

# Secreted protein acidic and rich in cysteine expression in human colorectal cancer predicts postoperative prognosis

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**Abstract.** – **OBJECTIVE:** Secreted protein acidic and rich in cysteine (SPARC) is an extracellular matrix glycoprotein involved in cell proliferation, migration and angiogenesis. The aim of this study was to assess its expression in colorectal cancer, see whether and how it correlates with clinicopathological features, and evaluate its potential prognostic significance.

**PATIENTS AND METHODS:** SPARC expression was detected by microarrays containing 847 immunohistochemically stained specimens, and further correlated with the clinicopathological and prognostic data. The prognostic significance of its expression was assessed using Kaplan-Meier survival with log-rank tests. Multivariate regression utilizing Cox's proportional hazard model was used to evaluate prognostic factors.

**RESULTS:** SPARC expression in the normal colorectal mucosa and colorectal cancer tissue was significantly different ( $p < 0.001$ ). Low SPARC expression was found to be associated with poor prognosis, and it was unfavorably correlated with overall survival and disease-free survival in colorectal cancer patients. In addition, SPARC expression in surrounding mesenchymal and stromal cells, bowel wall invasion, lymph node metastasis, and distant metastasis were independent prognostic factors for overall survival and disease-free survival.

**CONCLUSIONS:** Reduced expression of SPARC in colorectal cancer tissue is associated with poor prognosis and aggressive clinicopathological features. Therefore, SPARC expression could potentially be used as a prognostic predictor for colorectal cancer patients.

*Key Words:*

Colorectal cancer, Prognosis, Tissue microarray, SPARC.

## Introduction

Colorectal cancer (CRC) is the third most common malignant cancer in men and the second in women worldwide<sup>1</sup>. Surgery is the primary method for the treatment of CRC patients. However, a significant proportion of CRC patients developed postoperative recurrences and/or local or distant metastases, which often hinders optimal recovery in these patients, who had to receive postoperative chemo- and/or radiotherapy<sup>2,3</sup>. Clinicopathological tumor-node-metastasis (TNM) staging is the current gold standard for determining postoperative treatment and prognostication for CRC patients<sup>4</sup>. However, patients with the same TNM stage often have distinct postoperative prognoses, especially those with TNM stage II CRC. Additionally, why not all patients respond to chemo- and/or radiotherapy or have a satisfactory clinical outcome remains poorly understood<sup>5</sup>. Therefore, it is important to understand the mechanism of treatment failure and find out a valuable prognostic/predictive marker in the management of CRC patients. Certain genetic changes may play a prognostic role in CRC. However, only the prognostic value of KRAS mutations in patients with metastatic CRC and their response to cetuximab therapy has translated into clinical relevance<sup>6,7</sup>. Furthermore, BRAF mutations, chromosomal and microsatellite instability has been associated with the clinical outcome of CRC patients<sup>8,9</sup>. Despite great efforts to identify molecular markers that may allow the development of individualized CRC treatment, none of these markers are routinely

used in clinical practice. The aim of this study was to identify a potential diagnostic marker that could be used to predict the prognosis of CRC patients after curative surgery.

SPARC, an acronym for “secreted protein acidic and rich in cysteine”, also known as osteonectin or BM-40, belongs to the matricellular family of secreted proteins<sup>10</sup>. It was initially identified as osteonectin by Termine et al<sup>11</sup> as a bone-specific phosphoprotein that binds to collagen fibrils and hydroxyapatite at distinct sites. Later the same protein was identified as a serum albumin-binding glycoprotein secreted by endothelial cells<sup>12</sup>. It is known to play important roles in tissue remodeling, wound repair, cell migration and angiogenesis. In healthy tissues, SPARC production is largely restricted to areas undergoing tissue repair<sup>13,14</sup>, whereas in malignant tumors, SPARC expression is associated with disease progression and tumor growth<sup>15,16</sup>. Recently, SPARC expression has been found to associate with the prognosis of patients with invasive pancreatic and gastric cancers<sup>17,18</sup>. However, its value as a prognostic marker and its correlation with clinicopathology has been rarely studied in CRC patients. In the present study, tissue microarray (TMA) immunohistochemistry was used to assess SPARC expression in CRC patients. The main purpose of our study was to examine SPARC expression in surgically resected CRC specimens, explore the possible correlation between SPARC expression and clinicopathological variables, and determine the prognostic or predictive value of SPARC expression for CRC.

## Patients and Methods

### Patients Selection

This prospective tissue microarray-based study involved 847 subjects, including 56 patients with normal mucosa who underwent surgery for hemorrhoids, 51 patients with colon polyps, and 740 (stage I-IV) CRC patients who received colorectal surgery between 1999 and 2009 at Changhai Hospital of the Second Military Medical University (Shanghai, China). Patient eligibility criteria were as follows: (1) patients with a definitive pathological diagnosis of CRC or normal controls; (2) patients receiving no anti-cancer treatment prior to surgery; (3) patients undergoing curative resection with the cut surface being free of cancer as confirmed by pathology; (4) patients with availability of suitable paraffin-embedded

tissues; and (5) patients with complete clinicopathological and follow-up data. The study protocol was approved by the Medical Ethics Board of Changhai Hospital, and informed consent was obtained from all the patients. The ethical approval number is CHEC2011-146. The clinicopathological characteristics of the patients were recorded, including sex, age, tumor stage, bowel wall invasion, lymph node metastasis, distant metastasis, survival, carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) serum levels. Tumor stage was determined according to the 7<sup>th</sup> edition of TNM classification system of the American Joint Committee on Cancer (AJCC) for primary CRC. In our follow-up study, data from all patients were censored from the date of surgery to the date of the last follow-up visit (December 31, 2011) or death. Disease-free survival (DFS) was calculated from the date of surgery to the development of local recurrence or distant metastasis. Overall survival (OS) time was defined as the time from the date of surgery to the confirmed date of death or date of the last follow-up visit for deceased and surviving patients, respectively.

### Postoperative Follow-up of the Patients

Patients were evaluated every 3 months during the first postoperative year, every 6 months in the subsequent year, and once annually thereafter. The last follow-up date was December 31, 2011. During the follow-up period, the date of death and/or recurrence and the cause of death were recorded simultaneously. All patients were monitored for possible recurrence by detection of CEA and CA19-9 levels, colonoscopy, abdominal ultrasonography, and chest radiography. If recurrence was suspected, computed tomography (CT) of the abdomen, magnetic resonance imaging (MRI), or positron emission tomography (PET) was performed for confirmation. Of the 2618 CRC patients with complete clinical data, 810 patients were lost to follow-up because of changes in contact details; 897 patients lacked suitable pathological tissues; 150 patients on neoadjuvant therapy were excluded; 19 patients were not analyzed because of unfitness for TMA analysis; and 2 patients were lost in immunohistochemistry staining<sup>19</sup>.

### TMA Analysis and Immunohistochemistry

Paraffin sections used in this study were obtained from the Department of Pathology of Changhai Hospital. The samples were initially

reviewed by hematoxylin and eosin (H&E) staining, and representative areas were pre-marked on the paraffin blocks, avoiding necrotic and hemorrhagic areas. The formaldehyde fixed-paraffin embedded (FFPE) tissues corresponding to the selected histological sections were sampled from these marked regions using a specialized manual arraying instrument (Model MTA1, Beecher Instruments, Sun Prairie, WI, USA). With this device, 1.5 mm-diameter cylinders were obtained for each sample to ensure reproducibility and homogenous staining of the slides. The slides were then aligned in pre-arranged sequences according to TNM stages. The core samples were then placed on an empty paraffin block. After arraying was completed, the TMA blocks were completely sliced to 4  $\mu$ m sections, totaling 80 sections from each block. Six different TMA blocks were constructed, with each block containing a total of 160 specimens. Finally, 847 samples were aligned in 6 different TMA blocks.

Immunohistochemistry analysis was performed using rabbit anti-human SPARC monoclonal antibody (D10F10, dilution 1:200; Cell Signaling Technology, Danvers, MA, USA). Immunohistochemical staining of TMAs was carried out as follows: sections were deparaffinized in xylene, rehydrated, and washed in phosphate buffered saline (PBS) for 10, 5, and 10 min. After application of endogenous peroxidase for 10 min and antigen retrieval at 98°C for 25 min, the sections were pre-incubated with blocking serum for 30 min and then incubated with the SPARC monoclonal antibody at 4°C overnight. Subsequently, the sections were thoroughly rinsed with PBS, incubated with secondary antibodies, and treated with horseradish peroxidase-conjugated streptavidin. The immunohistochemical reaction was visualized using 3,3'-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. Specimens were not used if there was insufficient tumor tissue within the core, artifactual distortion of the tissue, or high background staining.

#### **Quantification of SPARC Expression by Immunohistochemistry**

The density of SPARC-positive staining was evaluated independently by two pathologists who were blinded to the patient characteristics using a Leica DMI 3000 microscope (magnification of  $\times 200$ ). SPARC was mainly expressed in the cytoplasm and stroma. SPARC expression was estimated according to the percentage and intensity

of the stained tumor cells<sup>20-22</sup>. Each photograph was evaluated for positive SPARC staining in the cancer and stromal cells. The staining intensity was scored as follows: 0 indicates negative staining; 1 indicates weak staining (light yellow); 2 indicates moderate staining (yellowish brown); and 3 indicates strong staining, (brown). The proportion of positive cells was then scored: 0 indicates 0-4% positive cells; 1 indicates 5-24% positive cells; 2 indicates 25-49% positive cells; 3 indicates 50-74% positive cells; and 4 indicates 75-100% positive cells. The final score for each specimen was obtained by multiplying the scores of staining intensity and the percentage of positive cells.

For statistical purposes, specimens were divided into four grades according to their overall scores: 0 indicates absent expression (-); 1-4 indicates weak expression (+); 5-8 indicates moderate expression (++); and 9-12 indicates strong expression (+++). For statistical convenience, specimens were further divided into two grades according to their overall scores: absent/low expression (- and +) and high expression (++ and +++)<sup>23</sup>. All samples were anonymized and independently scored by two investigators. In case of disagreement, the sections were re-examined until a final consensus was reached.

#### **Statistical Analysis**

Correlations between SPARC expression and the clinicopathological features were analyzed by Chi-square test or Fisher's exact test. For the analysis of the training set, the survival curves were estimated using the Kaplan-Meier method and compared using the log-rank test. To determine the independence of clinicopathological variables in predicting an individual's risk of survival, the validation set was analyzed using univariate analysis, followed by multivariate analysis in a Cox proportional-hazards model for prognostic predictors. All calculations were two-sided and performed using SPSS statistical package version 17.0 (SPSS, Chicago, IL, USA).  $p < 0.05$  was considered statistically significant.

## **Results**

### **Expression Patterns of SPARC in Colorectal Tissues**

SPARC was predominantly localized in the cytoplasm and was detected in cancer cells and the mesenchymal and stromal cells (MSC) of



CRC (Figure 1). SPARC expression in primary tumors in MSC and cancer cells was significantly different from that in the normal mucosa and adenoma ( $p < 0.001$  for both). The percentage of high-level SPARC expression in MSC was 3.6%, 7.8%, 44.2%, 54.0%, 35.7%, and 23.6% in normal mucosa, adenoma, stage I CRC, stage II CRC, stage III CRC, and stage IV CRC, respectively. In addition, the percentage of high-level SPARC expression in cancer cells was 3.6%, 3.9%, 23.1%, 50.5%, 37.0%, and 25.5% in normal mucosa, adenoma, stage I CRC, stage II CRC, stage III CRC, and stage IV CRC, respectively.

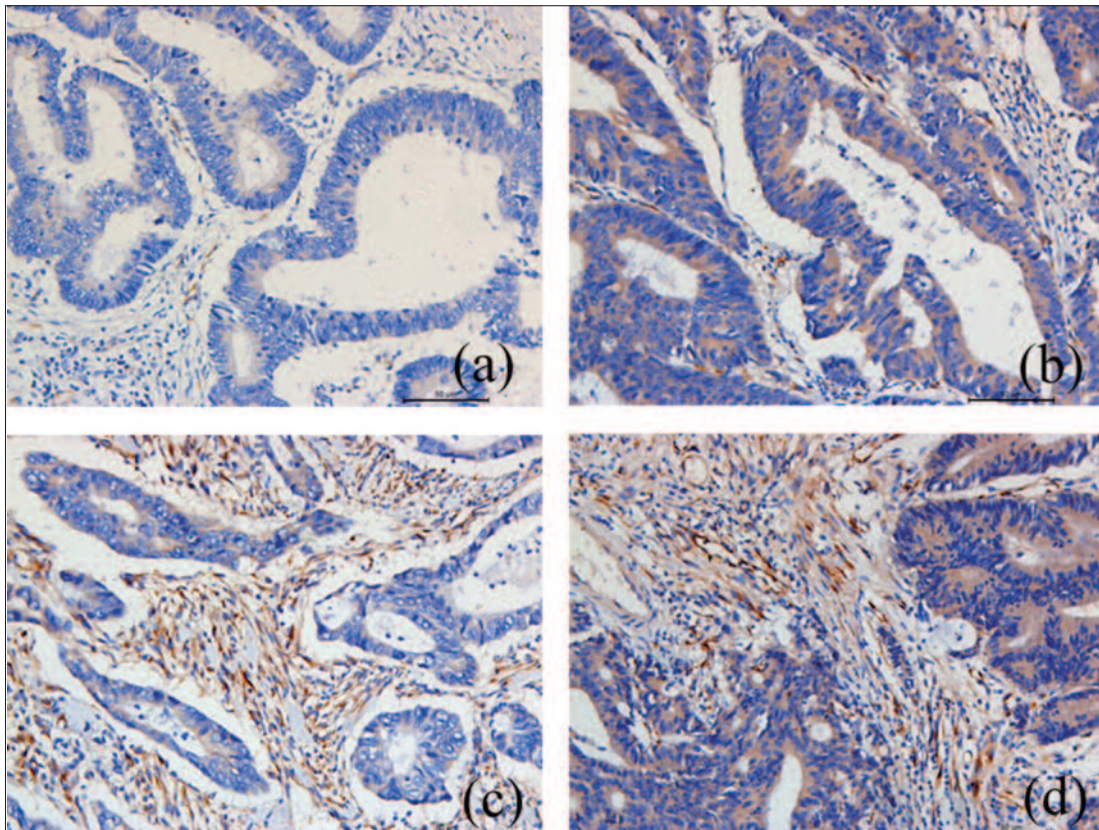
#### **Correlations Between SPARC Expression and the Clinicopathological Features**

To evaluate the correlation between SPARC expression and tumor biology, correlations of SPARC expression in MSC and cancer cells with the clinicopathologic features (sex, age, bowel wall invasion, lymph node metastasis, distant metastasis, CEA and CA19-9 serum levels) were

evaluated. Patients with low SPARC expression in MSC and cancer cells were more likely to exhibit aggressive clinicopathological features including lymph node metastasis, distant metastasis, high CEA and CA19-9 levels ( $p < 0.05$  for all features). Details are shown in Table I.

#### **Correlations Between SPARC Expression and Postoperative Survivals**

The median OS of the survivors was 51.9 months (range 4-122 months). Kaplan-Meier analysis with the log-rank test was used to evaluate the effect of SPARC expression on survival. The cutoff point of high SPARC expression was 5-12 scores of immunohistochemistry. Patients with high SPARC expression in MSC and cancer cells had a significantly longer DFS and OS than those with low SPARC expression (Figure 2). The 5-year survival rate was significantly higher in patients with a high SPARC expression in cancer cells ( $n = 302$ , 89.5%) than that in patients with a low expression ( $n = 438$ , 81.7%) ( $p = 0.005$ ). In addition, the 5 year survival rate was



**Figure 1.** Immunohistochemical staining for SPARC in colon cancer and MSC cells. Positive cells were stained brown. *(a)* The absence of expression in cancer and MSC cells. *(b)* High expression in cancer cells and low expression in MSC cells. *(c)*, High expression in MSC cells and low expression in cancer cells. *(d)* Both high expressions in cancer and MSC cells. Magnification,  $\times$

**Table I.** Correlations between SPARC expression and clinicopathological characteristics of CRC patients.

Characteristics	Cancer cells (%)		<i>p</i> value	MSC cells (%)		<i>p</i> value
	Low	High		Low	High	
Sex (n)			0.626*			0.644*
Male	253 (34.2%)	169 (22.8%)		237 (32.0%)	185 (25.0%)	
Female	185 (25.0%)	133 (18.0%)		184 (24.9%)	134 (18.1%)	
Age (years)			0.068*			0.084*
< 60	230 (31.1%)	138 (18.6%)		221 (29.9%)	147 (19.9%)	
≥ 60	208 (28.1%)	164 (22.2%)		200 (27.0%)	172 (23.2%)	
Bowel wall invasion			0.129**			0.769**
T1	8 (1.1%)	0 (0%)		5 (0.7%)	3 (0.4%)	
T2	47 (6.4%)	32 (4.3%)		42 (5.7%)	37 (5.0%)	
T3	379 (51.2%)	267 (36.1%)		369 (49.9%)	277 (37.4%)	
T4	4 (0.5%)	3 (0.4%)		5 (0.7%)	2 (0.3%)	
Lymph node metastasis			0.026*			< 0.001*
N0	202 (27.3%)	169 (22.8%)		178 (24.1%)	193 (26.1%)	
N1	150 (20.3%)	89 (12.0%)		154 (20.8%)	85 (11.5%)	
N2	86 (11.6%)	44 (5.9%)		89 (12.0%)	41 (5.5%)	
Distant metastasis			0.016*			0.002*
M0	397 (53.6%)	288 (38.9%)		379 (51.2%)	306 (41.4%)	
M1	41 (5.5%)	14 (1.9%)		42 (5.7%)	13 (1.8%)	
Serum CEA			0.032*			< 0.001*
< 5 ng/mL	254 (34.3%)	199 (26.9%)		227 (30.7%)	226 (30.5%)	
≥ 5 ng/mL	184 (24.9%)	103 (13.9%)		194 (26.2%)	93 (12.6%)	
Serum CA19-9			0.003*			0.004*
< 37 U/mL	351 (47.4%)	267 (36.1%)		337 (45.5%)	281 (38.0%)	
≥ 37 U/mL	87 (11.8%)	35 (4.7%)		84 (11.4%)	38 (5.1%)	

\*Chi-square test; \*\*Fisher’s exact test. *Abbreviations:* SPARC: secreted protein acidic and rich in cysteine, CRC: colorectal cancer, MSC: the mesenchymal and stromal cells, CEA: carcinoembryonic antigen, CA19-9: carbohydrate antigen19-9.

significantly higher in patients with a high SPARC expression in MSC (n = 319, 91.6%) than that in patients with a low expression (n = 421, 79.8%) (*p* < 0.001).

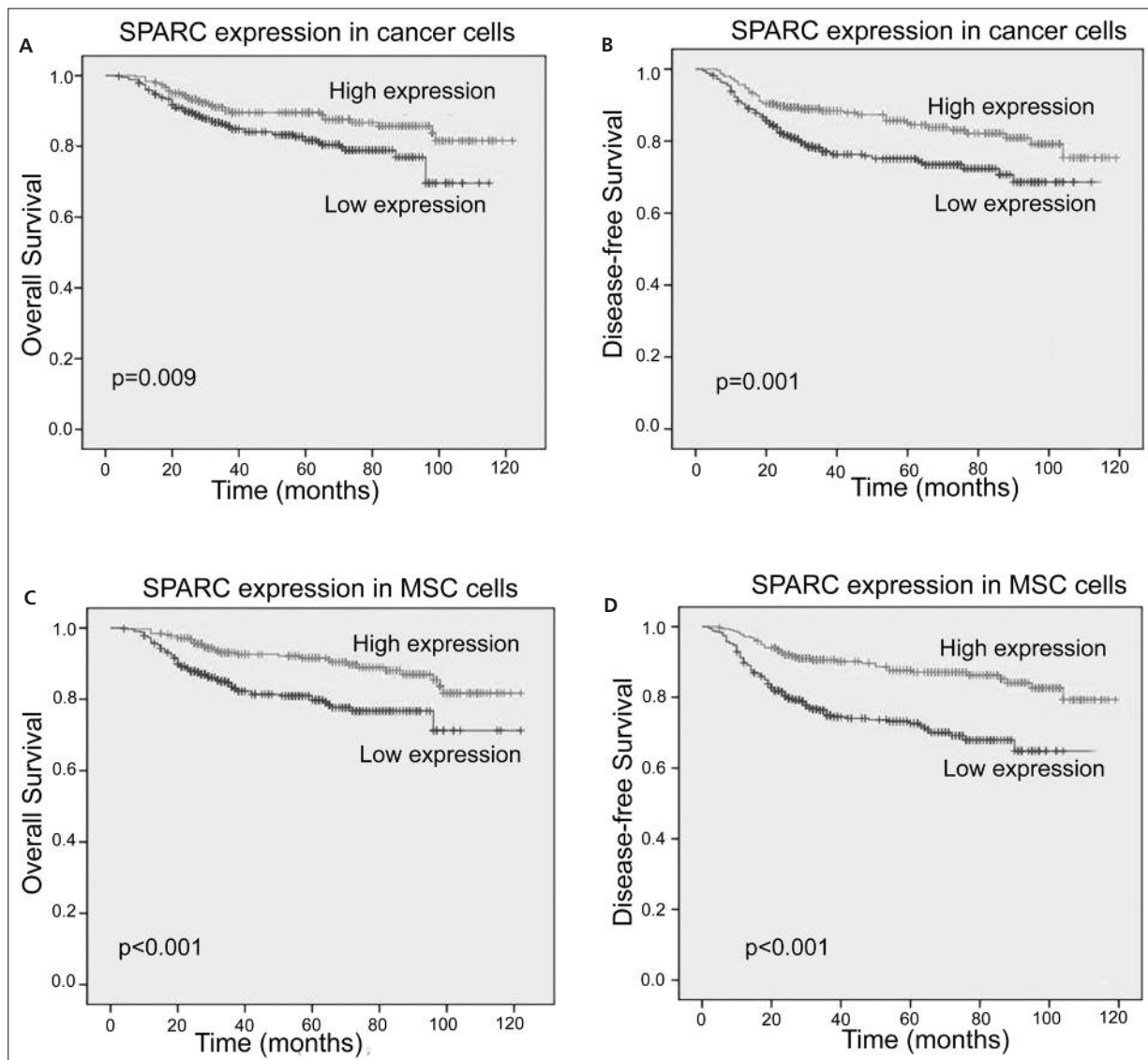
**Univariate and Multivariate Analysis of Factors for the Prediction of the Survivals**

A total of seven clinicopathological parameters, including bowel wall invasion, lymph node metastasis, distant metastasis, CEA and CA19-9 levels, and SPARC expression in MSC and cancer cells, were recorded for univariate analysis. All these parameters were significant predictors for OS and DFS (Table II), and entered into a multivariate Cox proportional hazards model for analysis. It was found that bowel wall invasion (hazard ratio [HR], 2.331; 95% confidence interval [CI], 1.153-4.711; *p* = 0.018), lymph node metastasis (HR, 2.059; CI, 1.566-2.707; *P* < 0.001), distant metastasis (HR, 4.263; CI, 2.672-6.802; *p* < 0.001), SPARC expression in MSC (HR, 0.654; CI, 0.409-1.048; *p* = 0.028), and the CA19-9 level (HR, 1.595; CI, 1.019-2.498; *p* = 0.041) were in-

dependent prognostic factors for OS. For DFS, bowel wall invasion (HR, 1.842; CI, 1.012-3.350; *p* = 0.045), lymph node metastasis (HR, 1.590; CI, 1.270-1.991; *p* < 0.001), distant metastasis (HR, 2.917; CI, 1.925-4.421; *p* < 0.001), and SPARC expression in MSC (HR, 0.536; CI, 0.359-0.802; *p* = 0.002) were all independent prognostic factors for DFS (Table III). Thus, bowel wall invasion, lymph node metastasis, distant metastasis, and CEA and CA19-9 levels were risk factors for CRC patients, and SPARC expression was a protective factor for CRC patients.

**Discussion**

The role of SPARC expression in CRC has generated considerable interest as a novel target candidate for cancer therapy<sup>24</sup>. To investigate the correlation between SPARC expression and CRC prognosis, SPARC expression in MSC and cancer cells was immunohistochemically investigated in our study. It was found that high SPARC expression in MSC and cancer cells was



**Figure 2.** Prognostic significance assessed using Kaplan–Meier survival estimates and log-rank tests stratified according to SPARC expression. High SPARC expression is associated with increased overall survival and disease-free survival. **(A)** and **(B)**, Kaplan–Meier survival curves showed a significantly increased overall survival and disease-free survival in patients with high SPARC expression as compared with patients with low expression in cancer cells.  $p < 0.05$ , log-rank test. **(C)** and **(D)**, Kaplan–Meier survival curves showed a significantly increased overall survival and disease-free survival in patients with high SPARC intensity scores as compared with patients with low scores in MSC cells.  $p < 0.001$ , log-rank test.

a good factor favorably influencing OS and DFS, suggesting that SPARC may act as a tumor suppressor gene. In addition, patients with low SPARC expression were more likely to exhibit aggressive clinicopathological features, including lymph node metastasis, distant metastasis, and high CEA and CA19-9 levels. A similar inhibitory effect of SPARC has been reported in pancreatic cancer<sup>25</sup>. One proposed mechanism underlying this tumor inhibitory effect may be the ability of SPARC to enhance tumor regression and in-

duce apoptosis<sup>16,26-28</sup>. Interestingly, other studies<sup>29,30</sup> have found that SPARC may regulate cell progression and proliferation in certain tumor types, and promote melanoma cell survival via the p53 pathway. Knockdown of SPARC expression significantly induced ovarian cell apoptosis and inhibited cell invasion and metastasis<sup>23</sup>. Thus, the role of SPARC in different tumor types needs to be further studied.

Our results also showed that SPARC was predominantly expressed in CRC tissues, and its low



**Table II.** Cox's univariate analysis for overall survival and disease-free survival.

Characteristics	OS		DFS	
	HR (95% CI)	p value	HR (95% CI)	p value
Bowel wall invasion (T1-T2 vs T3-T4)	3.269 (1.519-4.379)	0.039	2.800 (1.515-5.177)	0.001
Lymph node metastasis (No vs Yes)	2.225 (1.749-2.831)	<0.001	1.964 (1.603-2.405)	<0.001
Distant metastasis (No vs Yes)	5.524 (3.546-8.604)	<0.001	4.174 (2.793-6.238)	<0.001
SPARC cancer (Low vs High)	0.587 (0.390-0.882)	0.010	0.555 (0.392-0.785)	0.001
SPARC MSC (Low vs High)	0.431 (0.283-0.656)	<0.001	0.384 (0.267-0.552)	<0.001
CEA (< 5 vs > 5 ng/mL)	1.913 (1.311-2.792)	0.001	1.711 (1.246-2.350)	0.001
CA19-9 (< 37 vs > 37 U/mL)	2.410 (1.600-3.631)	<0.001	2.037 (1.420-2.924)	<0.001

*Abbreviations:* SPARC: secreted protein acidic and rich in cysteine; OS: overall survival; DFS: disease-free survival; MSC: the mesenchymal and stromal cells; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen19-9; HR: hazard ratio; CI: confidence interval.

expression was significantly associated with advanced-stage CRC. The intensity of high SPARC expression in MSC was lower in TNM stage III and IV cases than that in stage I and II cases (34.0% vs. 52.6%,  $p < 0.001$ ). The intensity of high SPARC expression in cancer cells was lower in TNM stage III and IV cases than that in stage I and II cases (35.3% vs. 46.6%,  $p = 0.002$ ). Recent studies<sup>31,32</sup> have proposed that this inhibitory effect may be related to tumor growth and progression, and that the loss of SPARC expression may be associated with aberrant hypermethylation of the CpG island in its promoter region. In addition, treatment with the demethylating agent 5-Aza-2'-deoxycytidine could reverse SPARC expression<sup>33</sup>. Based on these findings, we conclude that SPARC is an attractive target for therapeutic strategies in CRC patients.

Matricellular protein SPARC is expressed not only in cancer cells but in MSC surrounding the tumor<sup>34</sup>. Liang et al<sup>35</sup> showed that SPARC expression was significantly higher in MSC than

that in cancer cells, and that only the expression of SPARC in MSC significantly differed depending on the status of certain clinicopathological parameters including tumor differentiation and lymph node metastasis, confirming the finding of other studies that SPARC in MSC was associated with increased patient survival in the microenvironment of diffuse large B-cell lymphoma<sup>36</sup>. Similarly, we found in this study that SPARC expression in MSC was an independent prognostic factor for OS and DFS. The mechanism by which stromal SPARC expression confers a poor prognosis is unknown.

In this study, the expression of SPARC was verified by TMA immunohistochemistry. TMA is a useful technique for large-scale analysis of the expression level of genes of interest. When used for studies on clinical samples to compare normal and diseased tissue, TMA may help identify novel biomarkers. Such biomarkers may be candidates for establishing early diagnosis and designing therapeutic targets for cancer.

**Table III.** Cox's multivariate analysis for overall survival and disease-free survival.

Characteristics	OS		DFS	
	HR (95% CI)	p value	HR (95% CI)	p value
Bowel wall invasion (T1-T2 vs T3-T4)	2.331 (1.153-4.711)	0.018	1.842 (1.012-3.350)	0.045
Lymph node metastasis (No vs Yes)	2.059 (1.566-2.707)	<0.001	1.590 (1.270-1.991)	<0.001
Distant metastasis (No vs Yes)	4.263 (2.672-6.802)	<0.001	2.917 (1.925-4.421)	<0.001
SPARC cancer (Low vs High)	0.805 (0.517-1.255)	0.338	0.770 (0.530-1.120)	0.172
SPARC MSC (Low vs High)	0.654 (0.409-1.048)	0.028	0.536 (0.359-0.802)	0.002
CEA (< 5 vs > 5 ng/mL)	1.171 (0.771-1.781)	0.459	1.123 (0.794-1.588)	0.511
CA19-9 (< 37 vs > 37 U/mL)	1.595 (1.019-2.498)	0.041	1.433 (0.969-2.117)	0.071

*Abbreviations:* SPARC: secreted protein acidic and rich in cysteine; OS: overall survival; DFS: disease-free survival; MSC: the mesenchymal and stromal cells; CEA: carcinoembryonic antigen; CA19-9: carbohydrate antigen19-9; HR: hazard ratio; CI: confidence interval.

Although this study was initially based on a large number of samples, many of them were excluded because of a lack of information regarding the clinicopathological features. Second, the proportion of patients lost to follow-up was as high as 30.9% (810/2618) as per our CRC database. This might introduce a selection bias. Moreover, the uneven distribution of tumors by stage as this adds potential confounding bias. Because CRC patients in our surgery are mainly stage II and III, and our goal is to investigate the expression of SPARC in these people.

### Conclusions

The findings of this study may prove to be clinically significant. First, SPARC could be a novel predictor for clinical prognosis in CRC patients and may be able to distinguish between low- and high-risk CRC patients after surgery. Second, SPARC may mediate cancer progression and represent a promising therapeutic target for CRC molecular treatment. However, the role of SPARC and its potential as a clinical marker for CRC are not fully understood. Further studies are needed to clarify its role in CRC development and progression.

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### Conflict of Interest

The Authors declare that there are no conflicts of interest.

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