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Original Article

Roles of tumor microenvironment of metastasis and secreted protein acidic and rich in cysteine in human gastric cancer hematogenous metastasis

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Abstract: *Background and objectives:* Tumor microenvironment of metastasis (TMEM) is a crucial step of hematogenous metastasis of tumor. Secreted protein acidic and rich in cysteine (SPARC) can inhibit angiogenesis and tumor cell proliferation. This study aimed to investigate TMEM and SPARC expression in gastric cancer, and their relationships with hematogenous metastasis. *Methods:* 26 pairs of cases with gastric cancer were chosen, including 26 cases with developed distant organ metastases and 26 cases of gastric cancer without organ metastases as controls. TMEM and SPARC expression were determined. In addition, specimens of 48 gastric cancer patients were selected to detect the expression of SPARC, vascular endothelial growth factor (VEGF), tumor associated macrophages (TAMs), microvessel density (MVD), microvessel pericyte coverage index (MPI) and TMEM in tumor tissues. *Results:* TMEM count was significantly higher in the metastatic gastric cancer tissues than that in non-metastatic cancer tissues in case-control study ($P < 0.01$). On the contrary, SPARC expression in metastatic gastric cancer tissues was lower than that in non-metastatic cancer tissues. TMEM count, TAMs and MVD were significantly correlated with tumor 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 ($P < 0.05$). SPARC expression was significantly inversely correlated with VEGF expression in gastric cancer tissues ($P < 0.05$). *Conclusion:* TMEM is closely correlated with hematogenous metastasis of gastric cancer. SPARC may be involved in gastric cancer metastasis by effecting on tumor microenvironment.

Keywords: Gastric cancer, SPARC, VEGF, TMEM, TAMs, 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 occur in less-developed countries [2]. Metastasis is one of the most important reasons that causes treatment failure in gastric cancer, particularly hematogenous metastasis found in patients that experienced recurrence within 1 year radical resection for gastric cancer [3].

Tumor microenvironment of metastasis (TMEM), a microanatomic landmark which means a perivascular macrophage in contact with a tumor cell, plays an important role in tumor progression. In recent years, some reports have demonstrated that TMEM is significantly correlated with increased risk of distant metastasis [4]. TMEM is a crucial step of hematogenous metastasis, and is involved in the process of intravasation [5]. If vessels in tumor are immature, which are not covered totally by pericytes, they 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), the components of TMEM, are associated with a poor progression-free survival and overall survival [7]. TAMs promote angiogenesis by secreting pro-angiogenic cytokine vascular endothelial growth factor (VEGF), which has demonstrated to be a po-

tent inducer of vascular permeability [8]. Elimination of TAMs in the tumor stroma results in the reduction of tumor angiogenesis and markedly suppression of tumor growth and metastasis [9]. Researchers have shown that TAMs can 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, is closely related to the anti-angiogenic activity, pro-apoptosis and cell proliferation inhibition in some metastatic tumors [12, 13]. In gastric cancer, SPARC expression is down-regulated owning to the hypermethylation in its gene promoter region [14]. The low level of SPARC can promote angiogenesis through increasing the expression of matrix metalloproteinase-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 is still unclear. As far as known, no study has been reported about TMEM in human gastric cancer. This study aimed to explore the effects of SPARC in the tumor microenvironment on metastasis of human gastric cancer, and the relationship between TMEM and hematogenous metastasis.

Materials and methods

Sample collection

Specimens from 48 cases of gastric cancer tissues were collected from gastrectomy from January 2009 to January 2011. Twenty-four cases were classified as high- or moderate-differentiated adenocarcinomas and 24 as low- or un-differentiated adenocarcinomas. Total 48 patients including 34 male and 14 female patients (59.0±7.5 years, range, 39-82 years) were enrolled. 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 are 21 cases for stage I-II and 27 for III-IV of TNM. In addition, 20 noncancerous human gastric tissues were also 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 and VEGF.

Twenty-six pairs patients with gastric cancer were obtained between January 2002 and December 2011, including 26 cases with developed distant organ metastases and 26 cases of gastric cancer without organ metastases as controls. According to the case history, it was known 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. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Harbin Medical University. Written informed consent was obtained from all participants.

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 deparaffinized, endogenous peroxidase was inactivated as the previous study [16]. Two primary antibodies were used in the same section. Anti-CD68 monoclonal antibody CD68 (PG-M1, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China) and anti-CD34 monoclonal antibody (clone QBEnd/10, Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China) in sections were detected by DouSP™. Immunohistochemical double staining kit (Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China) 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

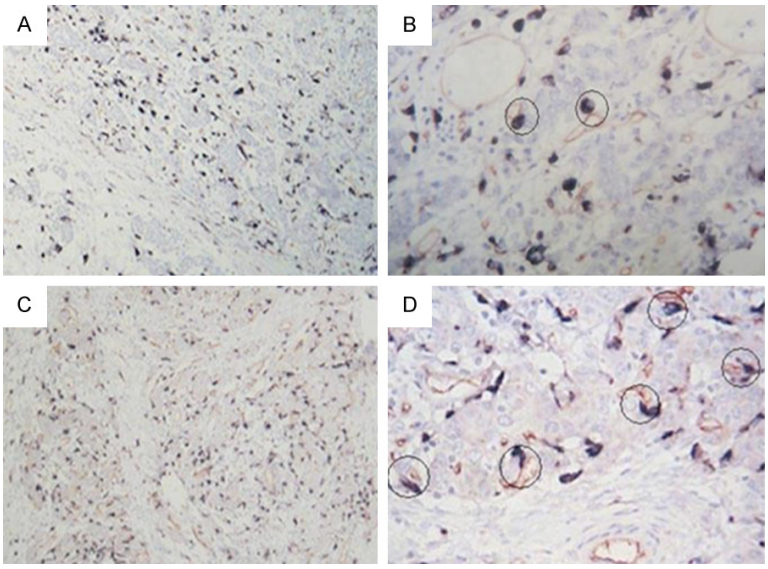

Figure 1. Identification of TMEM in gastric cancer tissues by double-labeling immunohistochemical staining. A: Non-metastatic, × 100; B: Non-metastatic, × 400; C: Metastatic, × 100; D: Metastatic, × 400. TMEM (in circles) was defined as the tripartite arrangement of a tumor cell (light blue), macrophage (dark blue), and endothelial cell (red) in direct apposition. It showed TMEM count in metastatic group increased than non-metastasis group. TMEM, tumor microenvironment of metastasis.

Table 1. SPARC expression of twenty-six pairs gastric cancer patients of case-control study

	SPARC		*r*	*P*
	High	Low		
Metastases	7	19	-0.274	0.048
Non-metastases	14	12		

SPARC, secreted protein, acidic and rich in cystein.

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

The immunohistochemistry of the samples was performed in our previous study. The primary antibodies used in this study were as follows: anti-CD68, anti-CD34, anti-SPARC (clone NCL-O-NECTIN, Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China), anti-VEGF (clone SP28, Beijing Zhongshan Golden Bridge Biotechnology Co., Ltd., Beijing, China), anti-SMA (Clone 1A4, 1:100 dilution, Fuzhou Maixin Biotechnology Development Co., Ltd., Fuzhou, China). Non-biotin detection system (anti-mouse/rabbit-HRP, EliVision, Dako Inc., Carpinteria, CA, 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 then incubated sequentially with primary antibodies at 4°C overnight and then incubated with labeled alkaline phosphatase conjugate for 30 min. Finally, the visualized color was shown in slides by bromochloroindolyl phosphate/nitro blue tetrazolium 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

Table 2. Relationship of TAMs, MVD and TMEM with clinicopathological characteristics

Parameters	n	TAMs	*P*	MVD	*P*	TMEM	*P*
Gender			0.689		0.963		0.834
Male	36	84.68±23.09		56.22±12.43		10.38±9.06	
Female	12	81.24±30.27		55.98±20.85		9.73±8.77	
Age (years)			0.893		0.879		0.590
< 50	10	83.09±17.74		56.69±14.82		9.08±4.80	
≥ 50	38	84.18±26.94		55.97±14.62		10.66±10.04	
Tumor size (cm)			0.586		0.721		0.503
≤ 2	8	75.60±29.15		54.30±15.31		4.25±5.45	
2-5	24	85.95±23.83		55.18±11.88		11.67±10.36	
≥ 5	16	84.94±24.21		56.16±14.52		10.23±8.90	
Lauren type			0.002		0.441		0.004
Intestinal	28	77.29±22.48		55.17±15.52		8.06±6.52	
Diffuse	20	101.65±21.71		58.85±11.50		16.08±11.81	
Histological type			0.001		0.006		0.001
High-moderate differentiation	28	71.02±19.58		51.41±11.30		6.86±6.12	
Low or un-differentiation	20	101.90±19.18		62.82±16.13		14.96±10.13	
Invasion depth			0.001		0.020		0.028
T1-T2	12	63.51±21.49		47.36±10.99		5.09±57.83	
T3-T4	36	89.95±22.31		58.78±14.52		11.76±8.72	
LN (number)			0.010		0.015		0.322
N0	11	68.43±21.82		47.43±12.31		8.00±8.16	
N1-N3	37	89.04±23.54		59.07±14.16		10.97±9.13	
Vascular tumor			0.177		0.198		0.152
Emboli negative	41	81.01±24.43		55.62±15.41		8.63±7.06	
Positive	7	94.49±21.09		59.74±5.29		12.71±5.19	
TNM stage			0.001		0.002		0.014
I-II	21	66.61±19.24		48.19±19.32		6.18±6.93	
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.

as follows: i) 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); ii) 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 microvessels were 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/total number of microvessels in the five fields of each slide.

TAMs assessment

TAMs were assessed according to Leek et al's study [21]. The densest area of TAMs in sections was selected under a microscope (× 400, the surface area of every vision field being

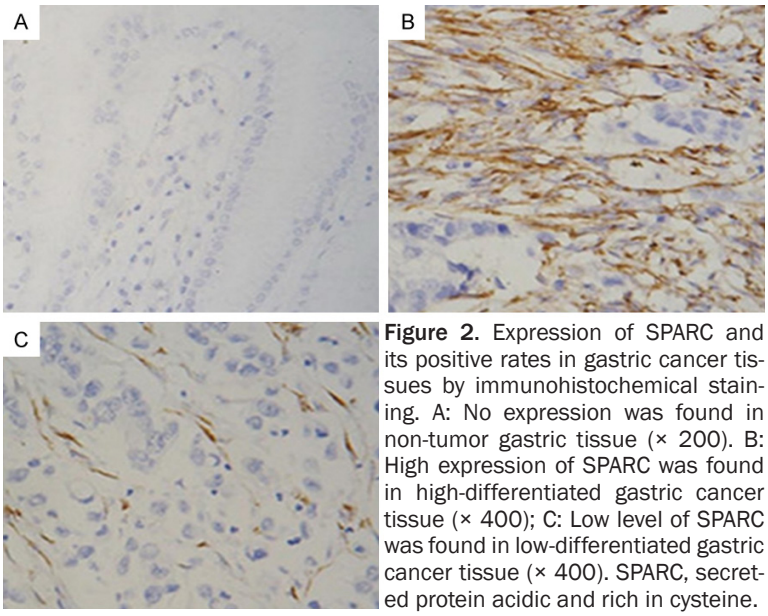

Figure 2. Expression of SPARC and its positive rates in gastric cancer tissues by immunohistochemical staining. A: No expression was found in non-tumor gastric tissue (× 200). B: High expression of SPARC was found in high-differentiated gastric cancer tissue (× 400); C: Low level of SPARC was found in low-differentiated gastric cancer tissue (× 400). SPARC, secreted protein acidic and rich in cysteine.

0.785 mm^2). The results were expressed an averaged number of TAMs in five fields each slide.

Statistical analysis

All statistical analyses were performed using the SPSS 17.0 software (IBM Inc., Chicago, IL). The difference in TMEM between metastatic and non-metastatic patients was evaluated 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*, chi-square test or One-way ANOVA, as appropriate. Correlation analysis was also performed through using Spearman's rank correlation coefficient test or Pearson product-moment correlation, as appropriate. Statistical significance was set at $P < 0.05$.

Results

TMEM and SPARC

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. Compared with patients without metastasis, TMEM count was increased in gastric cancer tissues with metastasis (**Figure 1**). SPARC expression was mainly localized in the cytoplasm 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 1**).

Relationship between TAMs, MVD, TMEM and clinicopathological characteristics

As shown in **Table 2**. TMEM count, TAMs and MVD were significantly correlated with invasion depth, histological type and TNM stage ($P < 0.05$ or $P < 0.01$). This meant 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 type ($P < 0.01$). The number of TAMs and MVD were significantly higher in cases with lymph node metastases than that in those without lymph node metastases ($P < 0.05$). These indicators had no significant relationship with other clinicopathological characteristics.

Expression of SPARC, VEGF and MPI with clinicopathological characteristics

Expression of SPARC protein was detected by immunohistochemistry staining in 48 cases of gastric cancer tissues and 20 cases of non-tumor mucosa (**Figure 2**). 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- or un-differentiated 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, VEGF and MPI with clinicopathological characteristics were shown in **Table 3**. 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,

Table 3. Relationship of SPARC, VEGF and MPI with clinicopathological characteristics

Parameters	n	SPARC		*P*	VEGF		*P*	MPI	*P*
		High	Low		(+)	(-)			
Gender				0.791			0.572		0.868
Male	34	16	18		24	10		45.34±23.33	
Female	14	6	8		11	3		46.35±23.85	
Age (years)				0.516			0.410		0.463
< 50	7	4	3		6	1		43.78±25.80	
≥ 50	41	18	23		29	12		45.99±22.79	
Tumor size (cm)				0.065			0.072		0.612
≤ 2	8	2	6		5	3		40.28±17.47	
2-5	24	15	9		15	9		48.97±24.19	
≥ 5	16	5	11		15	1		42.99±24.55	
Lauren type				0.024			0.003		0.280
Intestinal	20	13	7		10	10		48.44±23.94	
Diffuse	28	9	19		25	3		39.70±21.06	
Histological type				0.004			0.003		0.002
High-moderate differentiation	24	16	8		13	11		54.97±21.94	
Low or un-differentiation	24	6	18		22	2		35.27±20.28	
Invasion depth				0.028			0.010		0.437
T1-T2	13	5	14		13	0		49.63±20.45	
T3-T4	35	17	12		22	13		43.84±24.29	
LN (number)				0.024			0.037		0.215
N0	15	13	7		17	2		37.37±21.66	
N1	33	9	19		18	11		47.67±23.35	
Vascular tumor				0.070			0.081		0.002
Emboli negative	41	21	20		28	13		56.76±17.71	
Positive	7	1	6		7	0		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; LN, tumor lymph nodes metastasis.

Table 4. Correlating analysis of SPARC and VEGF expression

VEGF	SPARC (n)		*r*	*P*
	High	Low		
Positive (n)	22	10	-0.454	0.001
Negative (n)	4	12		

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

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

As shown in **Table 4**, SPARC expression was significantly inversely correlated with VEGF expression in gastric cancer tissues ($r = -0.454$, $P = 0.001$). SPARC expression in stromal cells of gastric cancer tissues was inversely correlated with TAMs, MVD, and positive correlated with MPI ($P < 0.05$). Expression of VEGF showed a positive correlation with TAMs and MVD ($P < 0.05$) (**Figure 3**).

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 4**). However, no rela-

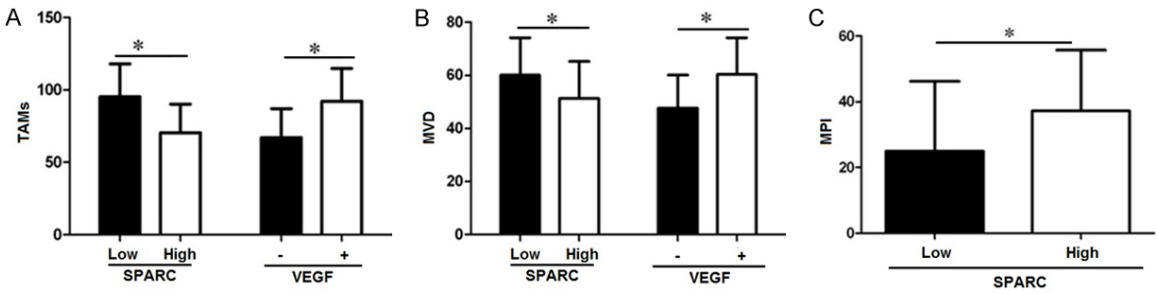

Figure 3. Relationship among expression of SPARC, VEGF, TAMs, MVD and MPI in gastric cancer tissues. SPARC and VEGF expression in gastric cancer tissues were significantly related with TAMs and MVD (A and B). SPARC expression in gastric cancer tissues showed a positive correlation with MPI (C). *$P < 0.05$ compared between two independent groups. SPARC, secreted protein acidic and rich in cysteine; VEGF, vascular endothelial growth factor; TAMs, tumor associated macrophages; MVD, microvessel density; MPI, microvessel pericyte coverage index.

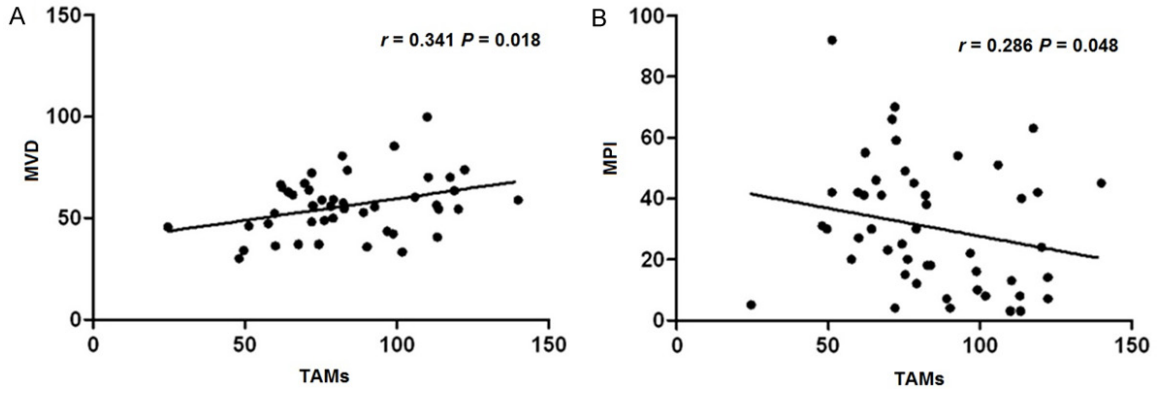

Figure 4. Correlated analyses of TAMs with MVD and MPI. TAMs had significant positive correlation with MVD (A) and significant negative correlation with MPI (B). TAMs, tumor associated macrophages; MVD, microvessel density; MPI, microvessel pericyte coverage index.

tionship was found between MVD and MPI (r = 0.243; P = 0.095).

Discussion

The present case-control study showed that, the TMEM at initial gastric cancer resection was associated with risk of hematogenous metastasis in gastric cancer patients. In other groups based on the differentiation, the expression of SPARC was related to clinicopathological 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 with VEGF expression in gastric cancer tissues. This indicates 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 is comprehensively considered as the inflammation microenvironment about interaction between the tumor cells and stromal cells, is 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, the results showed a high TMEM count in metastatic cases when compared with non-metastatic cases. This suggests that, high count of TMEM is associated with risk of metastasis, and can predict the development of metastases in gastric cancers. In another group study, the 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 can predict the development of hematogenous metastasis in gastric cancer. TAMs, one of the components of TMEM, play an essential role in tumor cell in-

travasation. Intravasation of a tumor cell is a crucial step in hematogenous metastasis. The role of macrophages in tumor cell migration and intravasation involves a loop in which tumor cells produce colony-stimulating factor-1 (CSF-1) recruited macrophages, which in turn secrete epidermal growth factor (EGF), and promote tumor cell migration [10, 11]. Using CSF-1 receptor inhibitors can reduce the metastatic through forbidding the macrophage recruitment to the tumor environment in pancreatic cancer [22]. Tumor cell intravasation is only observed in association with perivascular macrophages and is not shown in regions of blood vessels without perivascular macrophages [23]. Reduction of TAMs in the tumor stroma can decrease the formation of angiogenesis and markedly suppress tumor growth and metastasis [9]. Tumor cell intravasation is also related to MVD and vessel maturation (MPI). Our results displayed an increasing MVD and decreasing MPI in the low- or undifferentiated gastric adenocarcinoma. No relationship was observed between MPI and MVD. This indicates that, new immature microvessels can facilitate the intravasation and extravasation of tumor cells. Some researchers consider pericytes as "gatekeepers" in metastasis of tumor cell [24]. Lack of pericyte coverage can dramatically increase the tumor cell dissemination, and is significantly negative correlated with hematogenous metastasis [25, 26]. The lower MPI leads to the more vascular tumor emboli, suggesting that tumor cells can invade into immature microvessels more easily than into mature microvessles. Recovery of pericyte coverage can decrease invasion of tumor cells into blood vessels in prostate cancer xenografts [6]. Thus, the integrity of the vasculature is vital to the control of hematogenous metastasis. Targeting abnormal polarization of TAMs can inhibit metastasis partially by increasing pericyte coverage, and promote normalizes tumor vessels [27]. The results of our study showed that, TAMs correlated with the expression of VEGF and MVD. Another study showed that TAMs were significantly negative correlated with MPI in oral squamous cell carcinoma [28]. Our study also showed that there was a negative correlation between TAMs and MPI. It suggests that there may 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: i) by secreting some factor such as CSF-1/EGF to promote the invasion and metastasis; ii) by affecting the expression of VEGF and angiogenesis; iii) by inducing tumor cells into the blood vessels; iv) by reducing pericytes and disruption of vascular integrity.

In our study, the result showed that the number of TAMs was significantly correlated with 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 lymphatic metastasis. These results 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 is higher in cancer tissue than in non-tumor mucosa in gastric cancer [29]. Similar results were obtained in our study. SPARC is also lower expressed 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 is a result of hematological tumor cell dissemination augmented by the increase in TAMs and the loss of pericyte coverage [27].

Our study also showed that, the expression of SPARC correlated with MPI and inversely with MVD. Restoring SPARC expression in ovarian cancer cell lines can significantly decrease the recruitment of macrophages and down-regulation of the associated inflammation [30]. Recently, several studies show that SPARC has an anti-angiogenic activity [5, 31]. In our study, 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 about the mechanism of SPARC in

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, promote pericyte recruitment via inhibition of endoglin-dependent transforming growth factor-β activity and inhibit VEGF-mediated angiogenesis by altering matrix metalloprotein-9 expression [32-34]. In our study, SPARC expression was significantly reversely correlated with 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. 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 interaction in the mechanism of TMEM promotes gastric carcinoma metastasis, and SPARC may be involved in the metastasis.

In conclusion, TMEM density at initial gastric cancer resection is associated with risk of hematogenous metastasis in gastric cancer patients. TMEM can be a potential prognostic indicator for patients at initial gastric cancer resection. Expression of SPARC is significantly correlated with TAMs, MVD and MPI, and inversely correlated with VEGF expression in gastric cancer tissues. Both of them are related to clinicopathological 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 effect on gastric cancer metastasis.

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Disclosure of conflict of interest

None.

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