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Increased Expression of Serine Hydroxymethyltransferase 2 (SHMT2) is a Negative Prognostic Marker in Patients with Hepatocellular Carcinoma and is Associated with Proliferation of HepG2 Cells

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Serine hydroxymethyltransferase 2 (SHMT2) is a key enzyme in one-carbon cell metabolism, including in liver cancer. However, the associations between SHMT2 expression at the gene and protein level and prognosis in patients with hepatocellular carcinoma (HCC) remains unknown. This study aimed to investigate the expression levels of SHMT2 in tumor tissue samples from patients with HCC and clinical outcome and the effects of silencing the expression of the *SHMT2* gene in HepG2 cells.

Material/Methods: Expression levels of SHMT2 were evaluated in 144 cases of HCC using immunohistochemistry and correlated with clinicopathological factors using the chi-squared (χ^2) test. The prognostic significance of SHMT2 expression was analyzed by univariate analysis and multivariate analysis. Twenty pairs of HCC tissue and adjacent normal liver tissue were compared for *SHMT2* expression levels using quantitative reverse transcription polymerase chain reaction (qRT-PCR). HepG2 cells underwent *SHMT2* gene silencing and MTT and transwell assays investigated cell proliferation and migration. Western blot was used to detect the expression of markers of epithelial-mesenchymal transition (EMT).

Results: Expression levels of SHMT2 in HCC tissues were significantly correlated with tumor grade and hepatitis B virus (HBV) infection, and increased expression was an independent negative prognostic factor in patients with HCC (P=0.003). Increased expression of the *SHMT2* gene promoted the proliferation and migration of the HepG2 HCC cell line.

Conclusions: Increased expression of SHMT2 was a negative prognostic biomarker in patients with HCC. Expression of the *SHMT2* gene promoted the proliferation and migration of HepG2 HCC cells.

MeSH Keywords: **Carcinoma, Hepatocellular • Cell Proliferation • Epithelial-Mesenchymal Transition • Glycine Hydroxymethyltransferase • Neoplasm Invasiveness • Prognosis**

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Background

Worldwide, primary liver cancers are the second leading cause of cancer-related death and the fifth most common cancers, resulting in approximately 800,000 annual deaths [1]. The global incidence of hepatocellular carcinoma (HCC) is increasing and is expected to reach one million cases per year during the next decade [2]. Primary malignancy of the liver mainly includes HCC and intrahepatic cholangiocarcinoma, which have distinct molecular and clinical features [3]. HCC is the most prevalent primary malignant tumor of the liver, accounting for approximately 90% of all liver cancers [4]. The overall survival rate for patients with HCC remains very poor, despite recent breakthroughs in treatment, including surgical treatment and systemic treatments. The 5-year survival for patients with HCC following surgical resection is only 30% [5]. Targeted drug therapies for HCC are limited and include sorafenib and lenvatinib [6,7]. Therefore, compared with other malignant tumors, the prognosis for patients with HCC remains poor and because the development of targeted therapy for patients with HCC is limited, there is a need to discover new biomarkers and drug targets.

Deregulation of energy metabolism is one of the hallmarks of malignancy [8]. Several key metabolic changes have been reported in malignant tumors, including enhanced glucose uptake, aerobic glycolysis, and folate-dependent one-carbon metabolism, known as the Warburg effect, which are required for cancer growth and cell proliferation. One-carbon metabolism is essential as it provides cellular components that include nucleotides, lipids, and proteins for cell growth. In tumorigenesis and tumor progression, ectopic one-carbon metabolism is required for DNA synthesis in cells that are rapidly proliferating, and also for the biosynthesis of S-adenosyl methionine, which supplies methyl groups for DNA [9]. In one-carbon metabolism, serine hydroxymethyltransferase (SHMT) is a key enzyme that catalyzes the conversion from serine and hydrolyzed tetrahydrofolate (THF) to glycine and 5,10-methylenetetrahydrofolate (MeTHF) [10]. There are two iso-enzymes of SHMT, SHMT1 and SHMT2, and the former is mainly expressed in the cytoplasm and the latter is mainly found in mitochondria [11]. However, upregulation of SHMT2 and an association with tumor progression has been reported in several types of cancer [12–14].

A previously reported study showed that In HCC, SHMT2 was required for tumor cell metabolism but did not initiate malignant transformation [15]. Another study showed that the microRNA (miR), miR-615-5p prevented proliferation and migration by inhibiting the expression of SHMT2 of HCC cells [16]. Although SHMT2 has been shown to be involved in cell proliferation in HCC cells, the clinical significance of SHTM2 expression remains unknown.

Therefore, this study aimed to investigate the expression levels of SHMT2 in tumor tissue samples from patients with HCC and clinical outcome and the effects of silencing the expression of the *SHMT2* gene in HepG2 cells on cell proliferation, migration, and epithelial-mesenchymal transition (EMT).

Material and Methods

Patient cohort following surgical resection of hepatocellular carcinoma (HCC)

This retrospective clinical study included a primary cohort of 456 patients who underwent liver resection surgery for hepatocellular carcinoma (HCC) at Yidu Central Hospital and the Sixth Peoples' Hospital of Qingdao from 2006 to 2017. From the primary cohort, 144 patients were included who were diagnosed with HCC and who underwent liver resection surgery with sufficient tissue samples, and who had a postoperative follow-up of >3 months, and who had no severe complications associated with surgery. All tissue specimens were obtained with informed patient consent. The tumor TNM stage was identified according to the guidelines of the 7th American Joint Committee on Cancer and the Union for International Cancer Control (AJCC/UICC) staging system [17]. The study was approved and supervised by the Ethics Committee of Yidu Central Hospital of Weifang and the Sixth Peoples' Hospital of Qingdao.

Cells and reagents

The human HCC cell line, HepG2, was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Thermo Fisher Scientific, Waltham, MA, USA), supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 µg/ml streptomycin (Thermo Fisher Scientific, Waltham, MA, USA) in 5% CO_2. All reagents were obtained from Sigma-Aldrich Corporation (St. Louis, MO, USA). The primary antibody to serine hydroxymethyltransferase 2 (SHMT2) was purchased from Abcam (Cambridge, MA, USA) (Cat. No EPR3198).

Immunohistochemistry (IHC) and IHC scores

Immunohistochemistry (IHC) was performed using the streptavidin-peroxidase complex method for the detection of SHMT2. Paraffin-embedded and formalin-fixed tissue samples of HCC and adjacent normal liver tissue samples were prepared, as previously reported [18]. The IHC results underwent a semi-quantification method by light microscopy. The IHC score was defined as the product of the staining intensity multiplied by the percentage of positively-stained cells. The IHC scores were evaluated by two senior pathologists who were unaware of the patients' clinical information. The scores for the percentage of positive cells were defined as: 0, for <10% positive cells; 1, for 10–30% positive cells; 2, for 30–50% positive cells;

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and 3, for >50% positive cells. The scores for staining intensity were defined as: 0, for negative staining; 1, for weak staining; 2, for moderate staining; and 3, for strong staining. The total IHC score ranged from 0–9. The study cohort was divided into a low-expression group and a high-expression group of immunostaining for SHMT2 using the cutoff score, as previously described [19].

RNA interference and transfection

The short-interfering RNA (siRNA) and scrambled RNA of *SHMT2* were synthesized by GeneChem Inc., (Shanghai, China).
The sequences of *SHMT2* siRNA included:
sense: GGAGAGUUGUGGACUUUAUTT;
antisense: AUAAAGUCCACAACUCUCCTT.
The sequences of scrambled siRNA were:
sense: AUGUGUAGUGUACGGUUGATT;
antisense: UCAACCGUACACUACACAUTT.

Lipofectamine RNAiMAX Transfection Reagent (Invitrogen, Waltham, MA, USA) was used for siRNA transfection of *SHMT2*.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Twenty pairs of fresh HCC tissues and adjacent normal liver tissues were collected and stored in liquid nitrogen for quantitative reverse transcription polymerase chain reaction (qRT-PCR). The mRNA levels of *SHMT2* in HCC tissues, adjacent normal liver tissue, and the HepG2 HCC cell line were evaluated. Total mRNAs were extracted with TRIzol reagent (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. After reverse transcription, the StepOnePlus™ real-time PCR System (Applied Biosystems, Waltham, MA, USA) was used for qRT-PCR with SYBR Green qPCR Master Mix (Thermo Scientific, Waltham, MA, USA). Results were standardized by the $2^{-\Delta\Delta Ct}$ method with GAPDH as an internal control. The sequences of primers of GAPDH and SHMT2 were as follows:
SHMT2 forward: 5'-CGAGTTGCGATGCTGTACTT-3';
SHMT2 reverse: 5'-CTGCGTTGCTGTGCTGAG-3';
GAPDH forward: 5'-TGGAGAATGAGAGGTGGGATG-3';
GAPDH reverse: 5'-GAGCTTCACGTTCTTGTATCTGT-3'.

MTT assay

Cell proliferation of HepG2 cells was detected with the MTT assay. After successful *SHMT2* knockdown, cells were seeded into 96-well plates with about 3,000 cells per well and incubated for 48 hours. 10 µl MTT at 5mg/ml was added into each well and incubated for 4 hours at 37°C. After removal of the supernatant, 100 µl of dimethylsulfoxide (DMSO) was added each well to dissolve the crystals. The optical density (OD) at 570 nm was measured and the OD of the control group was set as the baseline. The proliferation index of other tested groups was standardized to the baseline.

Transwell cell migration assay

Transwell assays were performed to evaluate cell invasion capacity with pre-coated 8 µm core Matrigel chambers (BD Biosciences, Cambridge, MA, USA), as previously described [20]. Briefly, cells were then seeded into the upper chambers with DMEM containing 1% FBS, and lower chambers were supplemented with 10% FBS. After 24 hours to allow the cells to migrate, the cells in the lower chamber were fixed with paraformaldehyde and stained with 0.1% crystal violet. Migratory cells from five random visual fields were counted under the microscope. Data were analyzed from experiments performed in triplicate.

Western blot

Cells were lysed with RIPA lysis buffer (Beyotime, Shanghai, China) and the protein concentration was measured using the Bradford method. About 10 mg of protein was used for sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride (PVDF) membrane. After blocking in 5% BSA for 1 h, the membrane was incubated with the primary antibody to SHMT2, and the EMT biomarkers, including E-cadherin, N-cadherin, vimentin, and Snail, and incubated in secondary antibody for 2 hours in room temperature. Enhanced chemiluminescent (Pierce Biotechnology, Rockford, IL, USA) was used to visualize the proteins.

Statistical analysis

Data were analyzed with SPSS version 22.0 software (IBM, Chicago, IL, USA). Correlations between SHMT2 expression and clinicopathological factors were analyzed with the chi-squared (χ^2) test. Survival curves were calculated using the Kaplan-Meier method and the statistical differences between different subgroups were analyzed with the log-rank test. The Cox regression hazard model was applied to identify independent prognostic factors. The difference between groups for cell proliferation and migration were analyzed by Student's t-test. $P<0.05$ was considered to represent statistical significance.

Results

Expression of serine hydroxymethyltransferase 2 (SHMT2) in hepatocellular carcinoma (HCC) and adjacent normal liver tissue

The expression and subcellular location of SHMT2 in 144 cases of HCC were investigated by immunohistochemistry (IHC) (Table 1). SHTM2 was mainly expressed in the cytoplasm,

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Table 1. Demographic and clinicopathological data of the cohort of 144 patients with hepatocellular carcinoma (HCC) treated with surgical resection.

Clinicopathological factors	Number	Percent
Gender		
Female	55	38.19%
Male	89	61.81%
Age (yrs)		
<50	57	39.58%
≥50	87	60.42%
Tumor size (cm)		
≤5	60	41.67%
>5	84	58.33%
Tumor number		
Single	131	90.97%
Multiple	13	9.03%
Histopathological grade		
1	32	22.22%
2	76	52.78%
3	36	25.00%
HBsAg status		
Negative	44	30.56%
Positive	100	69.44%
Hepatitis C virus (HCV)		
Negative	131	90.97%
Positive	13	9.03%
Cirrhosis		
Negative	72	50.00%
Positive	72	50.00%
T stage		
I+II	80	55.56%
III+IV	64	44.44%
N stage		
N0	142	98.61%
N1	2	1.39%
TNM stage		
I	21	14.58%
II	59	40.97%
III	62	43.06%
IV	2	1.39%
SHMT2 level		
Low	80	55.56%
High	64	44.44%

which was consistent with its function as a regulator of energy metabolism. The retrospective cohort was divided into two subgroups with low expression and high expression of SHMT2 (Figure 1A, 1B). From the 144 cases, SHMT2 expression was determined in 33 patients with paired HCC tumor tissue and adjacent normal liver tissue and the IHC scores were compared. Tissues containing HCC tissues had significantly higher IHC scores compared with adjacent normal liver tissue (Figure 1B). Also, mRNA levels of *SHMT2* in the 20 pairs of fresh HCC tissues and adjacent normal liver tissues, using quantitative reverse transcription polymerase chain reaction (qRT-PCR), were similar to the IHC findings and showed that expression of *SHMT2* mRNA in HCC was significantly higher than in adjacent tissues (Figure 1C). These findings indicated that upregulation of SHMT2 at the gene and protein levels were present in HCC when compared with normal liver.

SHMT2 was correlated with the degree of HCC differentiation (tumor grade)

The correlation between SHMT2 expression and clinicopathological factors in patients with HCC were evaluated using the chi-squared (χ^2) test to identify possible prognostic factors (Table 2). The expression of SHMT2 was significantly correlated with HCC differentiation (P=0.001). Patients with poor HCC differentiation (high-grade tumors) had high expression levels of SHMT2, indicating a possible role of SHMT2 in HCC differentiation. Also, high expression of levels of SHMT2 were significantly associated with hepatitis B virus (HBV) infection, identified by measurement of HBsAg levels, which suggests a possible role for HBV in the progression of HCC associated with SHMT2 expression.

SHMT2 was an independent prognostic factor for HCC

The prognostic role of SHTM2 for HCC was estimated by univariate and multivariate analysis, respectively (Table 3).

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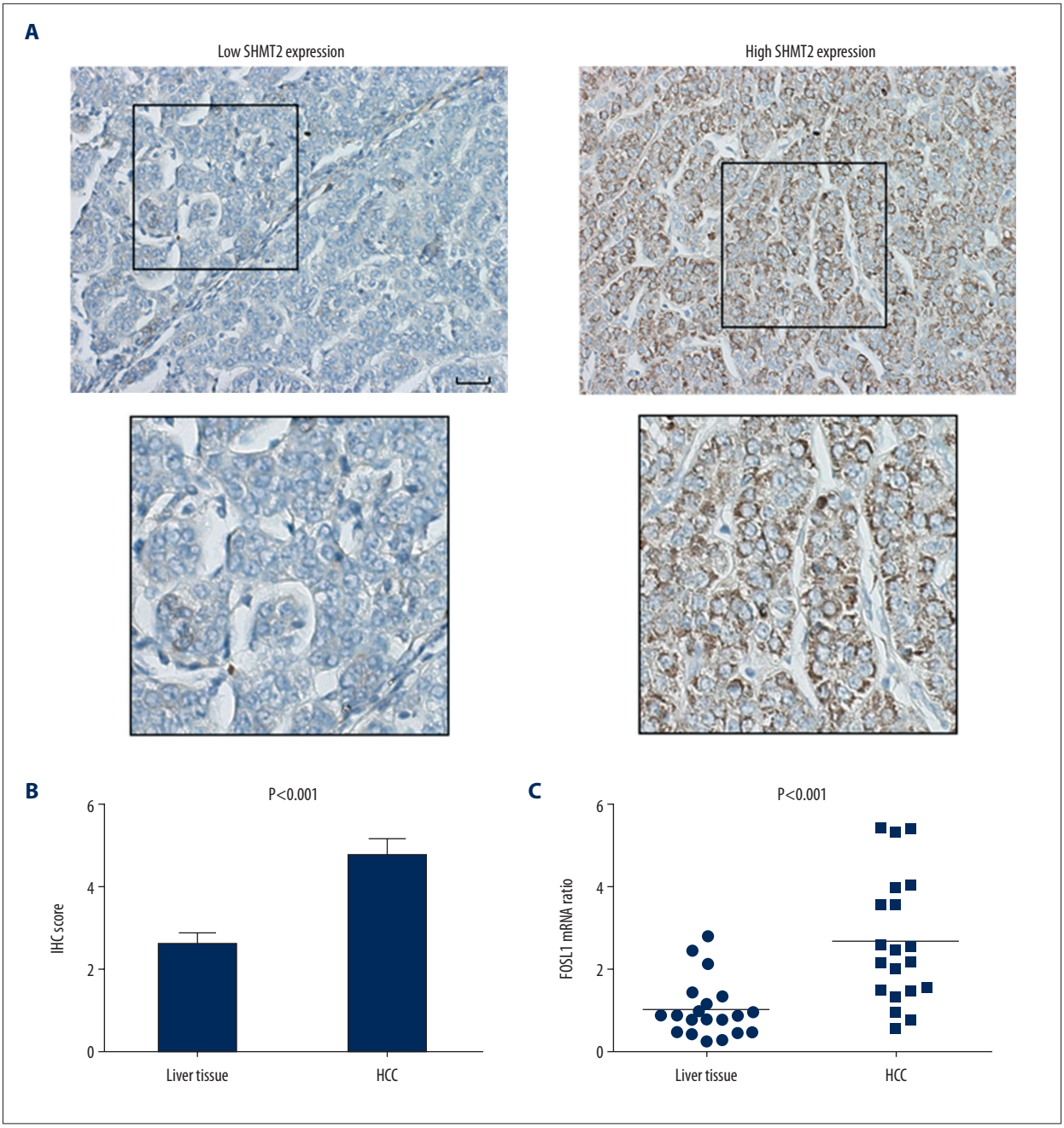

Figure 1. Immunohistochemistry (IHC) for expression of serine hydroxymethyltransferase 2 (SHMT2) in hepatocellular carcinoma (HCC) showed increased expression compared with adjacent normal liver tissue and was confirmed by *SHTM2* mRNA expression. (**A**) The patient cohort with HCC was divided into groups with low and high SHMT2 expression. Scale bar: 50 μm. (**B**) Tissue sections from HCC had higher IHC scores for SHMT2 compared with normal adjacent liver tissue. (**C**) The expression level of *SHTM2* mRNA in HCC was significantly higher compared with normal adjacent liver tissue.

Univariate analysis with the Kaplan-Meier method was performed to screen potential prognostic factors. SHMT2 had significant prognostic significance for HCC when overall survival (OS) was assessed (P<0.001). Patients with high expression of SHMT2 had a poorer prognosis than patients with low SHMT2 expression (5-year OS rate: 52.6% *vs.* 26.0%) (Figure 2A). Also, large tumor size (>5 cm), advanced T stage, and advanced TNM stage were also shown to be significantly associated with low OS rate in this study (Figure 2B–2D).

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Table 2. Expression levels of serine hydroxymethyltransferase 2 (SHMT2) and clinicopathological factors in patients with hepatocellular carcinoma (HCC).

Factor	SHMT2		P-value*
	Low	High	
Gender			
Female	32	23	0.730
Male	48	41	
Age (yrs)			
<50	35	22	0.252
≥50	45	42	
Tumor size (cm)			
≤5	37	23	0.237
>5	43	41	
Tumor number			
Single	71	60	0.386
Multiple	9	4	
Histopathological grade			
1	26	6	0.001
2	33	43	
3	15	21	
HBsAg			
Negative	30	14	0.043
Positive	50	50	
Hepatitis C Virus (HCV)			
Negative	70	61	0.145
Positive	10	3	
Cirrhosis			
Negative	40	32	1
Positive	40	32	
T stage			
I+II	50	30	0.061
III+IV	30	34	
N stage			
N0	79	63	1
N1	1	1	
TNM stage			
I	15	6	0.217
II	35	24	
III	29	33	
IV	1	1	

* Calculated with the chi-squared (χ^2) test.

SHMT2 expression was involved in cell proliferation, migration, and epithelial-mesenchymal transition (EMT) of HCC

Previous studies reported that SHMT2 down-regulation suppressed tumorigenesis in human HCC, which was supported by the findings from this study on the correlation between SHMT2 expression and poor prognosis [15]. In HepG2 HCC cells, *SHMT2* gene expression was silenced by short-interfering RNA (siRNA), and successful knockdown was validated by Western blot and qRT-PCR (Figure 3A, 3B). After silencing *SHMT2*, the proliferation and migration properties of HepG2 cells were evaluated with the MTT assay and the transwell assay, respectively (Figure 3C, 3D). *SHMT2* knockdown significantly decreased HepG2 cell proliferation and cell migration.

Epithelial-mesenchymal transition (EMT) is involved in tumor invasion and differentiation, so and EMT biomarkers, including E-cadherin, N-cadherin, vimentin, and Snail were detected by Western blot to be associated with SHMT2 expression. After silencing *SHMT2*, the expression of E-cadherin was upregulated and the other biomarkers, including N-cadherin, vimentin, and Snail were down-regulated (Figure 3E). These results suggested that SHMT2 may be involved in the process of EMT of HCC cells and that SHMT2 may influence tumor invasion and differentiation by promoting EMT.

Discussion

Cancer cells adapt their metabolic processes to support rapid cell proliferation, and one-carbon metabolism is essential for cancer cells to alter their metabolism to promote cell survival in a poorly vascularized microenvironment [21]. In one-carbon metabolism, serine hydroxymethyltransferase 2 (SHMT2) is a key regulator and plays an essential role in energy metabolism in tumorigenesis and tumor progression [22]. The *SHMT2* gene has been previously identified as a potential cancer driver gene in a comparative oncogenomics study [11]. However, the prognostic role of SHMT2 in hepatocellular carcinoma (HCC) has

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Table 3. The prognostic role of expression of serine hydroxymethyltransferase 2 (SHMT2) in patients with hepatocellular carcinoma (HCC) assessed by univariate analysis and multivariate analysis.

Factor	5-year survival rate (%)	P-value	HR	95% CI	P-value
Gender					
Female	37.9	0.442			
Male	42.5				
Age (yrs)					
<50	42.2	0.686			
≥50	39.8				
Tumor size (cm)					
≤5	53.2	0.001	1		
>5	32.1		0.94	0.59–1.76	0.939
Tumor number					
Single	39.2	0.538			
Multiple	53.8				
Histopathological grade					
1+2	41.5	0.603			
3	38.9				
HBsAg					
Negative	47.4	0.624			
Positive	37.6				
Hepatitis C Virus (HCV)					
Negative	40.1	0.999			
Positive	46.2				
Cirrhosis					
Negative	42.9	0.884			
Positive	39.5				
T stage					
I+II	60.2	<0.001	1		
III+IV	17.0		3.55	2.07–6.06	<0.001
N stage					
N0	41.3	0.239	1		
N1	0.0		1.28	0.31–5.32	0.731
TNM stage					
I	66.7	<0.001			
II	57.8				
III	17.6				
IV	0.0				
SHMT2					
Low	52.6	<0.001	1		
High	26.0		1.92	1.24–2.98	0.003

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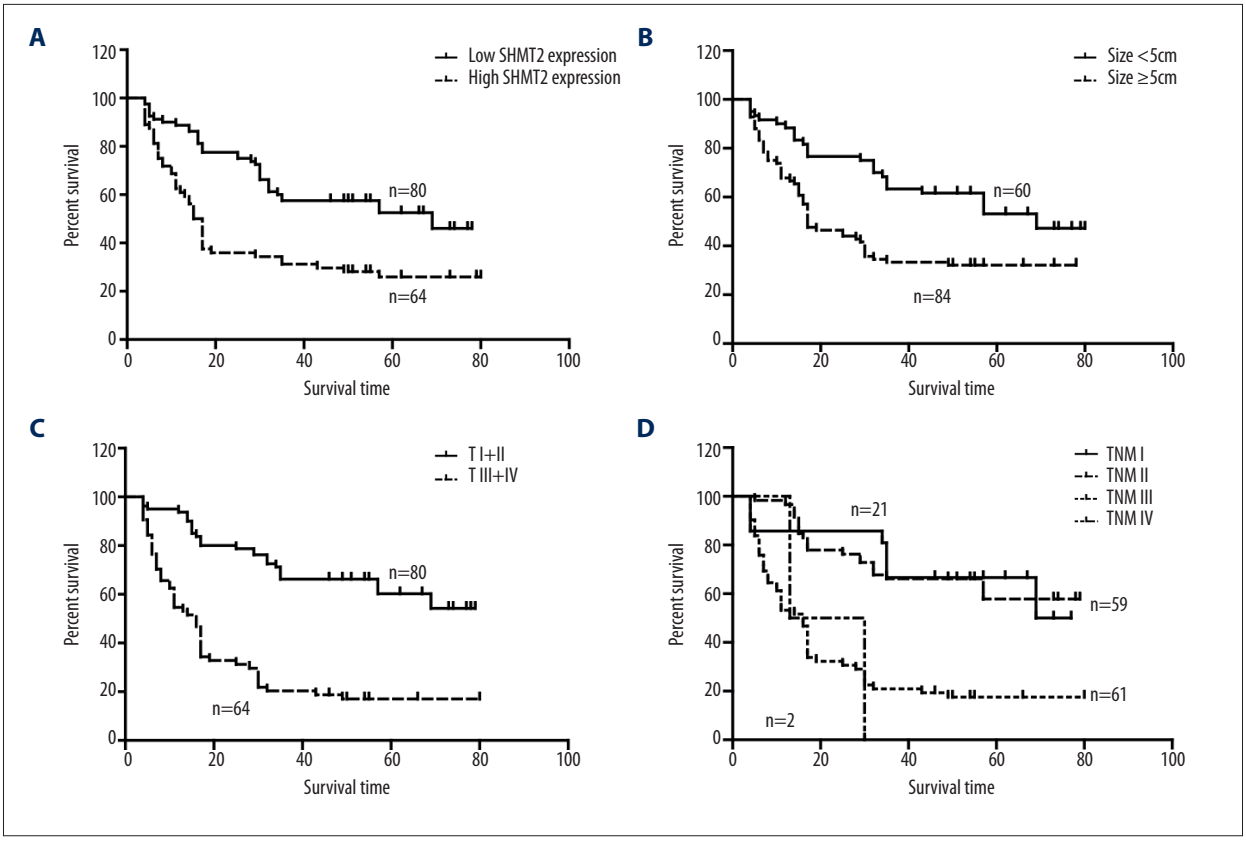

Figure 2. Correlations between overall survival (OS), SHMT2 expression, tumor size, T stage, and TNM stage in patients with hepatocellular carcinoma (HCC). (**A–D**) The overall survival (OS) rates for patients with HCC were stratified with SHMT2 expression (**A**), tumor size (**B**), T stage (**C**), and TNM stage (**D**). The Kaplan-Meier method was used for survival curves and statistical significance was analyzed with the log-rank test.

required further study because the expression and function of SHMT2 is tissue-specific and context-dependent. For example, experiments with different cell lines showed that *SHMT2* inhibition significantly suppressed the growth of HeLa cells but had little effect on A498 cells [23]. Down-regulation of *SHMT2* by short-hairpin RNA (shRNA) knockdown has previously been shown to inhibit tumorigenesis in HCC cells in two independent studies [15,16], but the prognostic value of SHMT2 in HCC was not previously evaluated. The findings of the present study showed that SHMT2 was an independent prognostic biomarker of HCC, following the detection of the protein expression using immunohistochemistry (IHC) in 144 tissue samples of HCC. The results suggested that SHMT2 was an effective prognostic biomarker in HCC and was associated with reduced patient prognosis in terms of overall survival (OS). The findings from the present study are the first to report the prognostic association between SHMT2 expression and HCC and the results expanded the understanding of the role of SHMT2 the progression and prognosis of HCC.

Humans have both cytosolic SHMT1 and mitochondrial SHMT2, but only SHMT2 has been shown to be upregulated in human cancer [10]. Although the oncogenic role of SHMT2 has been shown in several types of cancer, the underlying mechanism oncogenesis associated with expression of SHMT2 remains unclear. As an enzyme involved in energy metabolism, studies on the oncogenic role of SHMT2 have previously focused on tumor proliferation. However, the findings from this study showed, for the first time, that increased expression of SHMT2 was also associated with cell proliferation and migration of HepG2 HCC cells, and that SHMT2 may have a role in epithelial-mesenchymal transition (EMT) in HepG2 cells. Further studies are required to determine the HCC cell phenotype associated with upregulation of SHMT2 and investigate the mechanisms involved. Because patient prognosis in HCC is determined by the tumor's ability to invade and metastasize, and because invasion and metastasis of HCC are the main reasons for postoperative tumor recurrence, the findings of this study, although preliminary, are important and may be clinically relevant. These finding could expand the understanding of SHMT2 as an oncoprotein and help to reveal begin to identify the role of SHMT2 in the progression of HCC, possibly leading to studies on anti-SHMT2 or one-carbon metabolism therapy to inhibit invasion of HCC cells. Another finding from this study

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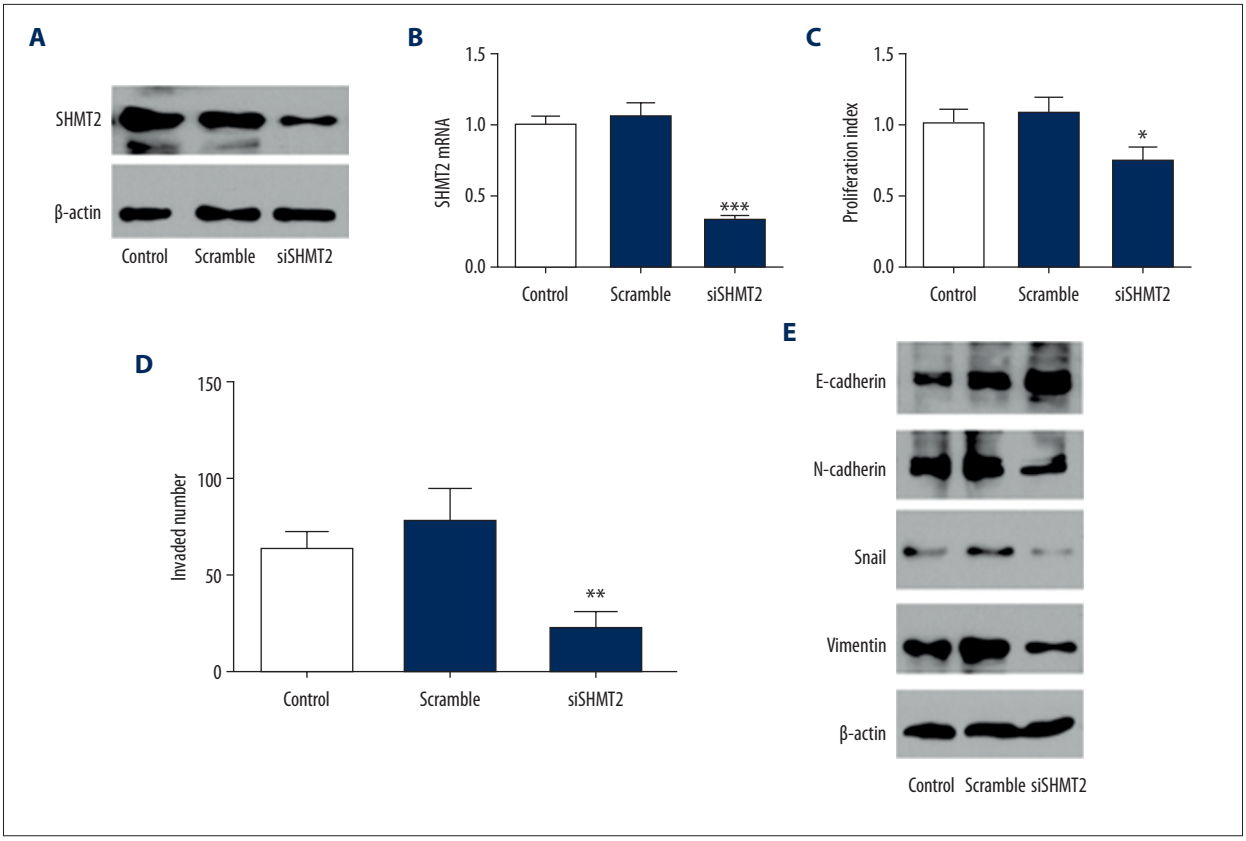

Figure 3. SHMT2 expression promoted cell proliferation, cell migration, and epithelial-mesenchymal transition (EMT) of HepG2 cells. (**A, B**) Successful knockdown of *SHMT2* was verified by Western blot (**A**) and quantitative reverse transcription polymerase chain reaction (qRT-PCR) (**B**) in HepG2 cells. (**C**) *SHMT2* knockdown significantly reduced cell proliferation of HepG2 cells. (**D**) *SHMT2* knockdown decreased cell migration of HepG2 cells. (**E**) SHMT2 promoted EMT of HepG2 cells. E-cadherin expression increased, and expression of N-cadherin, Snail, and vimentin decreased after silencing *SHMT2* expression. In (**B–D**) *, ** and *** are P<0.05, P<0.01, and P<0.001, respectively. Statistical significance was determined by Student's t-test and shown with ±SEM.

was the correlation between hepatitis B virus (HBV) infection and SHMT2 expression. Chronic inflammation, including fibrosis and cirrhosis, is a recognized risk factor for HCC and HBV infection is also associated with increased risk of developing HCC, and it would be of interest to investigate whether HBV infection might have a direct role in the progression of HCC induced by SHMT2. The preliminary findings from this study, which included a small patient cohort with HCC, should be followed with large-scale, controlled studies from several centers.

In China, HCC is the second most common cause of cancer-related death and results in a large healthcare and economic public health burden [24]. Currently, targeted therapy for patients with HCC is limited and includes sorafenib and lenvatinib, which are used to treat advanced-stage HCC and can only prolong patient survival by months [25]. New biomarkers and drug targets require continued investigation because of the poor outcome for patients with HCC. In 2016, Woo et al. showed that SHMT2 could suppress tumorigenesis in HCC and suggested that SHMT2 was a potential therapeutic target in the treatment of patients with HCC [15]. The findings of the present study identified, for the first time, that SHMT2 is a potential prognostic biomarker for HCC and showed that SHMT2 could promote proliferation, cell migration, and EMT of HepG2 HCC cells *in vitro*. SHMT2 is a potential prognostic biomarker for patients with HCC and further studies are needed to determine whether it has a role as a therapeutic biomarker, which might benefit patients with HCC who are at high risk of recurrence or metastasis after surgical resection for HCC.

Conclusions

This study aimed to investigate the expression levels of serine hydroxymethyltransferase 2 (SHMT2) in tumor tissue samples from patients with hepatocellular carcinoma (HCC) and clinical outcome, and the effects of silencing the expression of the *SHMT2* gene in HepG2 HCC cells. Increased expression

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of SHMT2, measured by immunohistochemistry (IHC), was a negative prognostic biomarker in patients with HCC, and expression of the *SHMT2* gene promoted the proliferation and migration of HepG2 HCC cells. The findings from this preliminary study are of clinical interest and it is hoped that further studies will be undertaken to validate the role of SHMT2 as a prognostic biomarker in HCC and to investigate its potential role as a therapeutic target in patients, particularly patients at high risk of tumor recurrence or metastasis following surgery.

Conflict of interest

None.

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