Expression of septin 2 and association with clinicopathological parameters in colorectal cancer

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Abstract. Septin 2 (SEPT2) is a tumor-related gene belonging to the SEPT family that affects the cellular processes of hepatoma carcinoma cells, glioblastoma cells and mesangial cells and is highly expressed in breast cancer, biliary tract cancer and acute myeloid leukemia. Colorectal cancer (CRC) is the third most common type of malignancy in humans. In the present study, Oncomine database was used to compare the expression pattern of SEPT2 mRNA between CRC and normal tissues. Additionally, protein expression in 90 pairs of CRC and paracancerous tissues was analyzed by western blotting and immunohistochemistry (IHC). The results showed that SEPT2 was highly expressed in CRC tissues at the mRNA and protein levels. SEPT2 expression quantified by IHC was associated with lymph node metastasis, the degree of differentiation and TNM staging. Increased SEPT2 wass associated with reduced overall survival (OS) according to Kaplan-Meier analysis. COX proportional hazard analysis indicated that SEPT2 was an independent factor that influenced the OS of patients with CRC. Therefore, SEPT2 was associated with the occurrence, progression and prognosis of CRC and thus, may be a marker and prognostic indicator of CRC.

Introduction

Between 1930 and 2014 in the United States, colorectal cancer (CRC) was the third most common cancer in both sexes and the second and third major cause of death in men and women, respectively (1-4). In China, CRC is one of the five most commonly diagnosed types of cancer resulting in mortality. The incidence patterns for CRC between males and females are similar, with rates declining by 3% per year between 2004 and 2013 due to early detection and treatment (5). The five-year relative survival rate of patients is 65%, followed by 58% after 10 years, leading to a 7% decline. Early detection and treatment are therefore important as they prolong the survival rate of patients with CRC. CRC has a better prognosis if it is limited to the intestinal wall and the patient is young. Patients with metastases, infiltration or complications have a poor prognosis (4,6).

Septins are proteins associated with GTP binding that are evolutionarily and structurally associated with the RAS oncogenes and participate in membrane interactions (7,8). Septins control actin remodeling during cell migration and thus may contribute to metastatic cancer cell dissemination and invasion; they also include highly conserved structural domains involved in various cellular processes such as cytoskeletal organization, membrane dynamics and cytokinesis (9-12). In addition, many septins are associated with a variety of neurodegenerative and hemorrhagic human diseases (13). Septins are highly expressed in liver cancer, lung cancer, brain tumors, breast cancer (BC), gastric cancer and other types of cancer (10,13-17). SEPT2 is part of the SEPT family and is also known as DIFF6, NEDD5, Pnutl3 and hNedd5. Elevated expression of SEPT2 has been reported in many types of cancer, including acute myeloid leukemia, pancreatic cancer, biliary tract cancer (BTC), Hodgkin's lymphoma and gastric cancer (16,18-21). In addition, SEPT2 may play a role in breast cancer cells by increasing cell migration and invasion through the MEK/ERK pathway (22). The expression of SEPT2 is upregulated in hepatoma carcinoma cells (HCCs), and the phosphorylation of SEPT2 stimulates the proliferation of HCCs (23). SEPT2 influences gene expression and proliferation in a BTC cell line (19). Additionally, suppression of SEPT2 may arrest glioblastoma (GBM) cells in the S phase and reduce viability by regulating the p53/p21 and MEK/ERK signaling pathways (24). The role of SEPT2 in maintaining the shape of Hodgkin and multinucleated Reed-Sternberg (H/RS) cells and the fact that SEPT2 is incorporated into the cytoskeleton and influences cytoskeleton structure have significant effects on H/RS cell differentiation (25). SEPT2 may promote angiogenesis and tumor growth through cancer-associated fibroblasts (CAF) (26). Overexpression of SEPT2 results in

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Abbreviations: SEPT2, septin 2; CRC, colorectal cancer; IHC, immunohistochemistry; OS, overall survival; IRS, immunoreactivity score

Key words: septin 2, colorectal cancer, tumorigenesis, prognosis, survival, biomarker

cytokinesis failure, aneuploidy, centrosome amplification and multipolar mitoses, which are all frequent in cancer cells (10). A study by Kremer *et al* (27) showed that the expression of SEPT2 was associated with the expression of F-actin, and these two proteins interact and participate in skeletal assembly in CRC cells. In the process of cell migration, the cytoskeletal front end protrudes from a sheet-like structure or filopodia, and maintains a stretch by establishing a new adhesion to the extracellular matrix; then, the cell regulates cell shrinkage by actin and myosin. The cells move forward and finally, the tail of the cell separates from the matrix and retracts, affecting the adhesion, invasion and metastasis of the tumor cells. Therefore, the cytoskeleton serves a very important role in cell migration (27-29).

Although many reports have focused on the function of SEPT2 in tumors, to the best of our knowledge the role of SEPT2 in CRC remains unclear. It has been reported that SEPT9, which is the most homologous to SEPT2 in the SEPT family, is differentially expressed in CRC and normal groups when measured using reverse transcription-quantitative polymerase chain reaction and immunohistochemistry (IHC) (30). Additionally, SEPT9 is involved in the occurrence of CRC based on a DNA methylation assay of the SEPT9 promoter (31,32). SEPT9 levels in peripheral blood can be used as a biomarker for early detection of CRC, with a sensitivity and specificity of up to 90 and 88%, respectively (16,31-34). In the present study, the expression of SEPT2 in clinical CRC specimens was analyzed, and the association of SEPT2 with OS in patients was investigated.

Materials and methods

Oncomine analysis. Oncomine (www.oncomine.org) is a web-based database and data-mining platform aimed at facilitating new discoveries from genome-wide expression analyses, in which exploration for differential expression analyses comparing most major types of cancer with respective normal tissues, as well as clinical-based and pathology-based analyses are available (35,36). Oncomine was used to analyze the individual gene expression levels of SEPT2 mRNA between CRC and adjacent tissues. To reduce the false discovery rate, the following thresholds were selected: 1.5-fold change in gene expression between CRC and normal tissues, P-value <0.001 and top 10% gene rank.

Clinical CRC specimens. The tissue used for western blotting was CRC tissues and adjacent tissues collected from 8 patients with CRC who had undergone surgery at the First Affiliated Hospital of Jinzhou Medical University (Liaoning, China) within a period of 11 days in November 2015. The patients were 52-66 years old, and included 5 males and 3 females. The experiment was reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University, and written informed consent was obtained from patients. Tissue microarray slides containing samples from 90 patients with CRC were purchased from Shanghai Outdo Biotech Co., Ltd. (chip no., HColA180su14). The tissue microarray contained CRC samples and adjacent samples for each patient. The clinicopathological features of the patient population included in the tissue microarray is presented in Table I.

Western blot analysis. Protein was extracted from tissue using RIPA buffer (Beyotime Institute of Biotechnology). Protein concentration was subsequently measured by Bradford assay. Proteins (20 μg) were then separated by SDS-PAGE (12% gels). The resolved proteins were transferred to PVDF membranes. Nonspecific reactivity was blocked with 5% skim milk in Tris-buffered saline with Tween-20 (TBST; 10 mM Tris-HCl, 150 mM NaCl, 0.1% Tween 20) for 1 h at room temperature. Membranes were then incubated overnight at 4˚C with rabbit monoclonal anti-SEPT2 (1:500; Abcam; cat. no. ab179436) and rabbit anti-GAPDH (1:2,000; Wanleibio Co., Ltd.; cat. no. WL03412) antibodies. The membranes were rinsed with TBST and incubated with the goat anti-rabbit immunoglobulin G (IgG) secondary antibody (1:5,000; Wanleibio Co., Ltd.; cat. no. WLA037a) for 1 h at room temperature. The bands were visualized with an LAS4010 imager (GE Healthcare Life Sciences) using ECL-Plus detection reagents (Santa Cruz Biotechnology, Inc.). The densitometric quantification of protein bands was performed with GAPDH as a control using the ImageJ software version 1.8.0 (National Institutes of Health).

IHC. The specimens on the tissue microarray that were only used for IHC were deparaffinized in xylene and sequentially dehydrated in alcohol. Endogenous peroxidase was eliminated by incubation with 3% H_2O_2 for 10 min at room temperature. Non-specific binding was blocked by incubation with 5% bovine serum albumin (Beijing Solarbio Science & Technology Co., Ltd.) in PBS for 30 min at room temperature. The sections were incubated with rabbit-anti-human SEPT2 monoclonal antibody (1:100; Abcam; cat. no. ab179436) without washing at 4˚C overnight in a humidity chamber. Next, the sections were incubated with horseradish peroxidase goat-anti-rabbit IgG (1:200; Wanleibio Co., Ltd.; cat. no. WLA037a) for 1 h at room temperature, and SEPT2 expression was detected using 3,3'-diaminobenzidine. Hematoxylin staining was performed for 1 min at room temperature to analyze the nucleus.

Evaluation of staining. The tissue specimens were viewed separately by two pathologists without prior knowledge of the clinicopathological status of the patients. Immunoreactivity score (IRS) intensity was calculated by multiplying staining score and staining percentage. Staining score was defined as follows: No staining, 0; weak staining, 1; moderate staining, 2; and strong staining, 3. Tissue staining percentage was classified as follows: No positive cell staining, 0; <25% positive cell staining, 1; 25-50% positive cell staining, 2; 50-75% positive cell staining, 3; and >75% positive cell staining, 4. The values for staining score and staining percentage were multiplied to obtain the IRS for each sample. IRS values were stratified as negative (-, 0 score), weakly positive (+, 1-4 score), moderately positive (++, 5-8 score) or strongly positive (+++, 9-12 score). Each sample on the tissue microarray was imaged using a microscope at x200 magnification, and scores were defined for a minimum of 3 non-overlapping areas.

Statistical analysis. Statistical analyses were performed using SPSS 20.0 software (IBM Corp.). Data were presented as the means ± standard deviation. Pearson's χ^2 test was used to compare differences between CRC and adjacent

Table I. Association of SEPT2 immunoreactivity scores and clinicopathological parameters of 90 colorectal cancer patients.

		SEPT2 expression				
Clinicopathological parameter	n	-	+	++	+++	P-value
Sex						0.545
Male	47	0	17	19	11	
Female	43	0	20	16	7	
Age, years						0.892
≥65	44	0	17	18	9	
<65	46	0	20	17	9	
Tumor size, cm						0.321
>5	41	0	18	13	10	
≤5	49	0	18	23	8	
Type						0.325
Apophysis	37	0	19	11	7	
Infiltration/ulcer	53	0	18	24	11	
Differentiation						<0.001[a]
Poor	25	0	2	12	11	
Moderate/well	65	0	35	23	7	
Infiltration depth						0.952
T1/T2	11	0	5	4	2	
T3/T4	79	0	32	31	16	
Lymph node metastasis						0.010[a]
With	29	0	6	13	10	
Without	61	0	31	22	8	
Distant metastasis						0.096
M0	87	0	37	34	16	
M1	3	0	0	2	1	
TNM Staging						0.003[a]
Phase I and II	59	0	31	21	7	
Phase III and IV	31	0	6	14	11	

[a]P<0.05. SEPT2, septin 2; TNM, Tumor Node Metastasis.

tissues and to compare SEPT2 IRS and clinicopathological parameters of CRC tissues. Survival curves were estimated using the Kaplan-Meier method, and differences in survival distributions were evaluated using the log-rank test. Cox proportional-hazard model was used to identify factors that may have a significant influence on survival. Expression levels of SEPT2 in a CRC and normal tissue dataset from the Oncomine database was compared using a permutation test with t statistics and singular value decomposition. In Fig. 1C, the statistical analysis was performed using one-way analysis of variance. Multiple comparison between the groups was performed using Bonferroni or Dunnett (2-sided) test. P<0.05 was considered to indicate a statistically significant difference.

Results

mRNA expression of SEPT2 in CRC tissue samples is significantly higher compared with that in adjacent tissues. The Oncomine database reported six databases that presented SEPT2 expression comparison between CRC and adjacent tissues (Fig. 1A). These databases were from previous studies published in PLOS One, Clin Exp Metastasis, etc. (37,38). The expression of SEPT2 in CRC tissues was increased in all 6 groups. To reduce the false discovery rate, the following thresholds were selected: 1.5-fold change, P<0.001 and top 10% gene rank. SEPT2 expression in 3 groups with 2 datasets (Fig. 1A; number 3, 5 and 6 boxes) are presented in Fig. 1B and C. In The Cancer Genome Atlas (TCGA) database, the relative expression of SEPT2 in colon tissues was -0.434, whereas SEPT2 relative values in the rectum, colon mucinous adenocarcinoma and colon adenocarcinoma were -0.346, 0.384 and 0.169, respectively. In the Skrzypczak 2 database, SEPT2 relative expression in colon and colon carcinoma tissues were 4.241 and 5.052.

Protein expression of SEPT2 in CRC tissues is significantly higher compared with that in adjacent tissues. Analysis of IHC results revealed that SEPT2 staining mainly localized in

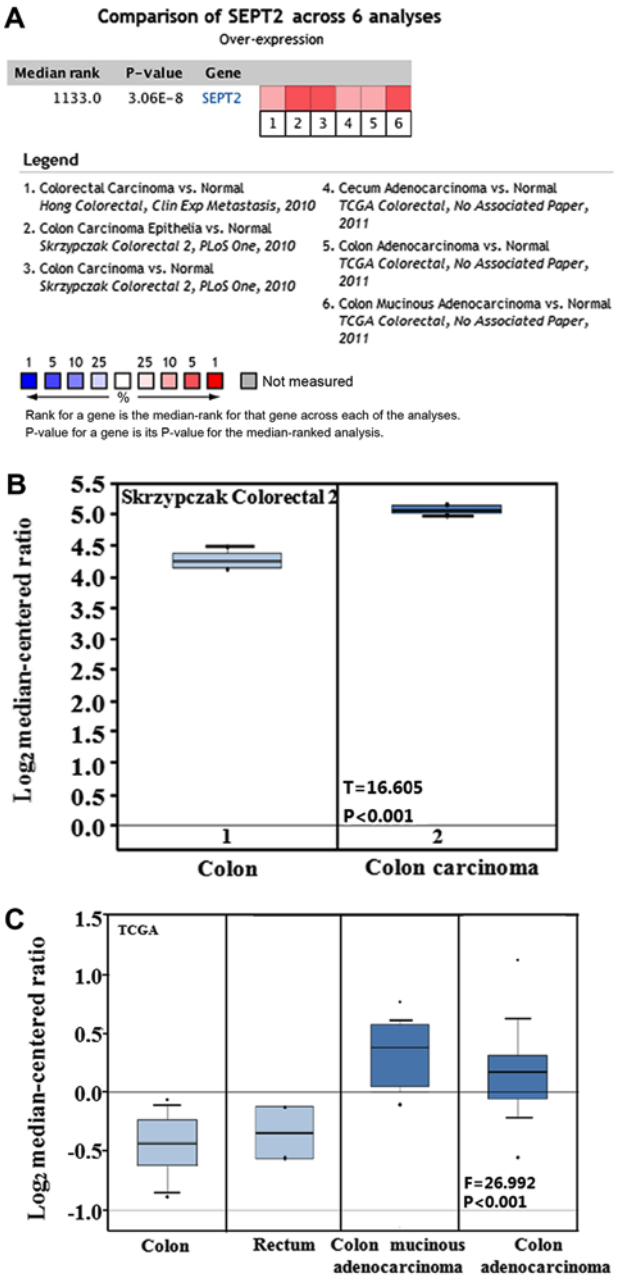

Figure 1. Gene expression levels of SEPT2 mRNA in datasets containing CRC patient samples from the Oncomine database. (A) Studies on the expression of SEPT2 in CRC are labeled 1-6; red represents increased expression, with deeper colors indicating higher expression. (B) Expression of SEPT2 in colon and colon carcinoma in the Skrzypczak Colorectal 2 dataset (P<0.001). (C) Differential expression of SEPT2 in colon, rectal, colon mucinous adenocarcinoma and colon adenocarcinoma tissues in the TCGA dataset (P<0.001). SEPT2, septin 2; CRC, colorectal cancer; TCGA, The Cancer Genome Atlas.

the cytoplasm and cytoskeleton of CRC cells (Fig. 2). Western blot analysis and IHC results demonstrated that expression of SEPT2 in tumor tissues was higher compared with that in adjacent tissues (Fig. 3 and Table II). The SEPT2 staining score according to Fig. 2 is divided into negative (-), weakly positive (+), moderately positive (++) and strongly positive (+++) categories. Among the 90 samples of CRC tissues, 18 were strongly positive (+++), 35 were moderately positive (++), 37 were weakly positive (+) and 0 were negative (-). However, in the paracancerous tissue samples, there were no strong positive or moderately positive results, weak positive results in 31 cases and negative results in 59 cases (Table II). The expression patterns of SEPT2 in CRC and paracancerous tissues were significantly different (P<0.001) and showed that the expression of SEPT2 was higher in CRC tissues than that in paracancerous tissues.

Association between SEPT2 staining intensity and clinicopathological data. Based on the IRS, the association between the staining intensity of SEPT2 in CRC and the clinicopathological features of patients was analyzed (Table I). The staining intensity of SEPT2 was associated with the metastasis of CRC, as higher SEPT2 staining was more frequent in CRC samples with lymph node metastasis compared with samples with no metastasis (P<0.05). In addition, the staining intensity of SEPT2 was associated with the differentiation degree of tumor tissue, as samples with poor tissue differentiation had higher IRS more frequently than samples were moderately or well differentiated (P<0.001). IRS was also associated with TNM staging (P<0.05). There were no significant associations between SEPT2 IRS and sex, age, tumor size, cancer type and infiltration depth. Analysis of the frequency of distant metastasis was hampered as there were only three positive cases and therefore, the statistical analysis was not convincing (Table I).

Association between SEPT2 expression and OS. Based on Kaplan-Meier survival curves, analysis was performed to compare the OS of patients with weak, moderate and strong SEPT2 expression based on IRS values (Fig. 4). Higher IRS values were significantly associated with reduced OS rates (P<0.001). The median OS rate for patients with strongly positive (+++) results was 20.0 months, while the median OS rates for those with moderately positive (++) and weakly positive (+) results were 71.0 and 72.5 months, respectively. The corresponding OS 25 and 75th percentiles were 11.0, 19.8 and 70.0 for moderately positive patients, and 40.5, 75.0 and 75.0 months for weakly positive patients.

To ascertain whether SEPT2 was an independent prognostic factor, univariate and multivariate analyses were performed. Cox analysis demonstrated that SEPT2 was an independent factor that affected patient OS (P<0.001; Table III). Age, TNM stage and other clinical features were incorporated into the Cox regression model. Moderately positive and strongly positive IRS values were associated with a higher risk of death compared with that of patients with weakly positive, with a hazard ratio (HR) of 0.031 and a 95% confidence interval (CI) of 0.007-0.143 for moderate and HR of 0.317 (95% CI, 0.151-0.666) for strongly positive groups (Table III).

Discussion

The present study analyzed the expression patterns of SEPT2 in CRC and adjacent tissues. The Oncomine database showed that mRNA expression of SEPT2 in cancerous tissues was increased compared with that of normal tissues. IHC revealed that protein expression of SEPT2 in CRC tissues was significantly higher than that in paracancerous tissues. The results of the current study are consistent with those of a previous report (17) found that the probability of observing a mutation in SEPT2 in colorectal tumors was significantly higher

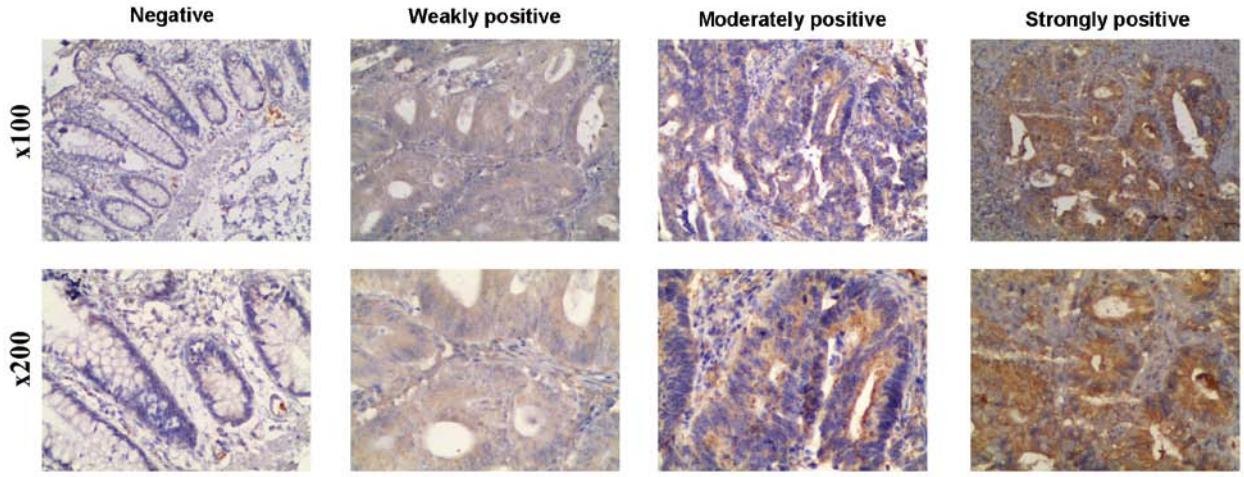

Figure 2. Analysis of SEPT2 protein expression based on immunohistochemistry. SEPT2 staining mainly localized in the cytoplasm and cytoskeleton of colorectal cancer cells. Samples with negative (-), weakly positive (+), moderately positive (++) and strongly positive (+++) staining are presented at x100 and x200 magnification. SEPT2, septin 2.

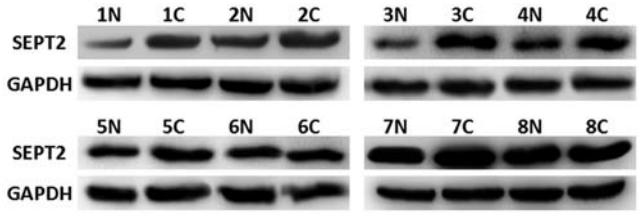

Figure 3. Western blot analysis of SEPT2 protein expression in colorectal cancer and adjacent normal tissue samples from 8 patients. SEPT2, septin 2; N, normal tissue; C, colorectal cancer tissue.

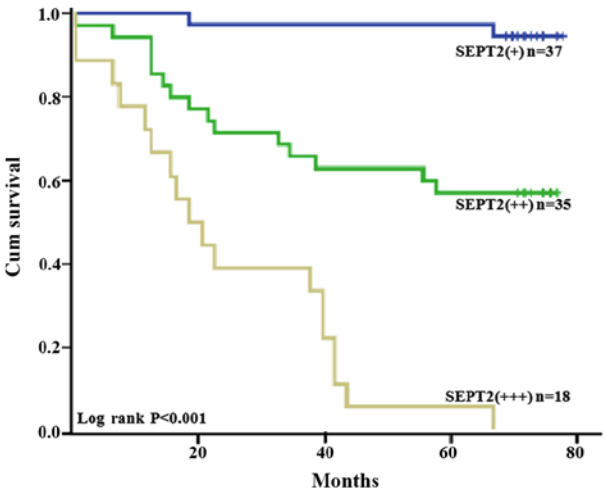

Figure 4. Kaplan-Meier curve analysis of OS rate in patients with colorectal cancer based on SEPT2 expression. The OS rate was highest in patients with weakly positive expression (+, n=37), but decreased for those with moderately positive expression (++; n=35) and was lowest for those with strongly positive expression (+++; n=18) (log-rank P<0.001). SEPT2, septin 2; OS, overall survival.

compared with that of finding a mutation in other family members in their corresponding tumor type (17). Other studies have also identified increased tumor tissue expression of SEPT2 compared with that in normal tissue (20,21,23,39). In primary astrocytic tumors, expression of SEPT2 was higher compared with that observed in the frontal and temporal lobes, as well as the cerebellum according to densitometry (39). In gastric cancer, high SEPT2 expression was observed in tumors compared to non-tumor tissue (20). SEPT2 protein was also found to be highly expressed in hepatoma tissues, but lowly or not expressed in adjacent normal liver tissues (23). SEPT2 has also been shown to promote the proliferation of HCC cells and inhibit cell apoptosis, indicating that it may function as an oncogene (21).

It has been reported that SEPT2 influences the invasion and metastasis of many types of tumor cells (19,21,40). Overexpression of SEPT2 accelerates proliferation in BTC cells (19). Upregulation of SEPT2 in HepG2, a human hepatoma cell line, leads to a significant decrease in apoptosis (21). SEPT2 increases BC cell migration and invasion through the MEK/ERK pathway (40). Based on these experimental results, it was hypothesized that patients with high expression of SEPT2 would be more likely to have metastatic events. Patients were divided into groups based on having distant metastasis, lymph node metastasis and no metastasis. Compared with patients without lymph node metastasis, those with metastases were more likely to have higher expression of SEPT2. Higher SEPT2 expression in CRC tissues of patients with metastasis indicates that SEPT2 may be associated with migration of CRC cells. The association between SEPT2 expression and prognosis was also analyzed, and it was revealed higher SEPT2 expression was associated with reduced OS rates in patients, and therefore a poorer prognosis. It was demonstrated, using the Cox proportional hazard regression model, that SEPT2 expression based on IRS values was an independent factor influencing the OS rate of patients.

The present study did not investigate what pathways are altered by SEPT2 expression in CRC tissues, and how these impact the regulation and metastasis of CRC. Previous studies have examined the role of SEPT2 in human BC cells (MDA-MB-231 and MCF7 cells) and GBM cells (24,40,41). A study by Zhang *et al* (40) confirmed that SEPT2 depletion impaired ERK1/2 phosphorylation in MCF7 cells. The activity

Table II. SEPT2 expression in cancer and adjacent tissues.

		SEPT2 expression					
Groups	n	-	+	++	+++	Positive rate, %	P-value
Paracancerous	90	59	31	0	0	34.4	<0.001[a]
Cancer	90	0	37	35	18	100.0	

[a]P<0.05. SEPT2, septin 2.

Table III. Association of SEPT2 expression and clinicopathological parameters with overall survival.

Feature	Unadjusted HR[a] (95% CI)	P-value	Adjusted HR[b] (95% CI)	P-value
SEPT2				
1 score	-	-	-	-
2 score	18.029 (8.143-27.914)	0.001[c]	0.031 (0.007-0.143)	<0.001[c]
3 score	45.944 (36.513-55.376)	<0.001[c]	0.317 (0.151-0.666)	0.002[c]
Sex				
Male	-1.859 (-12.813-9.095)	0.737	0.626 (0.296-1.322)	0.219
Female				
Age, years				
≥65	-0.335 (-12.805-9.115)	0.739	1.274 (0.642-2.529)	0.489
<65				
Differentiation				
Poor	-	-	-	-
Moderate	-14.484 (-28.599-0.369)	0.045[c]	1.186 (0.510-2.761)	0.692
Well	-8.630 (-28.916-11.656)	0.390	1.413 (0.427-4.681)	0.571
Infiltration depth				
T1/T2	10.170 (-6.162-26.503)	0.203	11.487 (1.003-131.598)	0.050[c]
T3/T4				
Lymph node metastasis				
With	-21.734 (-33.637-9.832)	0.001[c]	1.658 (0.133-20.644)	0.694
Without				
Distant metastasis				
M0	-37.724 (-44.668-0.781)	<0.001[c]	0.639 (0.077-5.331)	0.679
M1				
TNM Stage				
I	-	-	-	-
II	8.143 (-1.480-17.765)	0.094	0.008 (0.000-0.248)	0.006[c]
III	29.071 (16.217-41.926)	<0.001[c]	0.328 (0.084-1.281)	0.109
IV	51.667 (37.213-66.120)	<0.001[c]	0.560 (0.154-2.043)	0.380

[a]HR in univariate model. [b]HR in multivariable models. [c]P<0.05. HR, hazard ratio; CI, confidence interval; SEPT2, septin 2; TNM, Tumor Node Metastasis.

status of upstream kinases was also examined, and it was found that MEK1/2, but not Raf was inactivated in SEPT2-depleted cells, thus increasing BC cell proliferation, migration and invasion. Additionally, this study also demonstrated that the inhibition of septin activity via forchlorfenuron, an inhibitor of septin proteins, causes suppression of ERK1/2 phosphorylation without any influencing SEPT2 expression (40). A study by Xu *et al* (24) demonstrated similar findings in GBM cells; suppression of SEPT2 impaired MEK1/2 phosphorylation and the phosphorylation of downstream ERK1/2. The expression of cell cycle- and apoptosis-regulating proteins p53 and p21 were also examined, and following p53 accumulation, the protein level of p21 was also upregulated (41). Thus, it was concluded that SEPT2 may impact two parallel pathways (p53/p21 and

MEK/ERK) in the regulation of GBM cells and thus contribute to GBM cell proliferation, migration, invasion and tumor formation *in vivo* (24,41). Septins are cytoskeleton proteins that may enhance CRC migration and invasion by interacting with actin, tubulin and myosin. In addition, the KEGG network was used (http://www.genome.jp), and the query results show that the SEPT2 protein is related to the Bacterial Invasion of Epithelial Cells pathway, which is involved in the regulation of the actin cytoskeleton (data not shown).

In addition to an association with the occurrence and metastasis of tumors, there have been many reports describing other functions of SEPT2 (10,19,23,24,26,35,40). Using aphidicolin to arrest cells in the G2-M phase, SEPT2 was demonstrated to be a cell cycle-regulated protein that is essential for the process of cytokinesis in human astrocytoma cells and that it localizes to the actin-based contractile ring during cytokinesis (24). The expression of a dominant-negative mutant SEPT2 gene inhibits cytokinesis and results in multinucleated cells. Taken together, these observations suggest a conserved requirement for SEPT2 in U373 astrocytoma cell division. To date, only one mammalian septin, SEPT2, has been shown to play a role in cytokinesis (39). SEPT2 may promote angiogenesis and tumor growth by impacting cancer-associated fibroblasts (26). The overexpression of SEPT2 results in cytokinesis failure and causes aneuploidy, centrosome amplification and multipolar mitoses, which are frequent in cancer cells (10). The expression of SEPT2 is upregulated in HCCs, and the phosphorylation of SEPT2 stimulates the proliferation of HCCs (23).

SEPT2 may be a potential prognostic marker and therapeutic target for patients with CRC. This possibility provides the basis for the early diagnosis and treatment of CRC patients. However, the present study does not determine the biological function and signaling events of SEPT2 regulation in CRC, which will be the basis of future studies.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available in the Oncomine repository (www.oncomine.org) and the results from the datasets analysis in this study are available from the corresponding author on reasonable request.

Authors' contributions

HH, CY, ZK and JL designed the study. HH, JL and MX performed the experiments. HH and YG analyzed the data. HH and ZK drafted the manuscript. HH, JL and MX revised the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The relevant experiments of this subject have been approved by the Ethics Committee of the First Affiliated Hospital of Jinzhou Medical University and the Shanghai Outdo Biotech Ethics Committee. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that there have no competing interests.

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