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Role of secreted protein acidic in hematogenous metastasis of gastric cancer

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Abstract. – OBJECTIVE: To investigate tumor microenvironment of metastasis (TMEM) and the expression of SPARC (secreted protein acidic and rich in cysteine) in gastric cancer, and their relationships with hematogenous metastasis.

PATIENTS AND METHODS: Twenty-six pairs of cases with gastric cancer were enrolled, in which there were 26 cases with distant organ metastases and 26 cases of gastric cancer without organ metastases as controls. TMEM (by double-stained immunohistochemistry) and the expression of SPARC were determined in twenty-six pairs of cases. In addition, we selected 48 patients to detect the expression of SPARC, VEGF (vascular endothelial growth factor), and evaluated TAMs (tumor associated macrophages), MVD (the microvessel density), MPI (microvessel pericyte coverage index), and TMEM in gastric cancer tissues by immunohistochemistry.

RESULTS: TMEM count was significantly higher in the metastatic gastric cancer tissues than that in non-metastatic cancer tissues in a case-control study ($p<0.01$). On the contrary, SPARC expression was lower in the metastatic gastric cancer tissues than that in non-metastatic cancer tissues. TMEM count, TAMs, and MVD were significantly correlated with invasion depth, histological type and TNM stage ($p<0.05$ or $p<0.01$). Expression of SPARC and VEGF were significantly correlated with tumor histological types, invasion depth, differentiation and lymph node metastasis of patients ($p<0.05$). SPARC and VEGF expression in stromal cells of gastric cancer tissues were significantly correlated with TAMs, MVD and MPI ($p<0.05$). In addition, SPARC expression was significantly inversely correlated with VEGF expression in gastric cancer tissues ($p<0.05$).

CONCLUSIONS: TMEM was detected in initial gastric cancer resection and closely correlated with hematogenous metastasis. Furthermore, SPARC may be involved in gastric cancer metastasis by effecting on tumor microenvironment.

Key Words:
Gastric cancer, SPARC, VEGF, TMEM, TAMs, Pericytes, Angiogenesis, Hematogenous metastasis.

Introduction

Gastric cancer is the second leading cause of cancer-related mortality worldwide[1,2]. On the whole, 56% of new cases and deaths from gastric cancer occurred in less-developed countries[2]. Metastasis, one of the most important reasons that causes treatment failures in gastric cancer, particularly hematogenous metastasis found in patients that experienced recurrence within 1 year of radical resection for gastric cancer[3].

Tumor microenvironment of metastasis (TMEM), a microanatomic landmark that means a perivascular macrophage in contact with a tumor cell, plays an important role in tumor progression. In recent years, some reports demonstrated that TMEM was significantly correlated with increased risk of distant metastasis[4]. The formation of TMEM, as a crucial step of hematogenous metastasis, is in the process of intravasation[5]. If the vessel in tumor is immature, which is not covered totally by pericytes, it can be helpful for tumor cell invasion into blood vessels (intravasation)[6]. The integrity of the vasculature is vital to the control of hematogenous metastasis. Tumor associated macrophages (TAMs), one of the components of TMEM, was associated with a poor progression-free survival and overall survival[7]. TAMs promoted angiogenesis by secreting pro-angiogenic cytokine VEGF, which was demonstrated to be a potent inducer of vascular permeability[8]. Elimination of TAMs in the tumor stroma resulted in the reduction of tumor angiogenesis and markedly suppression of tumor growth and metastasis[9]. Researchers have shown that TAMs could guide tumor cells to blood vessels[10,11].

SPARC (secreted protein acidic and rich in cysteine), a group of non-structural components of the extracellular matrix (ECM), is closely related to anti-angiogenic activity, pro-apoptosis and cell proliferation inhibition in some metastatic tumors[12,13]. In gastric cancer, SPARC is down-regulated owning to the hypermethylation in SPARC

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gene promoter region[14]. The low level of SPARC can promote angiogenesis through increasing the expression of MMP-7 and VEGF[15]. SPARC, as a tumor suppressor, has been found in many kinds of cancers. Nevertheless, the relationship between SPARC and gastric cancer metastasis remains unclear.

As far as known, no studies have reported about TMEM in human gastric cancer. Thus, the aims of the present study were to explore the effects of SPARC in the tumor microenvironment on metastasis, and the relationship between TMEM and hematogenous metastasis.

Patients and Methods

Sample Collection

Specimens from gastric cancer tissues were collected from gastrectomy from January 2009 to January 2011 under the signed agreement of consent forms. Ethics approval was obtained from Third Military Medical University (Chongqing, China). Twenty-four of 48 cases were classified as high- or moderate- differentiated adenocarcinomas and 24 as low- or un-differentiated adenocarcinomas. A total of 48 patients were included, 34 male and 14 female patients (59.0 year, range, 39-82). Amongst these patients, there were 20 intestinal adenocarcinomas and 28 diffuse adenocarcinomas according to the WHO histological classification of gastric carcinoma formulated in 2010. There were 7 cases with vascular tumor emboli and 33 cases with lymph node metastasis. There were 21 cases for stage I-II and 27 for III-IV of TNM. In addition, 20 noncancerous human gastric tissues were obtained from gastrectomies of adjacent gastric cancer margins greater than 5 cm. These samples were used to evaluate TMEM, the microvessel density (MVD), TAMs, microvessel pericyte coverage index (MPI) and the expression of SPARC, vascular endothelial growth factor (VEGF).

Fifty-seven cases with gastric cancer were obtained between January 2008 and December 2016, including 23 cases with developed distant organ metastases and 34 cases of gastric cancer without organ metastases as controls. According to the case history we know whether the patients got distant organ metastases, such as liver, lung, brain, bone. The known prognostic factors such as sex (exactly), tumor grade (exactly), tumor size, presence or absence of lymph node metastasis, and clinical stage did not have significant difference between metastatic patients and controls. However, due to well-differentiated tumor seldom occurred in metastatic patients, only patients with moderately or poorly differentiated tumor were included in this study. TMEM and SPARC were evaluated in these samples.

TMEM Definition by Double-Labeling Immunohistochemical Staining

TMEM was defined as the tripartite arrangement of a tumor cell, a macrophage, and an endothelial cell in direct apposition. Representative sections from each case were stained by using double immunohistochemistry for CD68 (macrophage specific) and CD34 (endothelial cell specific). Sections (4 μm thick) were dewaxed, endogenous peroxidase was inactivated as a previous study[16]. Two primary antibodies were used in the same section. Anti-CD68 monoclonal antibody CD68 (PG-M1, ZhongShan, Beijing, China) and anti-CD34 monoclonal antibody (clone QBEnd/10, Boston, MA, USA) in sections were detected by DouSP™. Immunohistochemical double staining kit (Albany, NY, USA) was used according to the manufacturer's instructions. Hematoxylin was used as counterstain. The criteria of TMEM followed a previous study[17]. Briefly, the sections were reviewed to find 10 vision fields under the low-power microscope and then, switched to high power fields (×400) to identify TMEM. The total number of TMEM was counted for each vision field. TMEM in 10 vision fields was summarized and given a final TMEM count for each section. The results were expressed as the number of TMEM per section. If collagen fibers presented between a perivascular macrophage and the tumor cells or the endothelial cells and macrophages were not apposed, TMEM was not counted[18].

Immunohistochemistry

Our previous study has been performed these samples by immunohistochemistry. The primary antibodies used in this study were as follows: anti-CD68, anti-CD34, anti-SPARC (clone NCL-O-NECTIN, Albany, NY, USA), anti-VEGF (clone SP28, ZhongShan, Beijing, China), anti-SMA (Clone 1A4, 1:100 dilution, Denver, CO, USA). Non-biotin detection system (anti-mouse/rabbit-HRP, Denver, CO, USA) was used to analysis SPARC and VEGF. Double-labeling immunohistochemistry for TMEM was performed as mentioned above. In brief, deparaffinized and rehydrated sections were subjected to antigen retrieval and serum blocking treatments. Slides

were incubated sequentially with primary antibodies at 4ºC overnight and incubated with labeled alkaline phosphatase (AP) conjugate for 30 min. Finally, the visualized color was shown in slides by bromochloroindolylphosphate/nitro blue tetrazolium (BCIP/NBT) chromogen staining. All slides were counterstained with hematoxylin. Sections of known positive specimens were used as positive controls. The sections incubated with PBS instead of primary antibody were used as negative controls. All the results were presented by two independent observers without knowledge of the clinicopathological parameters of the patients.

SPARC Score

The immunostaining intensity of SPARC was reviewed and scored according to the location of cytoplasm[19]. The proportion of cells with SPARC expression was scored as follows: 0 (≤5% positive stromal fibroblast cells); 1 (6-25% positive cells); 2 (26-50% positive cells) and 3 (≥51% positive cells). The SPARC grade of staining intensity divided into 4 grades as follows: 0 (no staining), 1 (weak staining, light yellow), 2 (moderate staining, yellow brown) and 3 (strong staining, brown). The staining index was calculated as the staining intensity score and the proportion of positive stromal cells. The staining index with scores of 0, 1, 2, 3, 4, 6, or 9, a staining index score of ≥4 was used to define stromal cells with high SPARC, and a staining index score of ≤3 was used to indicate low SPARC.

VEGF Score

The results of VEGF were evaluated by scores from a previous study[20]. A score was set up as follows: 1) the percentage of cytoplasmic positive tumor cells (0 point, 0% immunopositive cells; 1 point, ≤25% positive cells; 2 points, 26-50% positive cells; 3 points, ≥51% positive cells); 2) the staining intensity (0 point, negative immunoreaction; 1 point, weak intensity; 2 points, moderated intensity; 3 points, strong intensity). The sum of the two parameters varied between 0 and 6: a negative immunoreaction (-) for a score 0-2 and a positive immunoreaction (+) for a score 3-6.

MPI and MVD Calculation

To analyze the frequency of pericyte-covered vessels, tissue sections were double-stained with CD34 and α-SMA to detect endothelial cells and pericytes simultaneously. The microvessel was counted according to the number of single endothelial cell or endothelial cell cluster showing red granules in the cytoplasm. MVD was expressed as the average number of microvessel in five random fields each slide. Pericytes were defined as a single layer of α-SMA-positive cells colocalized with CD34-positive microvessels. MPI was calculated by the number of microvessels associated with α-SMA-positive pericytes/the total number of microvessels in the five fields of each slide.

TAMs Assessment

TAMs were assessed according to Leek et al[21]. The densest area of TAMs in sections was selected under a microscope (×400, the surface area of every vision field being 0.785 mm^2). The results were expressed as an average number of TAMs in five fields each slide.

Statistical Analysis

All statistical analyses were performed using the SPSS17.0 software (SPSS Inc., Chicago, IL, USA). The difference in TMEM between metastatic and non-metastatic patients was evaluated via using the Wilcoxon signed-rank (matched pairs) test. The statistical significance of differences between two experiment groups or among three experiment groups was determined by Mann-Whitney test, Student's *t*-test, x^2 test or One-way ANOVA, as appropriate. Tukey's HSD (honestly significant difference) test was used in conjunction with an ANOVA to find means that are significantly different from each other. Correlation analysis was also performed through using Spearman rank correlation coefficient test or Pearson product-moment correlation, as appropriate. Statistical significance was set at $p<0.05$.

Results

TMEM and SPARC in 26 Pairs of Gastric Cancer Tissues

TMEM was identified by double-labeling immunohistochemistry. As shown in Figure 1, TMEM is an anatomical site consisting of a macrophage in direct contact with a tumor cell and an endothelial cell by circles. In the case-control study, the averaged count of TMEM in metastatic cases was (18.54 ± 10.76) (ranged from 5 to 47) and (10.69 ± 4.67) (ranged from 3 to 23) in the control group. TMEM count was significantly increased in gastric cancer tissues with metastasis (Figure 1C-D) when compared with patients without metastasis (Figure 1A and B) ($p<0.01$). SPARC expression was mainly localized in the cytoplasm

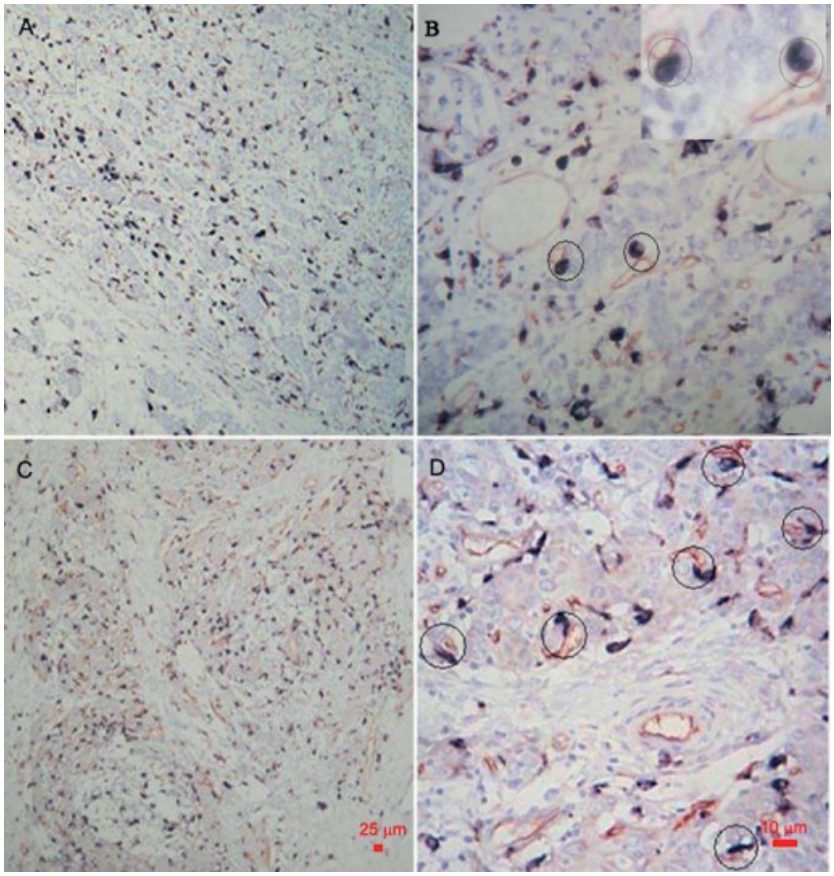

Figure 1. Definition of TMEM by double-labeling immunohistochemical staining (400X). TMEM (in circles) is defined as the tripartite arrangement of a tumor cell (light blue), macrophage (dark blue), and endothelial cell (red) in direct apposition ***(B)***. In this study, TMEM is shown in nonmetastatic (A and B) and metastatic ***(C-D)*** gastric cancer tissues.

of stromal cells surrounding gastric cancer cells. SPARC high expression was detected in 7 of 26 (26.92%) metastatic gastric cancer tissues, 14 of 26 (53.85%) non-metastatic gastric cancer tissues (r=-0.274, p=0.048) (Table I).

Relationship Between TAMs, MVD, TMEM and Clinicopathologic Characteristics

The correlations between TAMs, MVD, TMEM and clinicopathologic characteristics are shown in Table II. TMEM count, TAMs and MVD were significantly correlated with invasion depth, histological type and TNM stage (p<0.05 or p<0.01). This means that TMEM count, TAMs and MVD were different in different group of invasion depth, histological type and TNM stage. Furthermore, TMEM count and TAMs were significantly different between different Lauren types (p<0.01). The number of TAMs and MVD were significantly higher in cases with lymph node metastases than those without lymph node metastases (p<0.05). These indicators made no significant differences in the other groups of the other clinicopathologic characteristics.

Expression of SPARC, VEGF and MPI with Clinicopathologic Characteristics

Expression of SPARC protein was detected by immunohistochemistry staining in 48 cases of gastric cancer tissues and 20 cases of

Table I. SPARC expression of 26 pairs patients of case control study.

	SPARC			
	high	low	r	p
Metastases	7	19	-0.274	0.048
Non-metastases	14	12		

SPARC: secreted protein, acidic and rich in cysteine.

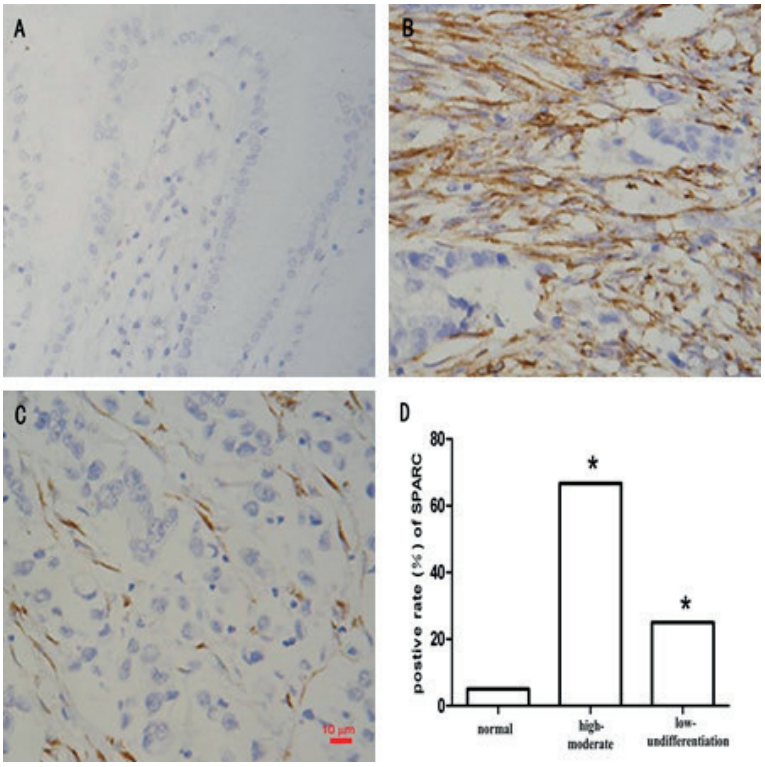

Figure 2. Expression of SPARC and its positive rates in gastric cancer tissues by immunohistochemical staining (400X). No expression was found in non-tumor gastric tissue ***(A)***. High expression of SPARC was found in high-differentiated gastric cancer tissue ***(B)*** and low level of SPARC in low-differentiated cancer tissue ***(C)***. The positive rates of SPARC expression are summarized in nontumor, moderate-high differentiated and low-undifferentiated adenocarcinoma of gastric tissues ***(D)***. $*p<0.05$, compared to the non-tumor gastric tissues.

non-tumor mucosa. SPARC high expression was detected in 1 of 20 (5%) non-tumor mucosa, 16 of 24 (66.7%) high-moderate adenocarcinoma and 6 of 24 (25%) low-undifferentiation adenocarcinoma. High expression of SPARC protein was detected in 22 (45.83%) cases of gastric cancer, and 26 (54.17%) cases with low SPARC expression. Expression of SPARC was significantly higher in gastric cancer than that in non-tumor tissues ($p<0.05$) (Figure 2D). Expression of SPARC, VEGF and MPI with clinicopathologic characteristics are shown in Table III. Expression of SPARC and VEGF in gastric cancer tissues were significantly related to tumor Lauren types, invasion depth, histological types and lymph node metastasis ($p<0.05$). However, SPARC expression was also correlated with TNM stage of patients ($p=0.049$). Pericytes were defined as a single layer of α-SMA-positive cells surrounding with CD34-positive microvessels. MPI was related to histological types of tumor ($p=0.002$) and inversely correlated with vascular tumor ($p=0.002$).

Relationship Among Expression SPARC and VEGF, with TAMs, MVD and MPI

SPARC expression was significantly inversely correlated with VEGF expression in gastric cancer tissues (r=-0.454, $p=0.001$) (Table IV). SPARC expression in stromal cells of gastric cancer tissues was inversely correlated with TAMs, MVD, and positive correlated with MPI ($p<0.05$) (Figure 3A-B, and C). Expression of VEGF showed a positive correlation with TAMs and MVD ($p<0.05$) (Figure 3A-B).

Effects of TAMs on MVD and MPI

The results showed that TAMs had a positive correlation with MVD and a negative correlation with MPI ($p<0.05$) (Figure 4A-B). However, no relationship was found between MVD and MPI ($r = 0.243$; $p = 0.095$).

Table II. Relationship of TAMs, MVD and TMEM with clinicopathologic characteristics.

Parameters	N	TAMs ($\bar{x}$±s)	p	MVD ($\bar{x}$±s)	p	TMEM ($\bar{x}$±s)	p
Gender							
male	36	84.68±23.09	0.689	56.22±12.43	0.963	10.38±9.06	
female	12	81.24±30.27		55.98±20.85		9.73±8.77	0.834
Age							
<50	10	83.09±17.74	0.893	56.69±14.82		9.08±4.80	
≥50	38	84.18±26.94		55.97±14.62	0.879	10.66±10.04	0.590
Tumor size (cm)							
<2	8	75.60±29.15		54.30±15.31		4.25±5.45	
2≤tumor size <5	24	85.95±23.83	0.586	55.18±11.88	0.721	11.67±10.36	
≥5	16	84.94±24.21		56.16±14.52		10.23±8.90	
Lauren type							
intestinal	28	77.29±22.48		55.17±15.52		8.06±6.52	
diffuse	20	101.65±21.71	0.002	58.85±11.50	0.441	16.08±11.81	0.004
Histological type							
High-moderate	28	71.02±19.58		51.41±11.30		6.86±6.12	
Low-undifferentiation	20	101.90±19.18	0.001	62.82±16.13	0.006	14.96±10.13	0.001
Invasion depth							
T1-T2	12	63.51±21.49	0.001	47.36±10.99		5.09±57.83	
T3-T4	36	89.95±22.31		58.78±14.52	0.02	11.76±8.72	0.028
LN (number)							
N0	11	68.43±21.82	0.01	47.43±12.31	0.015	8.00±8.16	0.322
N1-N3	37	89.04±23.54		59.07±14.16		10.97±9.13	
Vascular tumor							
emboli negative	41	81.01±24.43	0.177	55.62±15.41	0.198	8.63±7.06	0.152
positive	7	94.49±21.09		59.74±5.29		12.71±5.19	
TNM stage							
I-II	21	66.61±19.24	0.001	48.19±19.32	0.002	6.18±6.93	0.014
III-IV	27	94.22±22.1		61.32±13.91		12.73±9.20	

TAMs: tumor associated macrophages; TMEM: tumor microenvironment of metastasis; MVD: microvessel density; LN: tumor lymph nodes metastasis; TNM stage: TNM Classification of Malignant Tumors.

Discussion

In our investigation, our finding in a case-control study showed that the TMEM at initial gastric cancer resection was associated to risk of hematogenous metastasis in gastric cancer patients. In other groups based on the differentiation, our results showed that expression of SPARC was related to clinicopathologic characteristics such as tumor histological types, invasion depth and lymph node metastasis. It was also significantly correlated with TAMs, MVD and MPI. However, SPARC expression was significantly inversely correlated to VEGF expression in gastric cancer tissues. It indicated that SPARC may play some roles in the invasion and metastasis of gastric cancer by regulating the tumor microenvironment. Inflammation microenvironment plays a vital role in the progression of tumor. TMEM, which was comprehensively considered as the inflammation microenvironment about interaction between the tumor cells and stromal cells, was more important than the traditional prognostic factors such as the tumor size, differentiation, lymph node metastasis and vascular invasion because these were focused on tumor cell itself. In our case-control study of metastatic and non-metastatic gastric cancers, our results showed a high TMEM count in metastatic cases when compared with non-metastatic cases. It suggested that high count of TMEM was associated

Table III. Relationship of SPARC, VEGF and MPI with clinicopathologic characteristics.

		SPARC			VEGF			MPI	
Parameters	n	high	low	p	(+)	(-)	p		p
Gender	34	16	18	0.791	24	10	0.572	45.34±23.33	0.868
male	14	6	8		11	3		46.35±23.85	
female									
Age									
<50	7	4	3	0.516	6	1	0.410	43.78±25.80	0.463
≥50	41	18	23		29	12		45.99±22.79	
Tumor size (cm)									
<2	8	2	6		5	3		40.28±17.47	
2≤tumor size < 5	24	15	9	0.065	15	9	0.072	48.97±24.19	0.612
≥5	16	5	11		15	1		42.99±24.55	
Lauren type									
intestinal	20	13	7	0.024	10	10	0.003	48.44±23.94	0.280
diffuse	28	9	19		25	3		39.70±21.06	
Histological type									
High-moderate	24	16	8	0.004	13	11	0.003	54.97±21.94	0.002
Low-undifferentiation	24	6	18		22	2		35.27±20.28	
Invasion depth									
T1-T2	13	5	14	0.028	13	0	0.010	49.63±20.45	
T3-T4	35	17	12		22	13		43.84±24.29	0.437
LN (number)									
N0	15	13	7	0.024	17	2	0.037	37.37±21.66	
N1	33	9	19		18	11		47.67±23.35	0.215
Vascular tumor									
emboli negative	41	21	20		28	13		56.76±17.71	0.002
positive	7	1	6	0.070	7	0	0.081	29.94±18.30	
TNM stage									
I-II	21	13	8	0.049	15	6	0.838	27.50±22.61	0.386
III-IV	27	9	18		20	7		32.82±19.33	

SPARC: secreted protein, acidic and rich in cysteine; MPI: microvessel pericyte coverage index; VEGF: vascular endothelial growth factor.

with risk of metastasis and could predict the development of metastases in gastric cancers. In another group study, our results showed no relationship between TMEM and lymph node metastasis, indicating that tumor cell might invade into the blood vessels and form distant metastases. Therefore, TMEM could predict the development of hematogenous metastasis in gastric cancer. TAMs, one of the components of TMEM, played an essential role in tumor cell intravasation. Intravasation of a tumor cell is crucial to hematogenous metastasis. The role of macrophages in tumor cell migration and intravasation involved a loop in which tumor cells produced colony-stimulating factor-1 (CSF-1) recruited macrophages, which in turn secreted epidermal growth factor (EGF), and promoted

Table IV. Correlating analysis of SPARC and VEGF expression.

	SPARC			
VEGF	high	low	r	p
positive	10	22	-0.454	0.001
negative	12	4		

SPARC: secreted protein, acidic and rich in cysteine; VEGF: vascular endothelial growth factor.

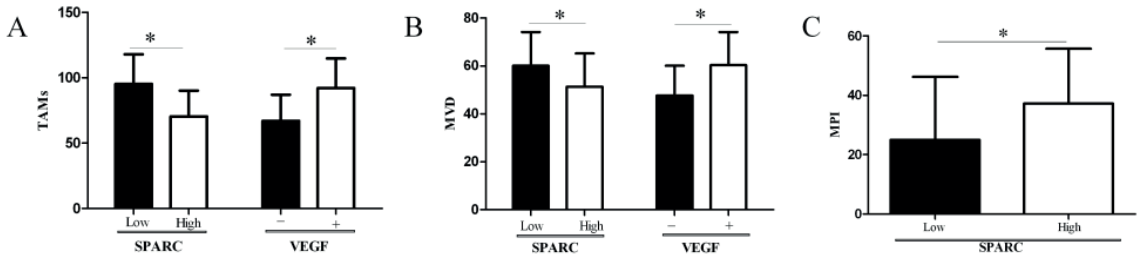

Figure 3. Relationship among expression of SPARC, VEGF, TAMs, MVD and MPI in gastric cancer tissues (400X). SPARC and VEGF expression in gastric cancer tissues were significantly related with TAMs and MVD (A and B). SPARC expression in gastric cancer tissues showed a positive correlation with MPI ***(C)***. $*p<0.05$, compared with two independent groups.

tumor cell migration[10,11]. Using the CSF1R inhibitors reduced metastatic through forbidding the macrophage recruitment to the tumor environment in pancreatic cancer[22]. Tumor cell intravasation was only observed in association with perivascular macrophages and was not shown in regions of blood vessels without perivascular macrophages[23]. Reduction of TAMs in the tumor stroma could decrease the formation of angiogenesis and markedly suppress tumor growth and metastasis[9]. Tumor cell intravasation is also related to vessel density (MVD) and vessel maturation (MPI). Our findings displayed an increasing MVD and decreasing MPI in the low- or undifferentiated gastric adenocarcinoma. No relationship was observed between MPI and MVD (data not shown). This indicates that new immature microvessels facilitated intravasation and extravasation of tumor cells. Gerhardt et al[24] considered pericytes as "gatekeepers" in metastasis of tumor cell. Lack of pericyte coverage dramatically increased tumor cell dissemination and was significantly negative correlated to hematogenous metastasis[25,26]. The lower MPI led to the more vascular tumor emboli, suggesting that tumor cells are intravasated into immature microvessels more easily than into mature microvessels. Recovery of pericyte coverage could decrease invasion of tumor cells into blood vessels in prostate cancer xenografts[6]. Thus, these results showed that the integrity of the vasculature was vital to the control of hematogenous metastasis. In some studies[27], targeting abnormal polarization of TAMs could inhibit metastasis in part by increasing pericyte coverage and promote normalizes tumor vessels. In our work, the results showed that TAMs correlated with the expression of VEGF and MVD. Another study showed that TAMs were significantly negative correlated to MPI in oral squamous cell carcinoma[28]. Our research also showed there was a negative correlation between TAMs and MPI. It suggests that there might present a complex crosstalk and synergistic effect between TAMs and pericytes in tumor invasion and metastasis. How do TAMs work in the development of gastric cancer as an important component of TMEM? Answers may be as follow: 1- by secreting some factor such as CSF-1/EGF to promote the invasion and metastasis; 2- by affecting the expression of VEGF and angioge-

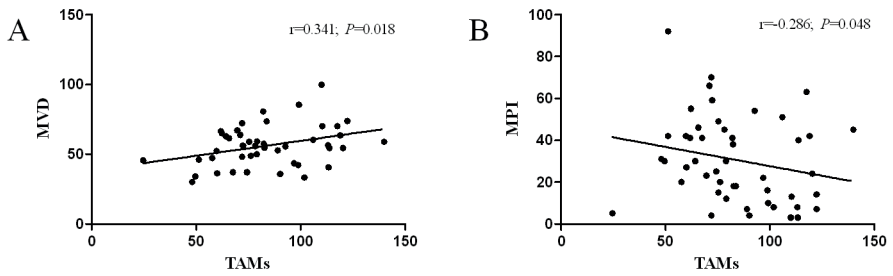

Figure 4. Correlated analyses of TAMs with MVD and MPI (400X). TAMs have a significant positive correlation with MVD ***(A)*** and a significant negative correlation with MPI ***(B)***.

nesis; 3- by inducing tumor cells into the blood vessels; 4- by reducing pericytes and disruption of vascular integrity. In our investigation, the results showed that the number of TAMs was significantly correlated to the histological type, differentiation, invasion depth and TNM stage. The number of TAMs was also significantly higher in cases with lymph node metastases than in those without. These data indicated that TAMs were closely related to the progression of gastric cancer. According to the differentiation, we selected gastric cancer specimens to evaluate TAMs, MPI and the expression of SPARC, and results showed that SPARC expression had a reversely correlation with TAMs and a positive correlation with MPI in gastric cancer tissues. The relative expression level of the SPARC gene was higher in cancer tissue than in non-tumor mucosa in gastric cancer[29]. Similar results were obtained in our research. SPARC also expressed lower in metastatic gastric cancer tissues than that in non-metastatic gastric cancer tissues. These consequences suggest that the microenvironment in which tumors grown in the absence of SPARC is immunosuppressive, protumorigenic and metastatic. Using orthotopic model of pancreatic cancer, the enhancement of metastatic progression in the absence of host SPARC was a result of hematological tumor cell dissemination augmented by the increase in TAMs and the loss of pericyte coverage[27]. Our paper also showed the expression of SPARC correlated to MPI and inversely with MVD. Restoring SPARC expression in ovarian cancer cell lines significantly decreased recruitment of macrophages and down-regulation of the associated inflammation[30]. Several studies showed that SPARC had an anti-angiogenic activity[5,31]. In our work, SPARC expression in tumor stromal cells was significantly negative related to MVD and VEGF, indicating that loss of SPARC might be effect on the angiogenesis of gastric cancer. Therefore, the infiltration of TAMs and the tumor vasculature in gastric cancer would be altered as a result of SPARC expression. However, it is still unclear the mechanisms of SPARC expression effects on TAMs, the pericytes coverage and angiogenesis in stromal cells of gastric cancer. In addition, based on available references and our results, SPARC could decrease tumor infiltration by macrophages through down-regulation of monocyte chemoattractant protein-1 (MCP-1), promote pericyte recruitment via inhibition of endoglin-dependent TGF-β activity and inhibit VEGF-mediated angiogenesis by altering MMP-9 expression[32-34]. In our study, SPARC expression was significantly reversely correlated to VEGF expression in gastric cancer. It suggests that low level of SPARC may increase VEGF expression during the process of new blood vessel growth by which indirect controlling the development, growth, invasion and metastasis of gastric cancer. A low level of SPARC expression promotes angiogenesis and infiltration of TAMs in gastric cancer. Plenty of TAMs increase the chance of formation of TMEM, then through a series of complex interactions of tumor cell-TAMs-endothelial cell promotes tumor metastasis. A lot of molecular interactions in the mechanism of TMEM promote gastric carcinoma metastasis, and our work proves SPARC may be involved.

Conclusions

We showed that TMEM density at initial gastric cancer resection was associated with risk of hematogenous metastasis in gastric cancer patients. TMEM could be a potential prognostic indicator for patients at initial gastric cancer resection. Expression of SPARC was significantly correlated to TAMs, MVD and MPI and inversely correlated to VEGF expression in gastric cancer tissues. Both of them were related to clinicopathologic characteristics such as tumor types, invasion depth and lymph node metastasis as well as TAMs, MVD and MPI. Thus, SPARC may alter the tumor microenvironment, and then effects on gastric cancer metastasis.

Conflict of interest

The authors declare no conflicts of interest.

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