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High expression of SHMT2 is correlated with tumor progression and predicts poor prognosis in gastrointestinal tumors

Y. LIU[1], C. YIN[1], M.-M. DENG[2], Q. WANG[3], X.-Q. HE[4], M.-T. LI[4], C.-P. LI[2], H. WU[1,5]

[1]Department of Gastroenterology, The Fifth People's Hospital of Chengdu, Chengdu, China
[2]Department of Gastroenterology, The Affiliated Hospital of Southwest Medical University, Luzhou, China
[3]Department of Orthopedics, Dalian Medical University, Dalian, China
[4]Department of Clinical Medicine, Zunyi Medical College, Zunyi, China
[5]Department of Hepatobiliary Surgery, The Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

Yan Liu, Chuan Yin, and *Mingming Deng* contributed equally to this work

Abstract. – OBJECTIVE: Gastrointestinal tumors are malignant tumors with high morbidity. Mitochondrial serine hydroxymethyltransferase 2 (SHMT2) is a key enzyme in the synthesis of serine and glycine, which has prognostic and therapeutic value for many malignant tumors. However, the role of SHMT2 in gastric cancer (GC), esophageal cancer (ESCC), and colorectal cancer (CC) has not been clarified.

PATIENTS AND METHODS: The expression of SHMT2 was detected in GC, ESCC, and CC by immunohistochemistry and reverse real time transcription-polymerase chain reaction. The relationships between SHMT2 expression and clinicopathologic characteristics, recurrence-free survival (RFS), and disease-specific survival (DSS) were analyzed by the survival analysis and correlation analysis.

RESULTS: The positive expression rate of SHMT2 in GC, ESCC, and CC was 74.1%, 69.2%, and 71.7%, respectively. Patients with high expression of SHMT2 had a worse prognosis. In GC, high SHMT2 expression had positive correlation with lymph node metastasis (p=0.005) and histological grade (p=0.002). In ESCC, high SHMT2 expression had positive correlation with pT classification (p=0.033) and pM classification (p=0.029). In CC, high SHMT2 expression had positive correlation with tumor size (p=0.004), lymph node metastasis (p=0.035), TNM stage (p=0.007), and histological grade (p=0.020). Notably, SHMT2 expression was an independent prognostic factor for RFS and DSS in GC, ESCC, and CC (p<0.05).

CONCLUSIONS: SHMT2 is upregulated in GC, ESCC, and CC. The high expression of SHMT2 is correlated with gastrointestinal tumors progression, and poor prognosis, which is a potential new target for the diagnosis and treatment of gastrointestinal tumors.

Key Words:
SHMT2, Gastric cancer, Esophageal cancer, Colon cancer, Prognostic significance.

Abbreviations

GC: gastric cancer; ESCC: esophageal cancer; CC: colorectal cancer; SHMT2: Mitochondrial serine hydroxymethyltransferase 2; CEA: carcinoembryonic antigen; HE: hematoxylin and eosin; NC: negative control; SDS-PAGE: sulfate polyacrylamide gel electrophoresis; RFS: recurrence-free survival; DSS: disease-specific survival.

Introduction

Gastrointestinal tumors are a leading cause of disability, short life expectancy, and death worldwide, and gastrointestinal cancer is the most frequent cause of cancer-related death[1]. GC, ESCC, and CC are the most common gastrointestinal cancer and have the second, sixth, and fourth highest tumor-related mortality, respectively[2-5]. GC, ESCC, and CC have the characteristics of concealed disease, easy recurrence, and high mortality, which bring a heavy burden to families and society[6-9]. Early diagnosis and treatment could improve the prognosis of these patients. However, except for the high value of carcinoembryonic antigen (CEA) in the diagnosis of colon cancer, there is no ideal biomarker for diagnosing and predicting the prognosis of these three types of gastrointestinal tumors.

Corresponding Authors: Chang-Ping Li, MD; e-mail: 506854209@qq.com
Hao Wu, MD; e-mail: whwh1hero@163.com

To find a break-through treatment, we need to find prognostic markers and therapeutic targets to improve the survival rate of cancer patients[10,11].

In cancer, oncogenes, and tumor-suppressor factors are often the driving factors of tumorigenesis. Therefore, the new therapies in oncology rely on the determination of new prognostic markers and therapeutic targets. Recently, integrated analysis has revealed that serine/glycine biosynthesis affects cellular antioxidative capacity, and supports cell homeostasis[12,13]. Excessive activation of the serine/glycine biosynthetic pathway promotes tumorigenesis[13]. Cancer cells are reprogrammed to overactivate glycolytic pathways in driving tumorigenesis[14]. SHMT2 is a mitochondrial enzyme that is mainly expressed in the mitochondria, but it has also been detected in the cytoplasm and nucleus[15]. SHMT2 is able to catalyse the conversion of serine to glycine with the transfer of the β-carbon from serine to tetrahydrofolate (THF) to form 5, 10-methylene-THF, which is a crucial factor for serine/glycine metabolism in proliferating cells[16]. SHMT2 has shown prognostic and therapeutic value for many malignant tumors, such as glioma, hepatic carcinoma, and breast cancer[17-23]. However, its role in gastrointestinal tumors, especially GC, ESCC, and CC is unclear.

This study aimed to explore the relationships between SHMT2 in GC, ESCC, and CC, and will provide a new target for the diagnosis and accurate treatment of gastrointestinal tumors.

Patients and Methods

Patients and Tissue Specimens

In this research, all samples were obtained from 2010 to 2013 in the Fifth People's Hospital of Chengdu. In total, we studied 183 patients, including 58 with gastric cancer, 65 with esophageal carcinoma, and 60 with colorectal cancer. After surgical resection or endoscopy, all specimens were fixed with 4% formalin (pH=7.0) and embedded in paraffin for less than 24 hours. Finally, the diagnosis of GC, ESCC, and CC was determined histologically by two experienced pathologists in the Department of Pathology Archives of the Fifth People's Hospital of Chengdu using hematoxylin and eosin (HE) staining. We collected data about the clinical and prognostic characteristics of every specimen. The diagnoses of GC, ESCC, and CC accorded with the criteria approved by the World Health Organization (WHO). All samples completed the follow-up before the deadline of May 2018. This investigation followed the tenets of the Declaration of Helsinki, was approved by the Ethics Review Committee of the Fifth People's Hospital of Chengdu, and written informed consent was obtained from all patients (IACUC-2010017, Chengdu, China).

Immunohistochemical Staining

Immunohistochemical staining was used to detect the expression of SHMT2 in paraffin-embedded sections (4 μm). We used xylene to dewax the specimens, which were hydrated in a graded alcohol series. Antigen repair was performed in 10 mmol/L sodium citrate solution (pH=6.0) at 100°C for ten minutes, and the specimens were cooled for twenty minutes. After rinsing in phosphate-buffered saline (PBS; pH=7.2), endogenous peroxidase activity was suppressed with 3% H_2O_2 for 15 minutes and stopped with goat serum for 15 minutes at external temperature to bind with other proteins. Hence, the slides were immersed with SHMT2 monoclonal antibody at a 1:100 dilution at 4°C overnight. Then, the specimens were rinsed three times in PBS (pH=7.2), soaked with biotinylated secondary antibody at 37°C for 30 minutes and incubated with avid in horseradish enzyme at 37°C for 20 minutes. We used 3,3-diaminobenzidine (DAB) and running water for 15 minutes to show the color and used 1% Mayer's hematoxylin to counterstain the slides. Next, we used a gradient ethanol series for dehydration and the specimens were covered with neutral gum. In the end, the slides were cleaned, and cover slipped.

Scoring Systems for Immunohistochemical Staining

The semi-quantitative assessment method of scoring was used for the expression of SHMT2. All slides were scored independently by two seasoned professionals. The parameters of scoring systems included staining intensity (range 0-3: 0, negative; 1, weak; 2, moderate; and 3, strong) and the percentage of positive cells (range 0-4: 0, negative or <5%; 1, 6%-25%; 2, 26%-50%; 3, 51%-75%; and 4, 76%-100%). We adopted the percentage of positive cells and the grade to calculate the final stain scores. The slides with a score <4 were regarded as low SHMT2 expression and the slides with a score ≥4 were regarded as high SHMT2 expression.

Cell Culture

One human gastric epithelial cell line (GES-1), four human gastric cancer cell lines (BGC-823, GC9811, MGC-807, and NCI-N87), a human esophageal squamous epithelial cell line (Het-1A), four

human esophageal cancer cell lines (KYSE150, KYSE30, TE-2, and EC109), a human colorectal mucosa cell line (FHC), and four human colorectal cancer cell lines (HT29, LOVO, HCT116, and SW620) were obtained from the Institute of Biochemistry and Cell Biology (Chinese Academy of Sciences, Shanghai, China). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Corning, Corning, NY, USA) containing 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA).

Reverse Transcription Real Time Polymerase Chain Reaction (RT-PCR)

Total RNA from cancer tissues, adjacent tissues, and cell lines was extracted using TRIzol reagent (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's protocol. PrimeScript RT Reagent (TaKaRa, Otsu, Shiga, Japan) was used for reverse transcription of cDNA, and RT-PCR was performed with SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan) using the SYBR Green Real-Time PCR Master Mix (TaKaRa, Dalian, Liaoning, China). Primers for SHMT2 (forward: 5'-ATG TCT GAC GTC AAG CGG AT-3'; and reverse: 5'-GGC CAG TTT TGG GGT TGA GC-3') and **β**-actin (forward: 5-TTC CAG CCT TCC TTC CTG GG-3'; and reverse: 5'-TTG CGC TCA GGA GGA GCA AT-3') were used. Reaction Cycle Conditions of PCR: 30 s of denaturation at 94°C, at 96°C for 15 s, and 65°C for 30 s (32 cycles). The mRNA expression of SHMT2 was analyzed using the $2^{-\Delta\Delta Ct}$ method. β-actin was used as the control.

Statistical Analysis

GraphPad Prism version 6.0 (San Diego, CA, USA) and SPSS 21.0 software (IBM, Armonk, NY, USA) were used for statistical analysis. All data was presented as the means ± standard deviation (SD). The correlation between SHMT2 expression and clinicopathological parameters in GC, ESCC, and CC were analysed by one-way analysis of variance (ANOVA), followed by the Newman-Keuls test. RFS was regarded as the period from removing the tumor tissue as cleanly as possibly to tumor recurrence, and DSS was regarded as the period from being diagnosed with cancer to dying from cancer or reaching the follow-up deadline. Next, we adopted the long-rank test to study the differences between the curves. Using Cox univariate and multivariate analysis, and the multivariate analysis, a panel of significant independent prognostic factors was derived. The relative importance of each prognostic factor in this panel was ranked from high to low based on its [–2 (log likelihood)]. Then, forward stepwise multivariable Cox regressive analysis and examination of partial *p*-value of [–2 (log likelihood ratio)] allowed us to identify predictive models. We regarded $p<0.05$ as the threshold for statistical significance.

Results

Clinical Pathological Features of GC, ESCC and CC, and the Expression of SHMT2

We used RT-PCR to detect SHMT2 expression in tumor tissues and paired normal adjacent tissues of GC, ESCC, and CC. RT-PCR results showed that SHMT2 was highly expressed in GC, ESSC, and CC (Figure 1A-C). Moreover, a higher expression level of SHMT2 in each cell line was correlated with higher malignancy (Figure 2 A-C).

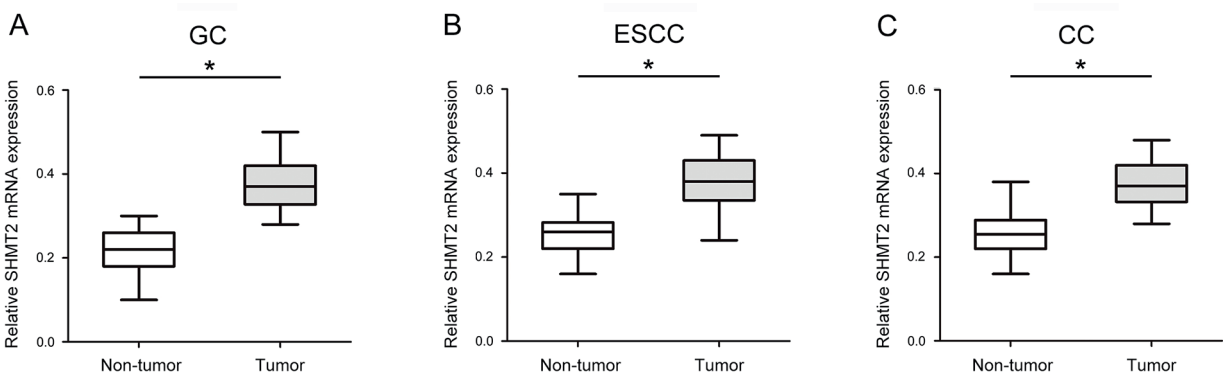

Figure 1. SHMT2 mRNA expression were quantified by RT-PCR in GC, ESCC, and CC. **A**, SHMT2 was significantly upregulated in GC compared with adjacent non-cancerous tissues. **B,** SHMT2 was significantly upregulated in ESCC compared with adjacent non-cancerous tissues. **C,** SHMT2 was significantly upregulated in CC compared with adjacent non-cancerous tissues. *$p<0.05$.

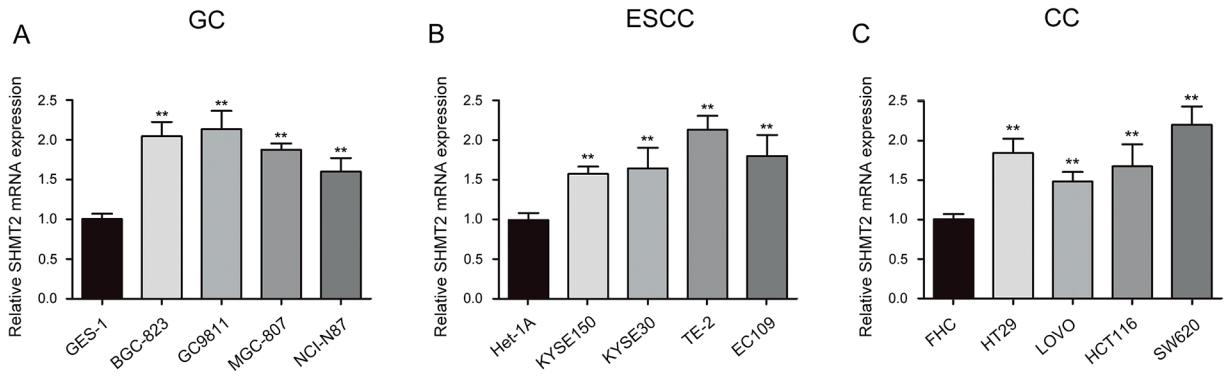

Figure 2. SHMT2 mRNA expression was quantified by RT-PCR in GC, ESCC, and CC cell lines. **A,** SHMT2 was significantly upregulated in GC cell lines. **B,** SHMT2 was significantly upregulated in ESCC cell lines. **C,** SHMT2 was significantly upregulated in CC cell lines. $^{**}p < 0.01$.

Clinical Pathological Features of Patients and the Expression of SHMT2 in GC, ESCC, and CC

At the time of this analysis, a total of 183 patients, including 58 GC, 65 ESCC, and 60 CC were investigated in our study. Moreover, the expression levels of SHMT2 were graded as negative/low and moderate/high. The results showed that the rate of high SHMT2 expression in GC was 74.1% (43/58), in ESCC was 69.2% (45/65), and in CC was 71.7% (43/60) (Figures 3-4).

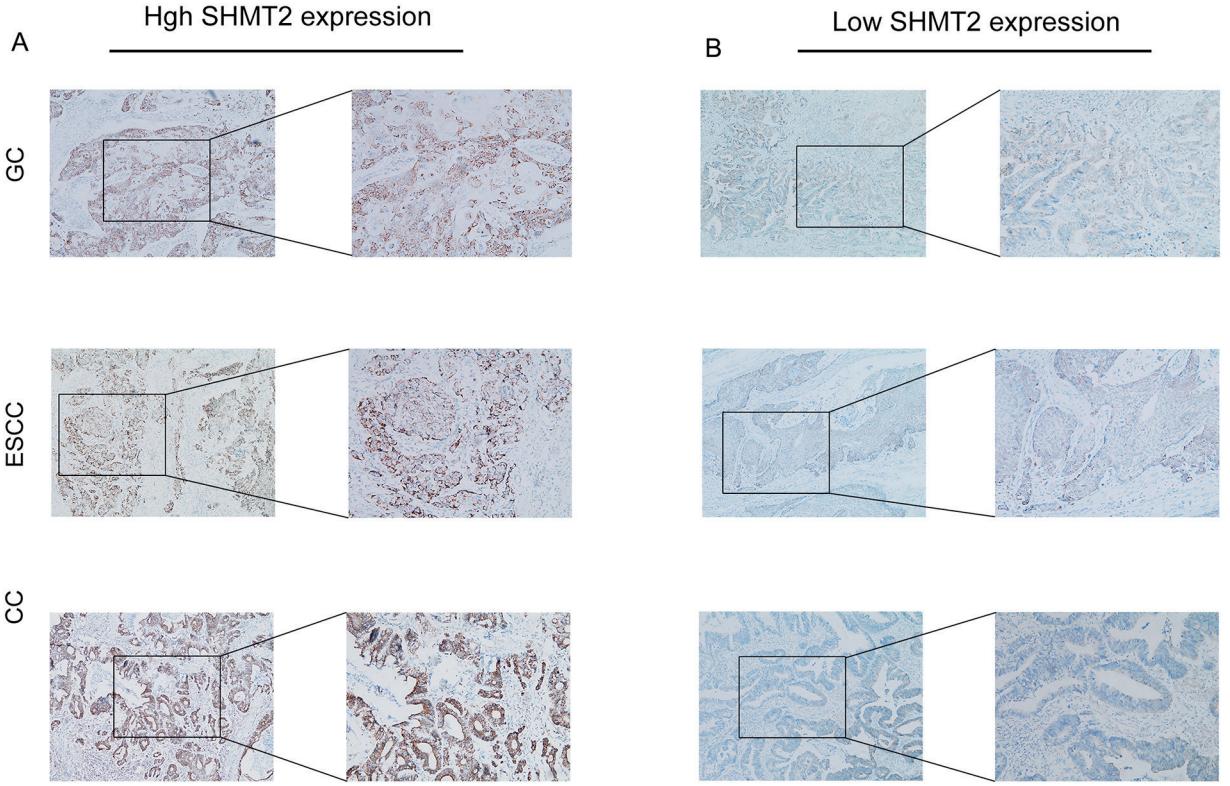

Figure 3. Expression of SHMT2 in GC, ESCC and CC. **A,** High expression of SHMT2 in GC, ESCC, and CC. **B,** Low expression of SHMT2 in GC, ESCC, and CC. Original magnification (x50 and x200).

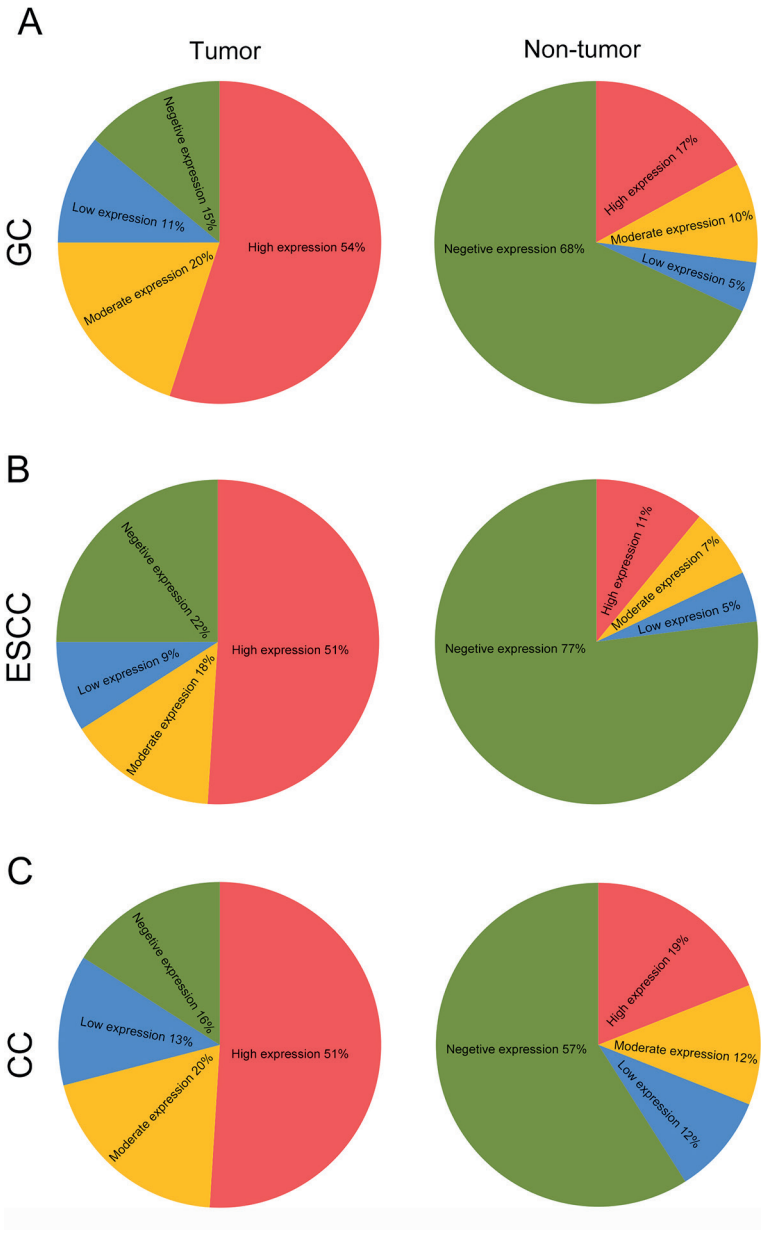

Figure 4. Statistical analysis of the expression of SHMT2 in GC, ESCC and CC. **A,** Expression of SHMT2 in GC. **B,** Expression of SHMT2 in ESCC. **C,** Expression of SHMT2 in CC.

Relationships Between SHMT2 Expression and the Clinicopathological Characteristics of Patients with GC, ESCC, and CC

We analyzed the associations between SHMT2 expression and the clinicopathological characteristics of patients with GC, ESCC, and CC. In GC, high SHMT2 expression had positive correlations with lymph node metastasis (p=0.005) and histological grade (p=0.002; Table I). In ESCC, high SHMT2 expression had positive correlations with the depth of tumor invasion (pT classification, p=0.033) and distant metastasis (pM classification, p=0.029; Table II). In CC, high SHMT2 expression had positive correlations with tumor size (p=0.004), lymph node metastasis (p=0.035), TNM stage (p=0.007), and histological grade (p=0.020; Table III). In addition, the TNM staging system is still a powerful prognostic predictive factor, and we analyzed the predictive capability of SHMT2 in the early-stage (I+II) and late-stage (III+IV) groups. The results showed that high

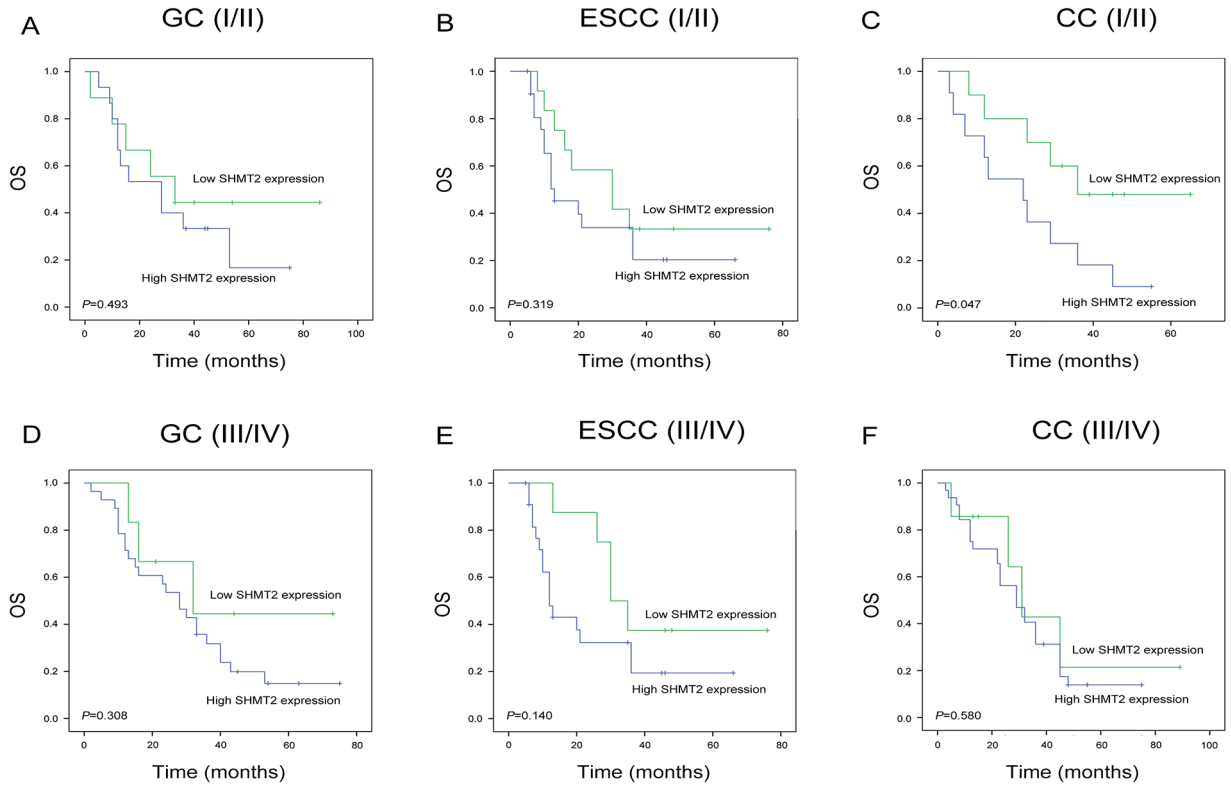

Figure 5. The relationship between SHMT2 and OS in the early-stage (I+II) and late-stage (III+IV) groups of GC, ESCC, and CC. **A,** Kaplan-Meier survival curves of OS in GC early-stage (I+II) patients according to SHMT2 expression. **B,** Kaplan-Meier survival curves of OS in ESCC early-stage (I+II) patients according to SHMT2 expression. **C,** Kaplan-Meier survival curves of OS in CC early-stage (I+II) patients according to SHMT2 expression. **D,** Kaplan-Meier survival curves of OS in GC (III+IV) patients according to SHMT2 expression. **E,** Kaplan-Meier survival curves of OS in ESCC (III+IV) patients according to SHMT2 expression. **F,** Kaplan-Meier survival curves of OS in CC (III+IV) patients according to SHMT2 expression.

Table I. Correlations of SHMT2 with clinicopathological features of GC.

Variables	No.	SHMT2 expression		*p*-value
		Low (n=15)	High (n=43)	
Age (year)				
<45	24	6	18	0.964
≥45	34	9	25	
Sex				
Male	29	8	21	0.938
Female	29	7	22	
Tumor size (cm)				
≤5	27	10	17	0.081
>5	31	5	26	
Lymph node metastasis				
Presence	35	4	31	0.005
Absence	23	11	12	
TNM stage				
I/II	24	9	15	0.129
III/IV	34	6	28	
Histological grade				
Well/moderately	25	12	13	0.002
Poorly	33	3	30	

Table II. Correlations of SHMT2 with clinicopathological features of ESCC.

Variables	No.	SHMT2 expression		*p*-value
		Low (n=20)	High (n=45)	
Age (year)				
<45	33	11	22	0.789
≥45	32	9	23	
Sex				
Male	35	12	23	0.938
Female	30	8	22	
pT classification				
T1/T2	32	14	18	0.033
T3/T4	33	6	27	
pN classification				
N0/N1	29	10	19	0.598
N2/N3	36	10	26	
pM classification				
M0	28	13	15	0.029
M1	37	7	30	
Cancer stage				
I/II	34	12	22	0.435
III/IV	31	8	23	
Tumor location				
Upper thoracic	18	5	13	0.753
Middle thoracic	22	7	15	
Lower thoracic	25	8	17	

Table III. Correlations of SHMT2 with clinicopathological features of CC.

Variables	No.	SHMT2 expression		*p*-value
		Low (n=17)	High (n=43)	
Age (year)				
<45	28	8	20	0.896
≥45	32	9	23	
Sex				
Male	27	7	20	0.779
Female	33	10	23	
Tumor size (cm)				
≤5	31	14	17	0.004
>5	29	3	26	
Lymph node metastasis				
Presence	34	6	28	0.035
Absence	26	11	15	
TNM stage				
I/II	21	10	11	0.007
III/IV	39	7	32	
Histological grade				
Well/moderately	27	12	15	0.020
Poorly	33	5	28	

SHMT2 expression exhibited significantly shorter overall survival (OS) compared with those with low SHMT2 expression in CC early-stage (I+II) groups (Figure 5C, $p<0.05$), and there is no difference in other groups (Figure 5A-B, Figure 5D-F, $p>0.05$).

Association of SHMT2 Expression with RFS in GC, ESCC, and CC

Kaplan-Meier survival analysis revealed that high SHMT2 expression with GC, ESCC and CC exhibited significantly shorter RFS compared with those with low SHMT2 expression (Figure 6A-C, $p<0.05$).

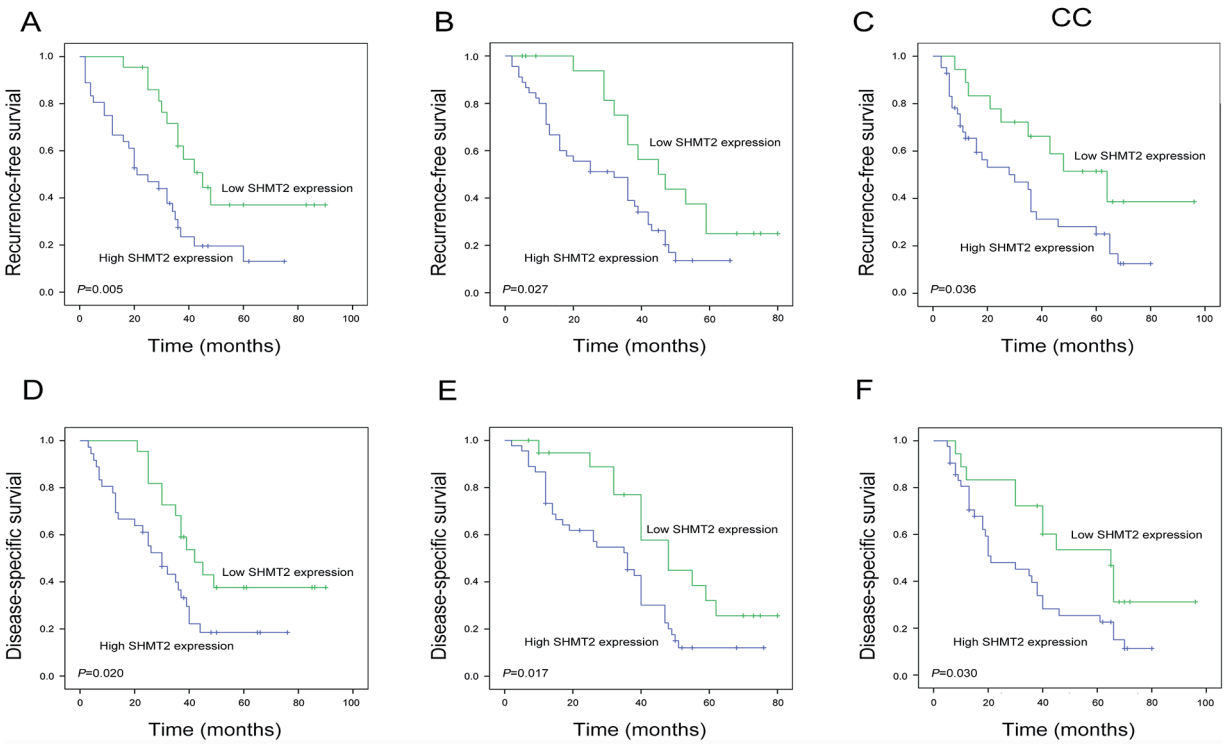

Figure 6. RFS and DSS in the patients with the high and low expression of SHMT2 in GC, ESCC, and CC. **A,** Kaplan-Meier survival curves of RFS in GC patients according to SHMT2 expression. **B,** Kaplan-Meier survival curves of RFS in ESCC patients according to SHMT2 expression. **C,** Kaplan-Meier survival curves of RFS in CC patients according to SHMT2 expression. **D,** Kaplan-Meier survival curves of DSS in GC patients according to SHMT2 expression. **E,** Kaplan-Meier survival curves of DSS in ESCC patients according to SHMT2 expression. **F,** Kaplan-Meier survival curves of DSS in CC patients according to SHMT2 expression.

Table IV. Univariate and multivariate analysis of different prognostic variables and RFS in GC.

		Univariate analysis		Multivariate analysis model	
Variables	**No.**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Sex		0.706 (0.940-2.014)	0.571		
Male	29				
Female	29				
Age (year)		1.204 (0.804-2.315)	0.684		
<45	24				
≥45	34				
Tumor size (cm)		0.548 (0.458-1.964)	0.764		
≤5	27				
>5	31				
Lymph node metastasis			0.030		<0.05
Positive	35				
Negative	23				
TNM stage		1.204 (1.397-2.754)	0.669		
I/II	28				
III/IV	30				
Histological grade		1.375 (1.018-3.018)	0.033		<0.05
Well/moderately	25				
Poorly	33				
SHMT2 expression		1.864 (0.480-2.657)	0.028		<0.05
High	43				
Low	15				

Table V. Univariate and multivariate analysis of different prognostic variables with RFS in ESCC.

		Univariate analysis		Multivariate analysis model	
Variables	**No.**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Sex		0.706 (0.940-2.014)	0.571		
Male	30				
Female	35				
Age (year)		1.288 (0.542-1.4996)	0.594		
<45	33				
≥45	32				
pT classification		1.079 (0.869-2.867)	0.030		
T1/T2	32				
T3/T4	33				
pN classification		1.021 (1.148 -3.841)	0.571		
N0/N1	29				
N2/N3	36				
pM classification		1.542 (0.967-3.075)	0.018		<0.05
M0	28				
M1	37				
Cancer stage		0.842 (0.948-3.007)	0.647		
I/II	34				
III/IV	31				
Tumor location		1.225 (0.756-2.396)	0.572		
Upper thoracic	18				
Middle thoracic	22				
SHMT2 expression		1.533 (0.957-2.275)	0.014		<0.05
High	45				
Low	20				

The univariate analysis showed that lymph node metastasis (HR=0.984, p=0.030), histological grade (HR=1.375, p=0.033), and SHMT2 expression (HR=1.864, p=0.028) were significantly associated with RFS in GC (Table IV). pT classification (HR=1.079, p=0.030), pM classification (HR=1.542, p=0.018), and SHMT2 expression (HR=1.533, p=0.014) were poor prognostic factors affecting RFS in ESCC after resection (Table V). Tumor size (HR=1.154, p=0.019), lymph node metastasis (HR=1.391, p=0.034), TNM stage (HR=0.533, p= 0.020), histological grade (HR=1.006, p=0.030), and SHMT2 expression (HR=1.563, p=0.012) were significantly associated with RFS in CC (Table VI).

In the multivariate analysis, we still use Cox models to analyze the significance of SHMT2 for RFS in GC, ESCC, and CC. For GC, Lymph node metastasis, histological grade, and SHMT2 expression (p<0.05) were independent prognostic factors for RFS in patients with GC (Table IV). pM classification and SHMT2 expression (p<0.05) were independent prognostic factors for RFS in patients with ESCC (Table V). Tumor size, TNM stage, and SHMT2 expression (p<0.05) were independent prognostic factors for RFS in patients with CC (Table VI).

Association of SHMT2 Expression with DSS in GC, ESCC, and CC

Kaplan-Meier survival analysis revealed that high SHMT2 expression with in GC, ESCC, and CC was associated with significantly shorter DSS compared with those with low SHMT2 expression (Figure 6D-F, p<0.05).

The univariate analysis showed that lymph node metastasis (HR=1.984, p=0.044), histological grade (HR=1.897, p=0.028), and SHMT2 expression (HR=1.338, p=0.041) were significantly associated with DSS in GC (Table VII). pT classification (HR=1.258, p=0.024), pM classification (HR=1.804, p=0.017), and SHMT2 expression (HR=1.871, p=0.024) were poor prognostic factors affecting DSS in ESCC (Table VIII). Tumor size (HR=1.174, p=0.017), lymph node metastasis (HR=1.084, p=0.031), TNM stage (HR=1.933, p=0.020), histological grade (HR=0.984, p=0.034), and SHMT2 expression (HR=1.047, p=0.010) were significantly associated with DSS in CC (Table IX).

Table VI. Univariate and multivariate analysis of different prognostic variables with RFS in CC.

		Univariate analysis		Multivariate analysis model	
Variables	No.	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Sex		1.668 (0.589-1.365)	0.503		
Male	33				
Female	27				
Age (year)		1.506 (1.095-2.630)	0.849		
<45	28				
≥45	32				
Tumor size (cm)		1.154 (1.508-2.203)	0.019		<0.05
≤5	31				
>5	29				
Lymph node metastasis		1.391 (1.238 -3.691)	0.034		
Positive	34				
Negative	26				
TNM stage		0.533 (1.580-2.618)	0.020		<0.05
I/II	21				
III/IV	39				
Histological grade		1.006 (1.239-2.663)			0.030
Well/moderately	27				
Poorly	33				
SHMT2 expression		1.563 (1.230-1.963)	0.012		<0.05
High	43				
Low	17				

The multivariate analysis showed that histological grade and SHMT2 expression ($p<0.05$) were significantly associated with DSS in GC (Table VII). pM classification and SHMT2 expression ($p<0.05$) were significantly associated with DSS in ESCC (Table VIII). Lymph node metastasis,

Table VII. Univariate and multivariate analysis of different prognostic variables and DSS in GC.

		Univariate analysis		Multivariate analysis model	
Variables	No.	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Sex		0.986 (1.125-1.994)	0.784		
Female	29				
Male	29				
Age (year)		1.044 (0.845-1.896)			0.722
<45	24				
≥45	34				
Tumor size (cm)		1.841 (1.429-3.047)			0.667
≤5	27				
>5	31				
Lymph node metastasis		1.984 (1.364 -2.640)			0.044
Positive	35				
Negative	23				
TNM stage		0.847 (1.087-2.607)			0.781
I/II	91				
III/IV	59				
Histological grade		1.897 (1.587-2.980)		0.028	<0.05
Well/moderately	25				
Poorly	33				
SHMT2 expression		1.338 (1.704-3.788)		0.041	<0.05
High	43				
Low	15				

Table VIII. Univariate and multivariate analysis of different prognostic variables with DSS in ESCC.

Variables	No.	Univariate analysis		Multivariate analysis model	
		HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Sex	1.653 (0.725-1.565)		0.723		
Female	30				
Male	35				
Age (year)	1.308 (0.427-1.596)		0.553		
<45	33				
≥45	32				
pT classification	1.258 (1.631-3.027)		0.024		
T1/T2	32				
T3/T4	33				
pN classification	0.775 (0.864 -1.894)		0.486		
N0/N1	29				
N2/N3	36				
pM classification	1.804 (1.086-2.486)		0.017		<0.05
M0	28				
M1	37				
Cancer stage	1.700 (1.059-2.980)		0.828		
I/II	34				
III/IV	31				
Tumor location	1.308 (1.228-2.816)		0.548		
Upper thoracic	18				
Middle thoracic	22				
Lower thoracic	25				
SHMT2 expression	1.871 (1.394-2.451)		0.024		<0.05
High	45				
Low	20				

TNM stage, and SHMT2 expression ($p<0.05$) were significantly associated with DSS in CC (Table IX).

Discussion

Gastrointestinal tumors include GC, ESCC, and CC, which are very common worldwide, with high mortality rates[24,25]. Due to the lack of early symptoms, these cancers are mostly diagnosed at an advanced stage, and treatment is difficult with poor prognosis and high mortality. Glycine consumption and the expression of the mitochondrial glycine biosynthesis pathway are closely related to the proliferation rate of cancer cells. SHMT2 is a key enzyme in serine/glycine synthesis pathway. In mammalian mitochondria, SHMT2 can catalyse the transformation of serine into glycine. In mitochondrial myc-dependent cells, interference with SHMT2 reduces the sNADPH/$NADP^+$ ratio, increases ROS, and induces hypoxia-induced cell death[17]. Serine catabolism by SHMT2 is required for proper mitochondrial translation initiation and maintenance of formylmethionyl-tRNAs, which indicates that SHMT2 can drive tumor growth[26]. Moreover, SHMT2 promotes cancer cell proliferation by catalysing serine catabolism[27].

Clinically, SHMT2 may serve as a prognostic factor and as a potential therapeutic target for human gliomas[19,20]. The downregulation of SHMT2 can reduce the occurrence of liver tumors[21]. Negatively regulating SHMT2 in hepatocellular carcinoma can prevent proliferation and migration[22]. Considering that SHMT2 is one of the metabolic building blocks of liver cancer, it may be a potential target for the treatment of liver cancer. By analyzing the relationships between metabolic proteins, pathological features, and survival in breast cancer, it was found that SHMT2 could be a valuable prognostic marker and a potential target for personalized breast cancer therapy[23]. Our study agrees with their conclusion. Of note, we demonstrated that SHMT2 was highly expressed in GC, ESCC, and CC, and patients with high SHMT2 expression had a worse prognosis. In the GC, ESCC, and CC cell lines, higher expression of SHMT2 in each cell line was correlated with

Table IX. Univariate and multivariate analysis of different prognostic variables with DSS in ESCC.

		Univariate analysis		Multivariate analysis model	
Variables	No.	HR (95% CI)	*p*-value	HR (95% CI)	*p*-value
Sex	1.538 (0.647-1.558)		0.612		
Female	33				
Male	27				
Age (year)	1.289 (0.589-1.886)		0.642		
<45	28				
≥45	32				
Tumor size (cm)	1.174 (1.347-2.894)		0.017		
≤5	31				
>5	29				
Lymph node metastasis	1.084 (0.684-2.885)		0.031		<0.05
Positive	34				
Negative	26				
TNM stage	1.933 (0.890-1.863)		0.020		<0.05
I/II	21				
III/IV	39				
Histological grade	0.984 (1.045-2.664)		0.034		
Well/moderately	27				
Poorly	33				
SHMT2 expression	1.047 (0.578-2.517)		0.010		<0.05
High	43				
Low	17				

higher malignancy. These results suggested that SHMT2 plays an important role in the malignant progression of GC, ESCC, and CC and predicts poor prognosis.

Malignant progression of tumors is a complex process involving many regulatory factors, such as gene mutations caused by epigenetic changes in tumor cells and normal hepatocytes, changes in cell surface signal transduction molecules and adhesion capacity, abnormal cell metabolism, and changes in tumor cells and the surrounding microenvironment[28,29]. The roles of SHMT2 in multisystem tumors have been observed in previous research. High SHMT2 expression is closely related to poor prognosis. SHMT2 can limit the activity of pyruvate kinase 2 (PKM2) and reduce oxygen consumption, promoting changes in metabolism that give glioma cells in poorly vascularized tumor areas a profound survival advantage[17]. In glioma patients, SHMT2 overexpression more frequently occurs in advanced-grade malignancy and tumor with poor prognosis. SHMT2 knockdown efficiently suppresses the proliferation and invasion of glioma cells *in vitro*, showing that it functions as an oncogene in glioma development and progression. Mitochondrial serine catabolism in gliomas supports tumor growth by maintaining mitochondrial redox balance and cell survival[18]. Remarkably, our results show that GC, ESCC, and CC patients with high SHMT2 expression exhibited increases in lymph node metastasis, histological grade, pT classification, pM classification, tumor size, and TNM stage. The above results indicate that SHMT2 can promote the malignant development of tumor by regulating the energy metabolism of tumor cells.

Taken together, these data strongly imply that SHMT2 participates in the tumor progression of gastrointestinal tumors. Survival analysis demonstrated that high SHMT2 expression was a poor prognostic factor for RFS and DSS in GC, ESCC, and CC. Our research showed that SHMT2 increased the degree of malignancy and decreased patient survival. Next, we will study the specific mechanism of SHMT2 in the malignant progression of gastrointestinal tumors, and provide new targets for the diagnosis and treatment of GC, ESCC, and CC.

Conclusions

The expression of SHMT2 is increased significantly in GC, ESCC, and CC, and correlates positively with gastrointestinal tumors progression and poor prognosis. SHMT2 may be involved in the malignant progression of GC, ESCC, and CC, making it a potential new target for the diagnosis and treatment of gastrointestinal tumors.

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Conflict of Interests

The Authors declare that they have no conflict of interests.

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