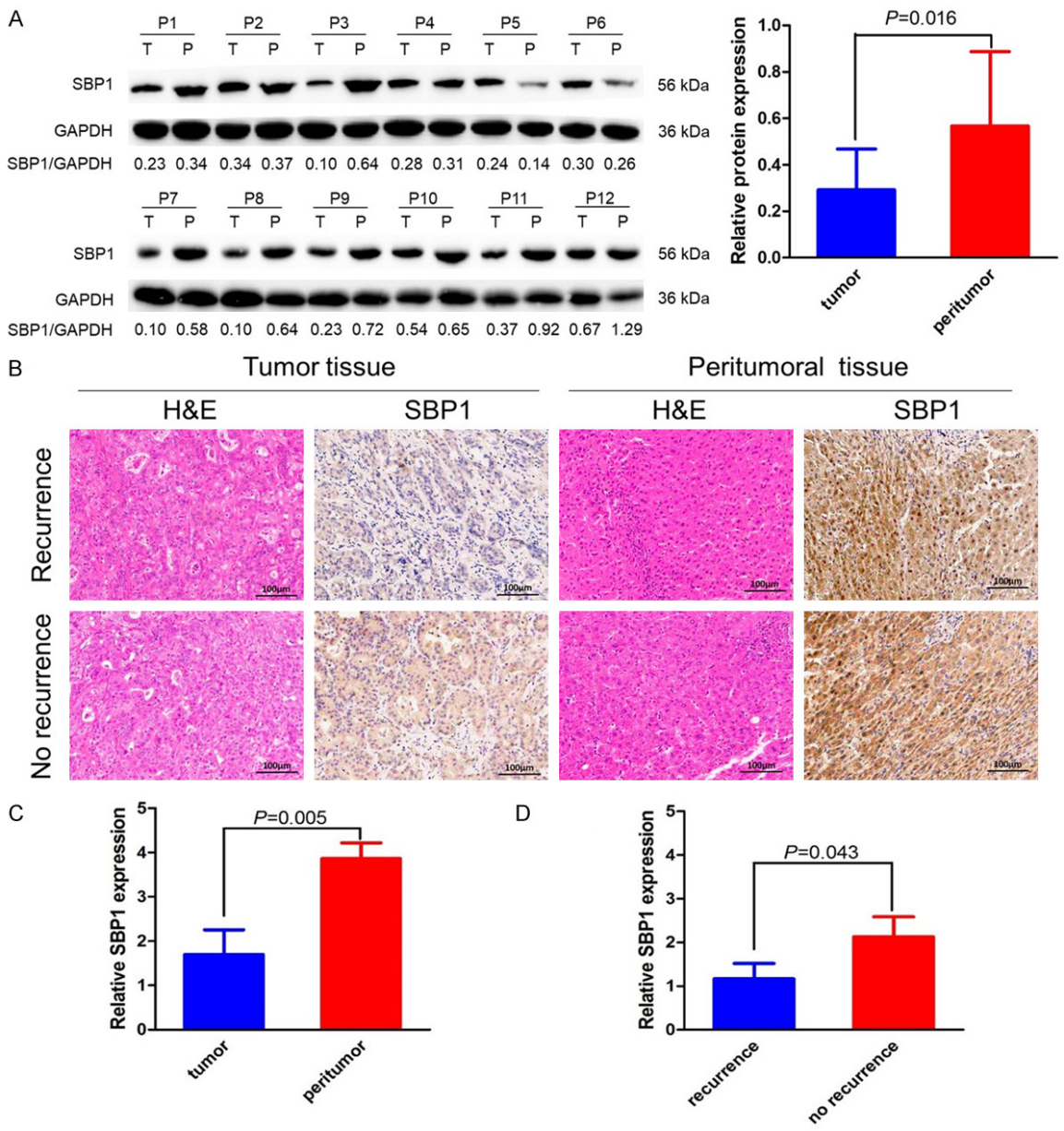

Figure 1. SBP1 expression was downregulated in ICC patients. A. Lower SBP1 protein was detected by Western blotting in ICC tumor tissues than in peritumoral tissues. GAPDH was used as the loading control. B-D. Representative images of H&E and SBP1 staining are shown for samples from ICC patients in the recurrence and nonrecurrence subgroups. *Scale bar* = 100 μm. SBP1 expression as determined by the staining intensity and area was significantly lower in ICC tumors and the recurrence group than in peritumoral tissues and the nonrecurrence group. *T* tumor, *P* peritumor, *H&E* hematoxylin-eosin.

tative analysis revealed that the SBP1 protein levels were significantly lower in tumor samples than in peritumoral samples (0.29 ± 0.05 vs 0.57 ± 0.09; *P* = 0.016; **Figure 1A**). We further analyzed SBP1 expression by immunohistochemistry in ICC TMAs that included 110 pairs of ICC tissue samples and matched peritumoral samples (**Figure 1B**). Lower SBP1 expression was found in tumor tissues than in the corresponding peritumoral tissues (1.82 ± 0.37 vs 3.87 ± 0.26; *P* = 0.005; **Figure 1C**).

To further investigate the relationships between SBP1 expression and clinicopathological features, we divided the ICC samples into two groups (SBP1 high and SBP1 low) based on

Table 1. SBP1 expression in relation to pathologic and clinical features of 110 patients with intrahepatic cholangiocarcinoma

Variables	SBP1 staining		*P* value[a]
	High expression	Low expression	
Age (years)			
≥ 53	24	38	0.723
< 53	17	31	
Sex			
Male	23	35	0.585
Female	18	34	
HBsAg			
Positive	14	30	0.334
Negative	27	39	
Serum CA19-9 (ng/mL)			
≥ 37	24	41	0.927
< 37	17	28	
Serum AFP (ng/mL)			
≥ 20	3	12	0.162[b]
< 20	38	57	
Microvascular invasion			
Yes	6	29	0.003
No	35	40	
Maximal tumor size (diameter, cm)			
≥ 5	36	55	0.311[b]
< 5	5	14	
Tumor number			
Multiple	7	9	0.562
Solitary	34	60	
Tumor differentiation			
III/IV	20	38	0.523
I/II	21	31	
Lymphatic metastasis			
Yes	8	27	0.033
No	33	42	
TNM stage			
III/IV	10	32	0.022
I/II	31	37	

AFP alpha-fetoprotein, *CA19-9* carbohydrate antigen 19-9, *HBsAg* hepatitis B surface antigen, *TNM* tumor-node-metastasis. [a]Chi-square test. [b]Fisher's exact test.

the immunohistochemistry scores. We found that low expression of SBP1 was markedly correlated with microvascular invasion, lymphatic metastasis, and tumor-node-metastasis (TNM) stage but not with age, tumor size and number, CA19-9 or other clinicopathological features (**Table 1**). Moreover, we compared SBP1 expression in tumor samples from ICC patients with recurrence and in those from patients with no recurrence and found lower SBP1 expression in tumor tissues in patients with recurrent ICC than in patients with no history of ICC recurrence (1.17 ± 0.20 vs 2.13 ± 0.26; $P = 0.043$; **Figure 1D**).

Low expression of SBP1 is correlated with vascular invasion in ICC patients

Because SBP1 expression has been previously reported to correlate with vascular invasion in hepatocellular carcinoma [24], we further explored the relationship between SBP1 expression and vascular invasion in ICC patients. Western blot analysis revealed that samples from the vascular invasion group had lower SBP1 expression than samples from the non-vascular invasion group (0.057 ± 0.011 vs 0.184 ± 0.044; $P = 0.020$; **Figure 2A**). We also observed a similar correlation through immunohistochemistry: lower SBP1 expression in tumor tissues was found in patients with vascular invasion than in patients without vascular invasion (0.567 ± 0.120 vs 1.502 ± 0.208; $P = 0.018$; **Figure 2B**).

Low SBP1 expression predicted an unfavorable prognosis in ICC patients

We next evaluated the predictive value of SBP1 for ICC prognosis. For the 110 ICC patients, the two- and five-year OS rates were 31.8% and 9.1%, respectively, and the two- and five-year cumulative recurrence rates were 65.5% and 72.7%, respectively. SBP1 expression showed marked heterogeneity in the different TMA samples; thus, we further divided the patients into two groups based on the SBP1 immunohistochemistry scores, examples of which are illustrated in **Figure 3A**. The group with low SBP1 expression showed a significantly less favorable prognosis (**Figure 3B**, **3C**), with two- and five-year OS rates lower than those of the SBP1 high-expression group (23.19% vs 51.22% and 2.90% vs 19.51%, respectively), while the two- and five-year cumulative recurrence rates were significantly higher in the SBP1 low-expression group than in the high-expression group (86.72% vs 67.25% and 97.10% vs 85.36%, respectively).

Using univariate analysis, we found that SBP1 expression, microvascular invasion, lymphatic

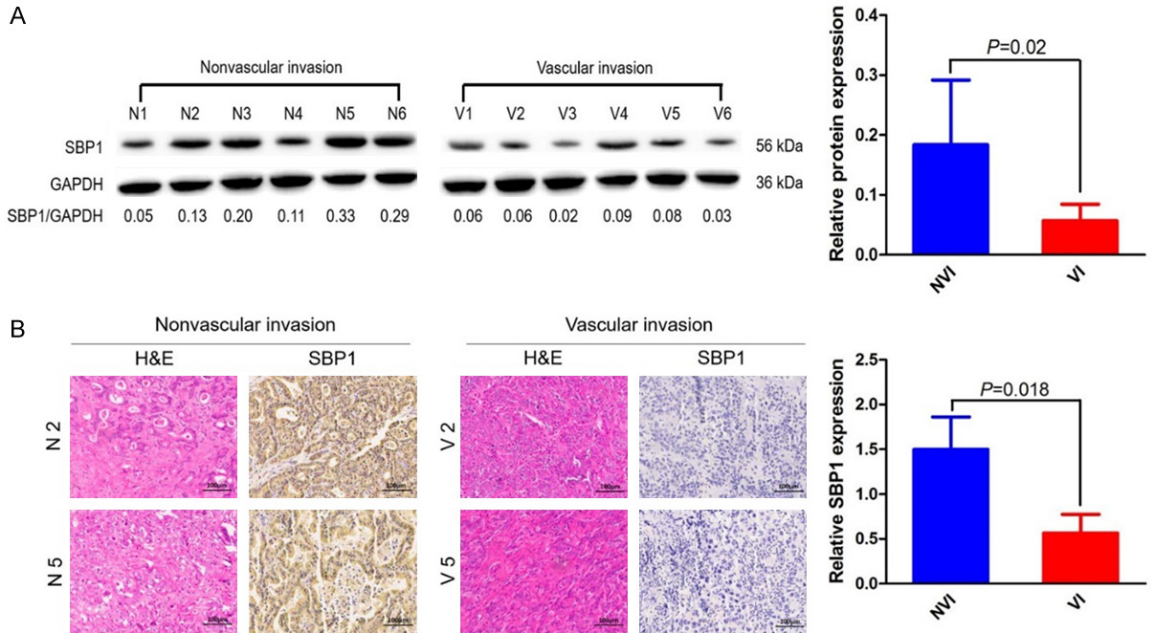

Figure 2. SBP1 expression was correlated with vascular invasion. A. Western blotting showed that SBP1 protein expression was markedly lower in the vascular invasion group than in the non-vascular invasion group. GAPDH was used as the loading control. B. Representative images of H&E and SBP1 staining are shown for samples from ICC patients with and without vascular invasion. *Scale bar* = 100 μm. *N/NVI* non-vascular invasion, *V/VI* vascular invasion, *H&E* hematoxylin-eosin.

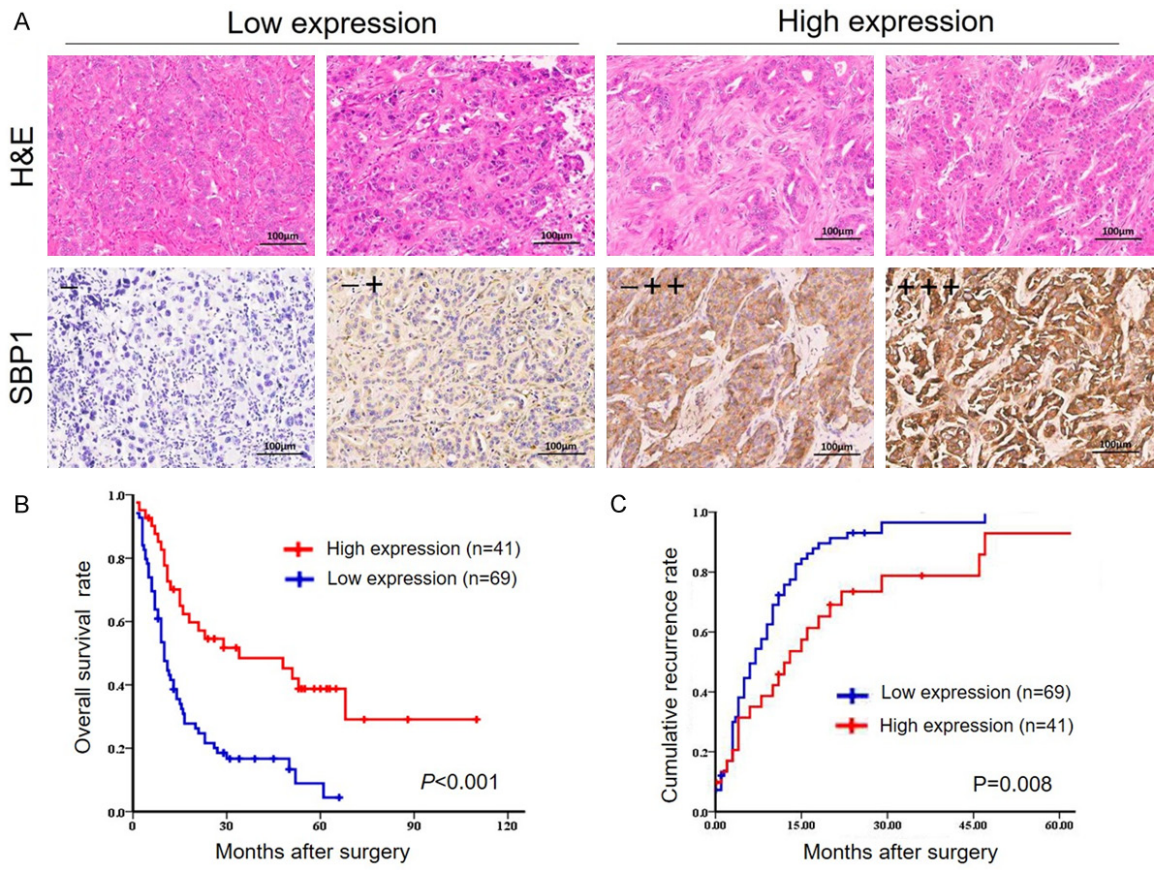

Figure 3. SBP1 had prognostic significance in patients with ICC. A. Representative images of H&E and SBP1 staining in the low-expression group (marked with "-" and "-+") and the high-expression group (marked with "-++" and "+++"). *Scale bar* = 100 μm. B. ICC patients with low SBP1 expression showed worse overall survival than patients with high SBP1 expression. C. Low SBP1 expression in ICC patients indicated a higher recurrence rate.

Table 2. Univariate and multivariate analysis of clinicopathological factors associated with overall survival and cumulative recurrence

Factor	Overall survival		Cumulative recurrence	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Univariate analysis				
Sex (male vs female)	0.733 (0.475 to 1.132)	0.161	0.988 (0.638 to 1.529)	0.955
Age, years (≥ 53 vs < 53)	1.350 (0.867 to 2.103)	0.184	1.194 (0.766 to 1.861)	0.434
Serum CA19-9 (< 37 vs ≥ 37, ng/mL)	0.852 (0.532 to 1.425)	0.532	0.892 (0.564 to 1.384)	0.485
AFP (≥ 20 vs < 20, ng/mL)	0.826 (0.426 to 1.602)	0.571	1.080 (0.555 to 2.103)	0.820
TNM stage (III/IV vs I/II)	1.605 (1.038 to 2.482)	0.033	1.557 (1.008 to 2.404)	0.046
Tumor differentiation (III/IV vs I/II)	1.042 (0.641 to 1.695)	0.867	0.823 (0.506 to 1.339)	0.433
Microvascular invasion (yes vs no)	1.759 (1.089 to 2.841)	0.021	1.765 (1.081 to 2.882)	0.023
Maximal tumor size (< 5 vs ≥ 5, cm)	1.081 (0.653 to 1.791)	0.762	1.158 (0.695 to 1.929)	0.574
Tumor number (single vs multiple)	0.983 (0.491 to 1.968)	0.961	0.915 (0.456 to 1.835)	0.802
Lymphatic metastasis (yes vs no)	1.636 (1.040 to 2.573)	0.033	1.769 (1.120 to 2.794)	0.014
SBP1 staining (high vs low)	0.377 (0.230 to 0.619)	0.001	0.551 (0.339 to 0.897)	0.016
Multivariate analysis				
TNM stage (III/IV vs I/II)	1.839 (1.169 to 2.894)	0.008	1.528 (0.974 to 2.397)	0.065
Microvascular invasion (yes vs no)	1.004 (0.618 to 1632)	0.986	1.607 (0.971 to 2.659)	0.065
Lymphatic metastasis (yes vs no)	1.640 (1.029 to 2.613)	0.038	1.896 (1.193 to 3.014)	0.007
SBP1 staining (high vs low)	0.375 (0.226 to 0.623)	0.001	0.532 (0.323 to 0.877)	0.013

Univariate and multivariate analysis: Cox proportional hazards regression model. *AFP* alpha-fetoprotein, *CA19-9* carbohydrate antigen 19-9, *TNM* tumor-node-metastasis, *CI* confidence interval, *HR* hazard ratio.

metastasis and TNM stage had predictive value for ICC patients concerning OS and cumulative recurrence. Using the Cox regression model, we further found that SBP1 expression was an independent prognostic factor for ICC patients (**Table 2**).

SBP1-knockdown ICC cells exhibited increased proliferation, migration and invasion ability

We performed RNA interference to knock down SBP1 expression in the ICC cell line RBE. Two shRNAs targeting SBP1 were designed and were found to potently inhibit SBP1 compared with the control shRNA in qRT-PCR and Western blot validation assays (**Figure 4A**, **4B**). The cell proliferation, migration and invasion ability of the three constructed RBE cell lines (RBE-sh-nc, RBE-sh-1, RBE-sh-2) were further assessed. We found that the proliferation rate of RBE cells was increased after SBP1 shRNA transfection for 72 hours in vitro (**Figure 4C**). Similarly, the colony formation ability of RBE cells was increased after SBP1 downregulation (**Figure 4D**). Furthermore, flow cytometry was performed for RBE cell cycle analysis after SBP1 RNA interference. The results showed that SBP1-knockdown RBE cells had a significantly greater G2/M population than the control cells (**Figure 4E**).

The migration ability of the SBP1-knockdown RBE cells was evaluated using a wound-healing assay. We found that reduced expression of SBP1 markedly enhanced the migration of RBE cells (**Figure 5A**). In addition, Transwell assay results revealed that the invasion ability of cells after SBP1 shRNA transfection was markedly greater than that in control shRNA-transfected cells (**Figure 5B**). All these data indicated that SBP1 downregulation led to increased proliferation, migration and invasion ability in ICC cells.

Inhibition of SBP1 expression enhanced the EMT process in ICC cells in vitro

Because reduced SBP1 was significantly correlated with vascular invasion and promoted migration and invasion ability in ICC cells, we fur-

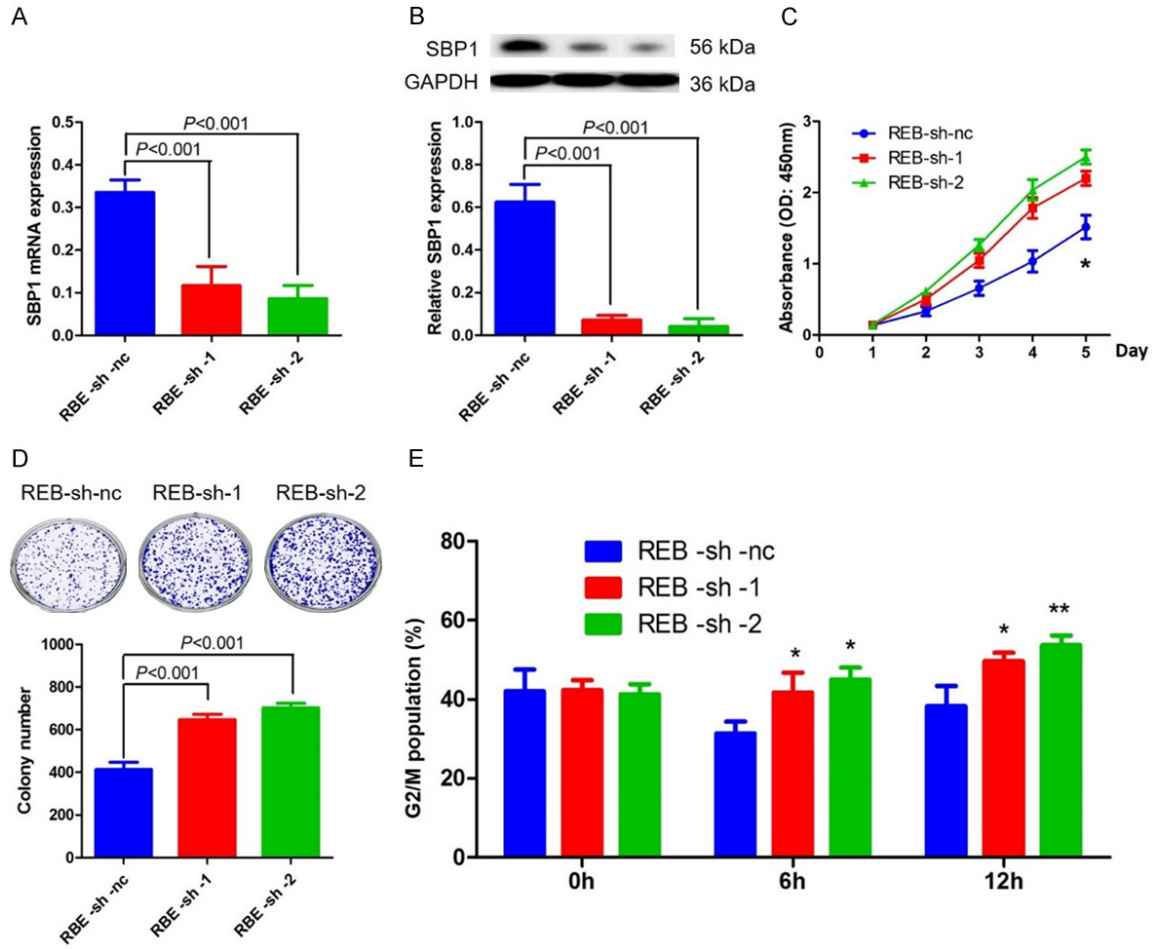

Figure 4. ICC cells showed increased cell viability and proliferation ability after SBP1 knockdown in vitro. (A) qRT-PCR and (B) Western blot assays of SBP1 expression confirmed the knockdown efficiency in RBE cells after SBP1 shRNA transfection. (C) A CCK-8 cell proliferation assay was carried out after SBP1 knockdown in RBE cells to analyze cell viability. (D) Colony formation assays showed increased colony formation ability after SBP1 knockdown in RBE cells. (E) The percentage of cells in the G2/M phase was measured by flow cytometry after SBP1 knockdown in RBE cells. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

ther investigated the regulation of SBP1 in the cell EMT process by examining the expression of EMT markers such as vimentin, snail, and E-cadherin in RBE cells with different levels of SBP1 expression. Western blotting revealed that reductions in SBP1 expression led to increased protein levels of mesenchymal markers, including snail and vimentin, while the expression of E-cadherin was downregulated (**Figure 6A**, **6B**). The IHC results further showed that SBP1 downregulation was positively correlated with vimentin ($P = 0.004$; $r = 0.458$) and snail ($P = 0.010$; $r = 0.314$) expression but was negatively correlated with E-cadherin ($P = 0.008$; $r = -0.256$) expression in the tumor tissues of ICC patients (**Figure 6C**). These results indicated that low expression of SBP1 could enhance the EMT process in tumor cells and subsequently promote ICC progression.

Discussion

Human selenium-binding protein 1 (SBP1), first cloned in 1997, has been detected in various tissue types, although the biological function of SBP1 remains unclear [25]. Recent studies have shown that this protein, which is targeted by hypoxia-inducible factor-1 alpha (HIF-1α), might be involved in the proteasomal degradation pathway and may directly interact with von Hippel-Lindau protein (pVHL) [26, 27]. Decreased expression of SBP1 is associated with a poor clinical outcome in several cancer types; this was first shown for lung adenocarcinoma

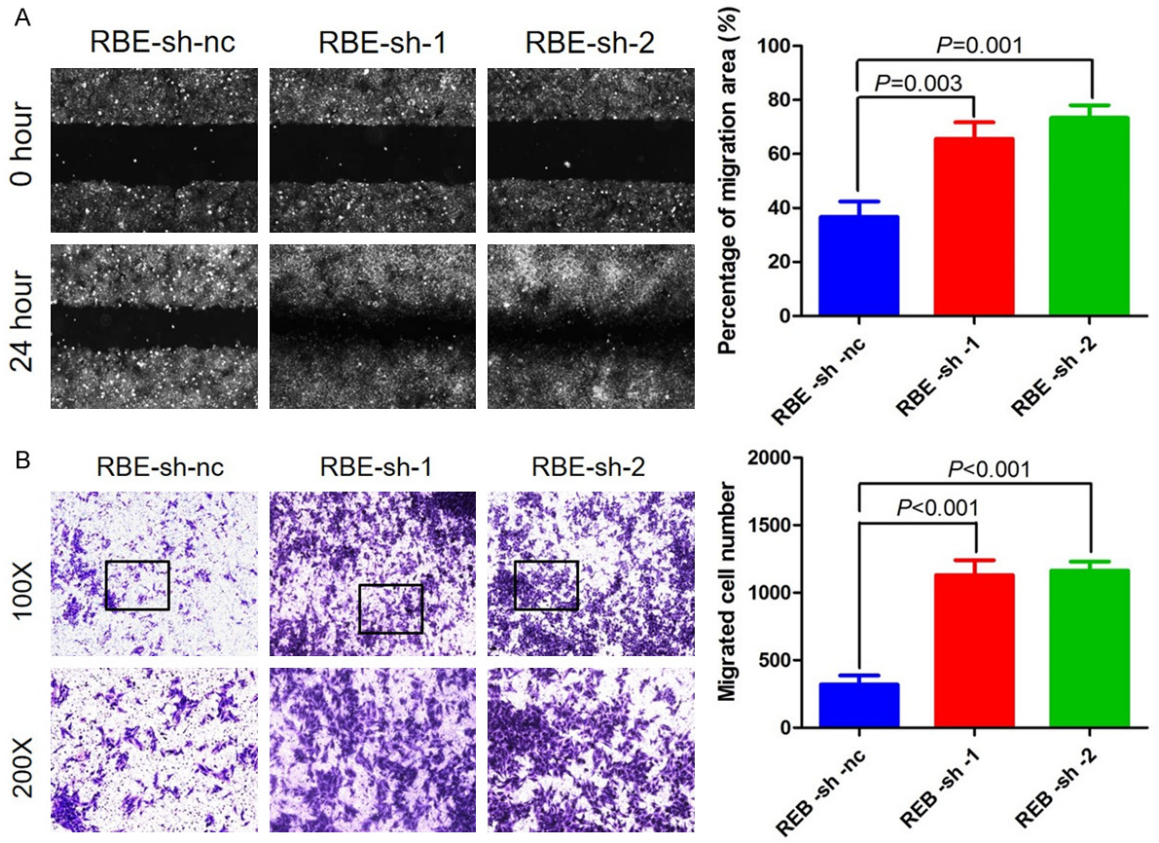

Figure 5. SBP1 knockdown resulted in stronger migration and invasion ability in ICC cells in vitro. A. A wound-healing assay showed greater numbers of migrating cells in the SBP1-knockdown group than in the control group. B. The invasion ability of SBP1-knockdown RBE cells was increased, as measured by a transwell assay with matrigel.

patients, in whom low expression of SBP1 was significantly related to unfavorable OS [28]. Subsequently, a similar poor clinical outcome with reduced SBP1 was observed in ovarian cancer [29], thyroid papillary carcinoma [30], and most recently, malignant melanoma [31]. However, no conclusions have been drawn concerning the clinical significance of SBP1 in ICC.

In the present study, our results revealed that SBP1 expression was significantly downregulated in ICC tumor samples, especially in those from ICC patients with recurrence or tumor vascular invasion, compared with that in peritumoral tissue samples. Furthermore, reduced expression of SBP1 was correlated with microvascular invasion, lymphatic metastasis, and TNM stage. In accordance with the results found in many other types of cancers, our data revealed that SBP1 expression level was an independent prognostic indicator for ICC patients.

Although the regulation of SBP1 expression in cancer and its biological function in tumor progression are largely unknown, several studies have reported that epigenetic changes may be involved in the downregulation of SBP1 expression. The methylation level of the SBP1 gene promoter is markedly greater in tumor tissues than in peritumoral tissues [32]. SBP1 has been indicated by several studies to be a putative tumor suppressor, as reduced SBP1 expression leads to enhanced cell proliferation, while exogenous SBP1 expression results in apoptosis and decreased cell migration ability in vitro [28]. Our data also revealed that ICC cells with reduced SBP1 expression exhibited decreased proliferation, migration and invasion ability in vitro. The results from our study showed that SBP1 expression might be involved in the EMT process through the regulation of EMT markers such as vimentin, snail and E-cadherin. We also demonstrated that the inhibition of SBP1 increased the migration and invasion ability of ICC cells in vitro. These data support the notion that reduced SBP1 may promote the EMT process and enhance tumor invasiveness during ICC progression. One recent study on SBP1

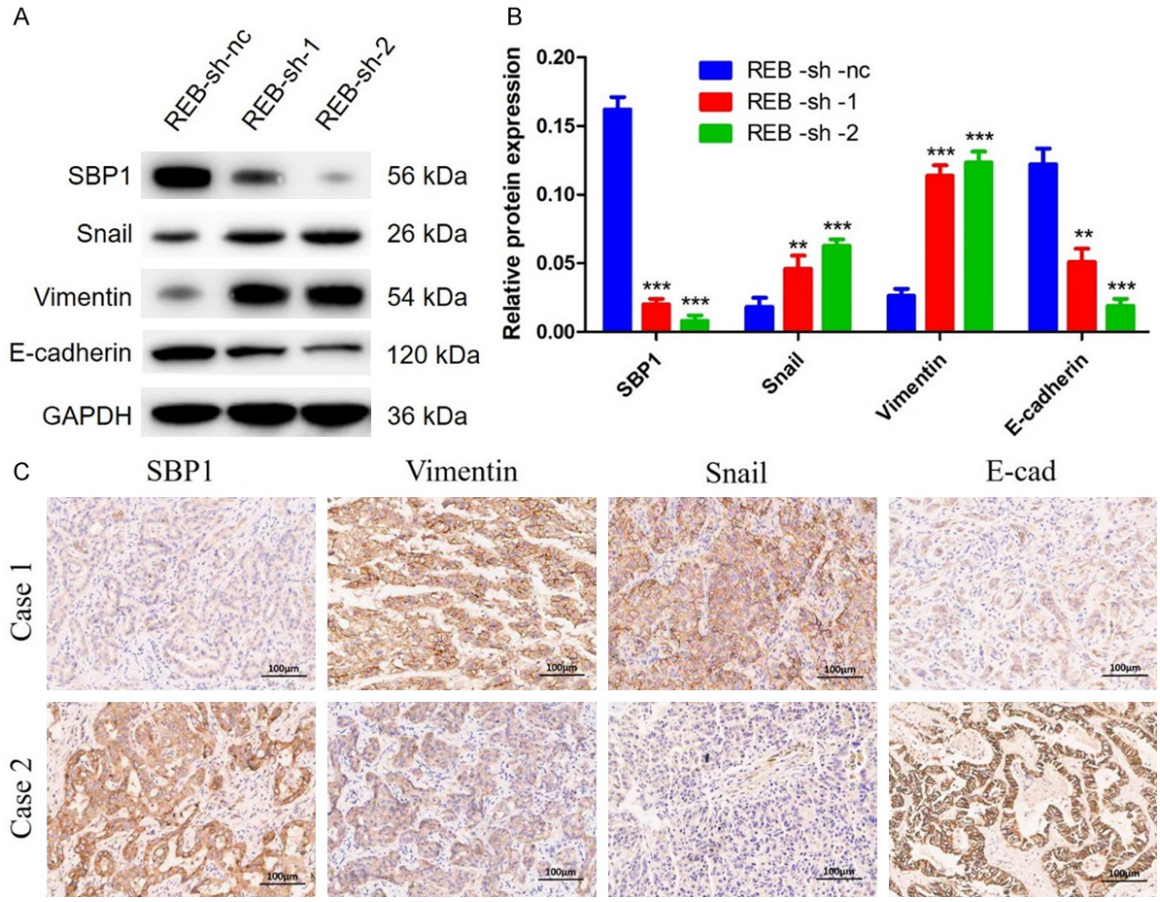

Figure 6. Reduced expression of SBP1 resulted in the activation of the epithelial-mesenchymal transition. A, B. Western blot analysis revealed that vimentin and snail were increased and E-cadherin was decreased by SBP1 interference in RBE cells. C. Representative IHC images of two ICC patients, one with SBP1^{low}, vimentin^{high}, snail^{high}, and E-cadherin^{low}, and another with SBP1^{high}, vimentin^{low}, snail^{low}, and E-cadherin^{high}. *Scale bar* = 100 μm.

in hepatocellular carcinoma showed that reduced expression of SBP1 led to a significant increase in glutathione peroxidase 1 (GPX1) activity followed by a decrease in HIF-1α expression [24]. Because SBP1 expression was significantly associated with vascular invasion in ICC patients in our study, similar mechanisms related to SBP1 and GPX1 interactions may exist in ICC and warrant further investigation.

For patients with ICC, surgery provides the only possibility for a cure. However, only a minority of ICC patients are considered candidates for resection [33, 34]. Systemic adjuvant therapy is increasingly being applied in cases of advanced ICC, but the effectiveness of systemic therapy is difficult to foresee [35]. Several lines of evidence have indicated a promising possibility of using SBP1 expression as a treatment response predictor. For example, reduced SBP1 may have a marked negative impact on the ability of chemotherapeutic agents to kill tumor cells in esophageal cancer [24], while restoration of SBP1 makes colon cancer cells more vulnerable to H_2O_2 therapy, with increased apoptosis and decreased migration and growth [36]. Therefore, the effects of differential SBP1 expression on responses to chemotherapy in ICC tumor tissues are worth investigating in the future.

In the present study, our results revealed that SBP1 is a critical player in ICC tumor progression as well as a potential indicator of ICC patient prognosis, and we showed that reductions in SBP1 may enhance cell migration and invasion through the activation of the EMT process. Despite the fact that the precise mechanisms by which SBP1 affects tumor development remain unclear, our study confirmed SBP1 as a

potential biological marker and therapeutic target of ICC.

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Disclosure of conflict of interest

None.

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